

frontiers

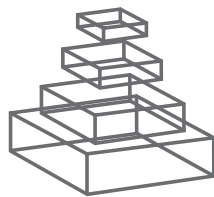
RESEARCH TOPICS

STRIATAL SIGNALING: TWO DECADES OF PROGRESS

Hosted by
Emmanuel Valjent



frontiers in
NEUROANATOMY



frontiers

FRONTIERS COPYRIGHT STATEMENT

© Copyright 2007-2012
Frontiers Media SA.
All rights reserved.

All content included on this site, such as text, graphics, logos, button icons, images, video/audio clips, downloads, data compilations and software, is the property of or is licensed to Frontiers Media SA ("Frontiers") or its licensees and/or subcontractors. The copyright in the text of individual articles is the property of their respective authors, subject to a license granted to Frontiers.

The compilation of articles constituting this e-book, as well as all content on this site is the exclusive property of Frontiers. Images and graphics not forming part of user-contributed materials may not be downloaded or copied without permission.

Articles and other user-contributed materials may be downloaded and reproduced subject to any copyright or other notices. No financial payment or reward may be given for any such reproduction except to the author(s) of the article concerned.

As author or other contributor you grant permission to others to reproduce your articles, including any graphics and third-party materials supplied by you, in accordance with the Conditions for Website Use and subject to any copyright notices which you include in connection with your articles and materials.

All copyright, and all rights therein, are protected by national and international copyright laws.

The above represents a summary only. For the full conditions see the Conditions for Authors and the Conditions for Website Use.

Cover image provided by Ibbl sarl, Lausanne CH

ISSN 1664-8714

ISBN 978-2-88919-070-6

DOI 10.3389/978-2-88919-070-6

ABOUT FRONTIERS

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

FRONTIERS JOURNAL SERIES

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing.

All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

DEDICATION TO QUALITY

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.

Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.

By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

WHAT ARE FRONTIERS RESEARCH TOPICS?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area!

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

STRIATAL SIGNALING: TWO DECADES OF PROGRESS

Hosted By:

Emmanuel Valjent, Inserm—Institut national de la santé et de la recherche médicale, CNRS—Centre national de la recherche scientifique, and Université Montpellier, France

This Research Topic aims to highlight and cover recent understanding on striatal signaling pathways, which are activated by a variety of therapeutic agents or drugs of abuse in physiological and pathological context. The recent development of different mouse models allowing the identification of specific cell types and neuronal circuits in which a given signaling pathway is activated in various physiological and pathological conditions provides essential information and allowed to untangle the complexity of study signal transduction in the brain in vivo.

I believe this Research Topic is very timely because of the recent flurry of research papers on this topic. This special issue represents a unique opportunity to bring together leading experts in the field to provide a deep overview of current striatal signaling knowledge.

Table of Contents

- 05 *Striatal Signaling: Two Decades of Progress***
Emmanuel Valjent
- 07 *Mechanisms for the Modulation of Dopamine D1 Receptor Signaling in Striatal Neurons***
Akinori Nishi, Mahomi Kuroiwa and Takahide Shuto
- 17 *Identification of a Specific Assembly of the G Protein Golf as a Critical and Regulated Module of Dopamine and Adenosine-Activated cAMP Pathways in the Striatum***
Denis Hervé
- 26 *The Dopamine D1–D2 Receptor Heteromer in Striatal Medium Spiny Neurons: Evidence for a Third Distinct Neuronal Pathway in Basal Ganglia***
Melissa L. Perreault, Ahmed Hasbi, Brian F. O’Dowd and Susan R. George
- 34 *Adenosine A2A Receptors and A2A Receptor Heteromers as Key Players in Striatal Function***
Sergi Ferré, César Quiroz, Marco Orru, Xavier Guitart, Gemma Navarro, Antonio Cortés, Vicent Casadó, Enric I. Canela, Carme Lluís and Rafael Franco
- 42 *Control of Striatal Signaling by G Protein Regulators***
Keqiang Xie and Kirill A. Martemyanov
- 56 *Role of Beta-Arrestin 2 Downstream of Dopamine Receptors in the Basal Ganglia***
Thomas Del’Guidice, Morgane Lemasson and Jean-Martin Beaulieu
- 67 *Signaling from the Cytoplasm to the Nucleus in Striatal Medium-Sized Spiny Neurons***
Miriam Matamalas and Jean-Antoine Girault
- 80 *Beyond the Dopamine Receptor: Regulation and Roles of Serine/Threonine Protein Phosphatases***
Sven Ivar Walaas, Hugh Carroll Hemmings, Paul Greengard and Angus Clark Nairn
- 97 *The Role of Striatal-Enriched Protein Tyrosine Phosphatase (STEP) in Cognition***
Christopher James Fitzpatrick and Paul J. Lombroso
- 108 *Striatal Signal Transduction and Drug Addiction***
Scott D. Philibin, Adan Hernandez, David W. Self and James A. Bibb
- 123 *Post-Translational Modification Biology of Glutamate Receptors and Drug Addiction***
Li-Min Mao, Ming-Lei Guo, Dao-Zhong Jin, Eugene E. Fibuch, Eun Sang Choe and John Q. Wang

- 134 The Striatal Balancing Act in Drug Addiction: Distinct Roles of Direct and Indirect Pathway Medium Spiny Neurons**
Mary Kay Lobo and Eric J. Nestler
- 145 Targeting Neuronal Populations of the Striatum**
Pierre F. Durieux, Serge N. Schiffmann and Alban de Kerchove d'Exaerde
- 154 Striatal Signaling in L-DOPA-Induced Dyskinesia: Common Mechanisms with Drug Abuse and Long Term Memory Involving D1 Dopamine Receptor Stimulation**
Mario Gustavo Murer and Rosario Moratalla
- 166 Deciphering the Actions of Antiparkinsonian and Antipsychotic Drugs on cAMP/DARPP-32 Signaling**
Alessandra Bonito-Oliva, Michael Feyder and Gilberto Fisone
- 175 Huntington's Disease and Striatal Signaling**
Emmanuel Roze, Emma Cahill, Elodie Martin, Cecilia Bonnet, Peter Vanhoutte, Sandrine Betuing and Jocelyne Caboche
- 191 Basal Ganglia Disorders Associated with Imbalances in the Striatal Striosome and Matrix Compartments**
Jill R. Crittenden and Ann M. Graybiel



Striatal signaling: two decades of progress

Emmanuel Valjent^{1,2,3,4*}

¹ Institut de Génomique Fonctionnelle, Montpellier, France

² Inserm U661, Montpellier, France

³ CNRS UMR 5203, Montpellier, France

⁴ Université Montpellier I & II, France

*Correspondence: emmanuel.valjent@igf.cnrs.fr; emmanuel.valjent@gmail.com

Edited by:

Javier DeFelipe, Cajal Institute, Spain

Reviewed by:

Javier DeFelipe, Cajal Institute, Spain

The striatum and its ventral extension the nucleus accumbens (NAcc) are the main inputs structure of the basal ganglia circuit (**Figure 1**). While the striatum appears to be essential for the selection and initiation of actions and for the learning of habits and skills, the NAcc seems to be more involved in motivation and reward. Interestingly, dysfunctional plasticity of these structures has been associated with prominent neurological and psychiatric disorders such as Parkinson's disease, obsessive-compulsive disorder, Tourette's syndrome, and drug addiction.

The aims of this Special Topic was to propose a series of reviews covering our recent understanding on striatal signaling pathways, which are activated by a variety of therapeutic agents or drugs of abuse in physiological and pathological context. The 17 articles provide a deep overview of our current knowledge and highlight how recent advances in cell-type specific technologies, including fluorescent reporter mice, conditional knockout, and viral-mediated gene transfer allow to untangle the complexity of studying signal transduction in the brain *in vivo*. The content of each of these articles is briefly summarized below.

The first article, by Nishi et al. (2011) summarizes some of the mechanisms involved in the modulation of dopamine D1R signaling. The authors review both the canonical and non-canonical D1R signaling cascade that coupled to G α olf/adenylyl cyclase/PKA signaling and to Gq/phospholipase C or Src family kinase, respectively. They also extensively discuss the role played by Phosphodiesterases (PDEs) in the control of striatal signaling. In the second article, Hervé (2011) reviews the critical role of the highly specialized G-protein subunit heterotrimer containing G α olf/ β 2/ γ 7 that couples D1R and A2aR to adenylyl cyclase 5. The physiological consequences of the alterations of G α olf levels and/or activity are also extensively discussed. The third article by Perreault et al. (2011) reviews the current knowledge on D1R-D2R heteromers. Although, it is commonly accepted that D1R and D2R are preferentially expressed in striatonigral and striatopallidal MSNs, respectively, the authors put forward the hypothesis that MSNs co-expressing both receptors display unique signaling properties supporting the existence of a "novel third pathway". Ferré et al. (2011) focus on the role of the different subpopulation of striatal adenosine A2aR. Postsynaptically, A2aR are localized in striatopallidal MSNs where they form heteromers with dopamine D2R, cannabinoid CB1R, and mGluR5R. The authors also highlight how presynaptic A2aR-A1R heteromers can impact on striatal signaling. After a brief overview of the major G-protein-coupled receptors (GPCRs) and their downstream effectors, Xie and Martemyanov (2011) discuss

the progress made in understanding the roles of RGS proteins in controlling striatal G-protein signaling. A particular attention has been paid on the role of the striatal-enriched RGS9-2 protein on dopamine and opioid signaling. The sixth article by Del'Guidice et al. (2011) deals with the role of β -arrestin 2 on the regulation of dopamine receptor desensitization as well as the involvement of this scaffolding protein in the control of the Akt/GSK3 signaling pathways. Potential molecular targets of β -arrestin 2/Akt/GSK3 signaling are also reviewed. After an outline of the current knowledge of the signaling cascades that target the nucleus, Matamales and Girault (2011) describe how through the regulation of protein kinases, phosphatases, and transport through the nuclear pore, a signal initiated at the plasma membrane is amplified in the cytosol and then relayed to the nucleus. Identified mechanisms involved in transcription regulation and chromatin re-modeling in MSNs are also discussed. In the eighth article Walaas et al. (2011) provide a comprehensive review on the regulation and roles of the three major subclasses of serine/threonine protein phosphatases, PP1, PP2B, and PP2A. Their direct or indirect regulation by the striatum-enriched phosphoproteins DARPP32, RCS, and ARPP-16 is extensively discussed. The ninth article by Fitzpatrick and Lombroso (2011) also deals with the role of protein phosphatases focusing more specifically on the striatal-enriched protein tyrosine phosphatase (STEP). The structure and the regulation of STEP phosphorylation are described, as are the functional consequences of STEP dysfunctions in various neurological and neuropsychiatric disorders.

The dysregulation of striatal signaling induced by the drugs of abuse leads to an abnormal neuronal plasticity that under normal circumstances serves to shape appropriate reward-related behaviors. After a rapid update on the role played by dopamine receptors in drug addiction, Philibin et al. (2011) perform an in-depth review of intracellular signaling cascades, involving specific kinases and phosphatases affected by cAMP and Ca²⁺ that modulate neuroplasticity to affect behavioral outcome. Particular emphasis is placed on PKA, cdk5, ERK, CamKII, and PKC. Glutamate receptors cooperate closely with dopamine to regulate striatal signaling. In the article of Mao et al. (2011) several major types of post-translational modifications of glutamate receptors, including phosphorylation, palmitoylation, ubiquitination, and sumoylation are discussed. The impact of such modifications on striatal signaling and drug-induced neuronal plasticity is also addressed. The article by Lobo and Nestler (2011) discuss extensively how recent advances in cell-type-specific technologies have advanced the field toward a more comprehensive understanding

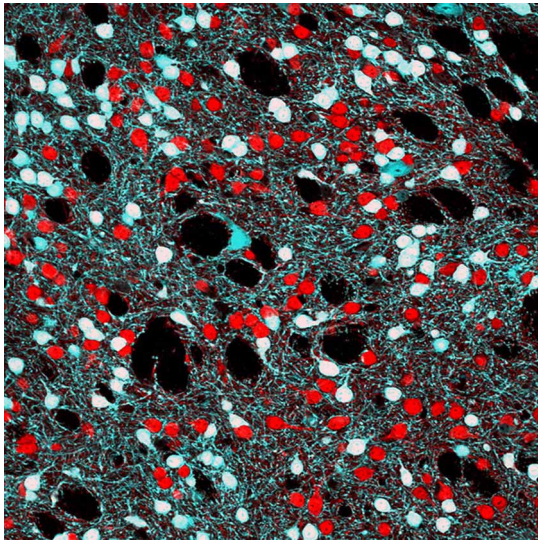


FIGURE 1 | Single confocal image showing DARPP-32 immunoreactivity (red) and EGFP fluorescence (cyan) in *Drd2-EGFP* BAC transgenic mice. DARPP-32 allows the identification of all striatal medium-sized spiny neurons (MSNs) while EGFP labels striatopallidal MSNs and large aspiny cholinergic interneurons. Picture by E. Valjent.

of the distinct molecular and functional contributions of striatonigral and striatopallidal MSNs in drug addiction. The article by Durieux et al. (2011) also deals with the development of new models and techniques allowing the selective targeting of striatal MSNs and interneurons. Although these approaches represent

invaluable tools, the authors point out the need of an extensive characterization to avoid overstated conclusions.

Altered striatal signaling in neurological disease has been also covered in this Special Issue. Thus, the contribution of Murer and Moratalla (2011) deals with the abnormal striatal signaling observed in response to L-DOPA in the context of Parkinson's disease. Transcriptional and translational molecular mechanisms that may translate enhanced D1 signaling into dyskinetic movements are discussed, as are the role of D2/D3R-mediated signaling. Bonito-Oliva et al. (2011) review the key role played by DARPP-32 in the actions of antiparkinsonian and antipsychotic drugs. The emerging picture indicating that L-DOPA-induced dyskinesia and extrapyramidal syndrome are linked to abnormal cAMP signaling in striatonigral and striatopallidal MSNs is also discussed. After a brief overview of the clinical aspects, genetics, and neuropathology, Roze et al. (2011) summarize our current knowledge on the molecular mechanisms underlying Huntington's disease. Changes in axonal transport and synaptic dysfunction have been discussed, as are transcriptional dysregulation observed at the striatal level. Finally, the last article by Crittenden and Graybiel (2011) provides a comprehensive review on the current state of knowledge of the details of striosome-matrix compartmentalization on the striatum and alterations in various disease states.

I am grateful for the enthusiasm and willingness shown by so many colleagues in the field of striatal signaling to contribute an article in this Special Issue. Some of the contributors also served as expert reviewers for articles. In addition, I would like to thank external reviewers for their valuable criticisms and suggestions that contributed to the success of this Special Issue.

REFERENCES

- Bonito-Oliva, A., Feyder, M., and Fisone, G. (2011). Deciphering the actions of antiparkinsonian and antipsychotic drugs on cAMP/DARPP-32 signaling. *Front. Neuroanat.* 5:38. doi: 10.3389/fnana.2011.00038
- Crittenden, J. R., and Graybiel, A. M. (2011). Basal ganglia disorders associated with imbalances in the striatal striosome and matrix compartments. *Front. Neuroanat.* 5:59. doi: 10.3389/fnana.2011.00059
- Del'Guidice, T., Lemasson, M., and Beaulieu, J. -M. (2011). Role of beta-arrestin 2 downstream of dopamine receptors in the basal ganglia. *Front. Neuroanat.* 5:58. doi: 10.3389/fnana.2011.00058
- Durieux, P. F., Schiffmann, S. N., and de Kerchove d'Exaerde, A. (2011). Targeting neuronal populations of the striatum. *Front. Neuroanat.* 5:40. doi: 10.3389/fnana.2011.00040
- Ferré, S., Quiroz, C., Orru, M., Guitart, X., Navarro, G., Cortés, A., et al. (2011). Adenosine A2A receptors and A2A receptor heteromers as key players in striatal function. *Front. Neuroanat.* 5:36. doi: 10.3389/fnana.2011.00036
- Fitzpatrick, C. J., and Lombroso, P. J. (2011). The role of striatal-enriched protein tyrosine phosphatase (STEP) in cognition. *Front. Neuroanat.* 5:47. doi: 10.3389/fnana.2011.00047
- Hervé, D. (2011). Identification of a specific assembly of the G protein Golf as a critical and regulated module of dopamine and adenosine-activated cAMP pathways in the striatum. *Front. Neuroanat.* 5:48. doi: 10.3389/fnana.2011.00048
- Lobo, M. K., and Nestler, E. J. (2011). The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. *Front. Neuroanat.* 5:41. doi: 10.3389/fnana.2011.00041
- Mao, L.-M., Guo, M.-L., Jin, D.-Z., Fibuch, E. E., Choe, E. S., and Wang, J. Q. (2011). Post-translational modification biology of glutamate receptors and drug addiction. *Front. Neuroanat.* 5:19. doi: 10.3389/fnana.2011.00019
- Matamalas, M., and Girault, J.-A. (2011). Signaling from the cytoplasm to the nucleus in striatal medium-sized spiny neurons. *Front. Neuroanat.* 5:37. doi: 10.3389/fnana.2011.00037
- Murer, M. G., and Moratalla, R. (2011). Striatal signaling in L-DOPA-induced dyskinesia: common mechanisms with drug abuse and long term memory involving D1 dopamine receptor stimulation. *Front. Neuroanat.* 5:51. doi: 10.3389/fnana.2011.00051
- Nishi, A., Kuroiwa, M., and Shuto, T. (2011). Mechanisms for the modulation of dopamine D1 receptor signaling in striatal neurons. *Front. Neuroanat.* 5:43. doi: 10.3389/fnana.2011.00043
- Perreault, M. L., Hasbi, A., O'Dowd, B. F., and George, S. R. (2011). The dopamine D1–D2 receptor heteromer in striatal medium spiny neurons: evidence for a third distinct neuronal pathway in basal ganglia. *Front. Neuroanat.* 5:31. doi: 10.3389/fnana.2011.00031
- Phillips, S. D., Hernandez, A., Self, D. W., and Bibb, J. A. (2011). Striatal signal transduction and drug addiction. *Front. Neuroanat.* 5:60. doi: 10.3389/fnana.2011.00060
- Roze, E., Cahill, E., Martin, E., Bonnet, C., Vanhoutte, P., Betuing, S., et al. (2011). Huntington's disease and striatal signaling. *Front. Neuroanat.* 5:55. doi: 10.3389/fnana.2011.00055
- Walaas, S. I., Hemmings, H. C. Jr., Greengard, P., and Nairn, A. C. (2011). Beyond the dopamine receptor: regulation and roles of serine/threonine protein phosphatases. *Front. Neuroanat.* 5:50. doi: 10.3389/fnana.2011.00050
- Xie, K., and Martemyanov, K. A. (2011). Control of striatal signaling by G protein regulators. *Front. Neuroanat.* 5:49. doi: 10.3389/fnana.2011.00049

Received: 20 September 2012; accepted: 30 September 2012; published online: 16 October 2012.

Citation: Valjent E (2012) Striatal signaling: two decades of progress. *Front. Neuroanat.* 6:43. doi: 10.3389/fnana.2012.00043

Copyright © 2012 Valjent. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.



Mechanisms for the modulation of dopamine D₁ receptor signaling in striatal neurons

Akinori Nishi^{1,2*}, Mahomi Kuroiwa^{1,2} and Takahide Shuto^{1,2}

¹ Department of Pharmacology, Kurume University School of Medicine, Kurume, Fukuoka, Japan

² Core Research of Evolutional Science and Technology, Japan Science of Technology Agency, Tokyo, Japan

Edited by:

Emmanuel Valjent, Université Montpellier 1 and 2, France

Reviewed by:

Jean-Martin Beaulieu, Université Laval/CRULRG, Canada

Cristina Missale, University of Brescia, Italy

*Correspondence:

Akinori Nishi, Department of Pharmacology, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan.
e-mail: nishia@med.kurume-u.ac.jp

In the striatum, dopamine D₁ receptors are preferentially expressed in striatonigral neurons, and increase the neuronal excitability, leading to the increase in GABAergic inhibitory output to substantia nigra pars reticulata. Such roles of D₁ receptors are important for the control of motor functions. In addition, the roles of D₁ receptors are implicated in reward, cognition, and drug addiction. Therefore, elucidation of mechanisms for the regulation of dopamine D₁ receptor signaling is required to identify therapeutic targets for Parkinson's disease and drug addiction. D₁ receptors are coupled to G_{s/olf}/adenylyl cyclase/PKA signaling, leading to the phosphorylation of PKA substrates including DARPP-32. Phosphorylated form of DARPP-32 at Thr34 has been shown to inhibit protein phosphatase-1, and thereby controls the phosphorylation states and activity of many downstream physiological effectors. Roles of DARPP-32 and its phosphorylation at Thr34 and other sites in D₁ receptor signaling are extensively studied. In addition, functional roles of the non-canonical D₁ receptor signaling cascades that coupled to G_q/phospholipase C or Src family kinase become evident. We have recently shown that phosphodiesterases (PDEs), especially PDE10A, play a pivotal role in regulating the tone of D₁ receptor signaling relatively to that of D₂ receptor signaling. We review the current understanding of molecular mechanisms for the modulation of D₁ receptor signaling in the striatum.

Keywords: dopamine, D₁ receptor, signaling, DARPP-32, phosphodiesterase, striatum

INTRODUCTION

Dopamine plays critical roles in the regulation of psychomotor functions in the brain (Bromberg-Martin et al., 2010; Cools, 2011; Gerfen and Surmeier, 2011). The dopamine receptors are a superfamily of heptahelical G protein-coupled receptors, and are grouped into two categories, D₁-like (D₁, D₅) and D₂-like (D₂, D₃, D₄) receptors, based on functional properties to stimulate adenylyl cyclase (AC) via G_{s/olf} and to inhibit AC via G_{i/o}, respectively (Kebabian and Calne, 1979; Jackson and Westlind-Danielsson, 1994; Missale et al., 1998). In the striatum, dopamine D₁ and D₂ receptor expressions are segregated in two types of medium spiny neurons, striatonigral/direct and striatopallidal/indirect pathway neurons, respectively (Hersch et al., 1995; Surmeier et al., 1996; Valjent et al., 2009; Bertran-Gonzalez et al., 2010). In the striatonigral/direct pathway neurons, D₁ receptors are coupled to G_{s/olf}/AC/PKA signaling, and activation of PKA induces the phosphorylation of PKA substrates such as DARPP-32, a dopamine- and cAMP-regulated phosphoprotein of M_r 32 kDa, and a transcription factor, CREB, leading to alterations of neuronal functions (Greengard et al., 1999; Hyman and Malenka, 2001). In this review, the roles of DARPP-32 and its phosphorylation in D₁ receptor signaling and the recent findings on the modulation of D₁ receptor/G_{s/olf}/AC/PKA signaling by phosphodiesterase (PDE) inhibitors are discussed. In addition, the non-canonical D₁ receptor signaling cascades that couple to G_q/phospholipase C (PLC) or Src family kinase (SFK) are overviewed.

ROLES OF DARPP-32 AND ITS PHOSPHORYLATION IN D₁ RECEPTOR/G_{s/olf}/AC/cAMP/PKA SIGNALING

ROLE OF THE PKA PHOSPHORYLATION-SITE AT Thr34 OF DARPP-32

Dopamine, acting on D₁ receptors, stimulates cAMP/PKA signaling via G_{s/olf}-mediated activation of AC (Herve et al., 2001). In postsynaptic striatal neurons, DARPP-32 is a major target for the cAMP/PKA signaling cascade (Greengard et al., 1999; Svenningsson et al., 2004). DARPP-32 is expressed in D₁ receptor-enriched striatonigral neurons as well as D₂ receptor-enriched striatopallidal neurons (Bateup et al., 2008). Phosphorylation at Thr34 by PKA converts DARPP-32 into a potent inhibitor of protein phosphatase-1 (PP-1; **Figure 1**). The inhibition of PP-1 thereby controls the phosphorylation state and activity of many downstream physiological effectors, including various neurotransmitter receptors (e.g., AMPA receptor GluR1 subunit, NMDA receptor NR1 subunit), ion channels and pumps (e.g., N/P-type Ca²⁺ channels, Na⁺ channel, Na⁺, K⁺-ATPase), and transcription factors (e.g., CREB, c-Fos, ΔFosB). Especially in cases of dual substrates for PKA and PP-1 such as GluR1 at Ser845 and NR1 at Ser897, activation of PKA signaling can efficiently increase the phosphorylation states of such substrates. Mice lacking DARPP-32 are deficient in their molecular, electrophysiological, and behavioral responses to dopamine, drugs of abuse, and antipsychotic medication, indicating an essential role for DARPP-32 in dopaminergic signaling (Fienberg et al., 1998; Fienberg and Greengard, 2000). Recently, a study using DARPP-32 conditional knockout mice, in which

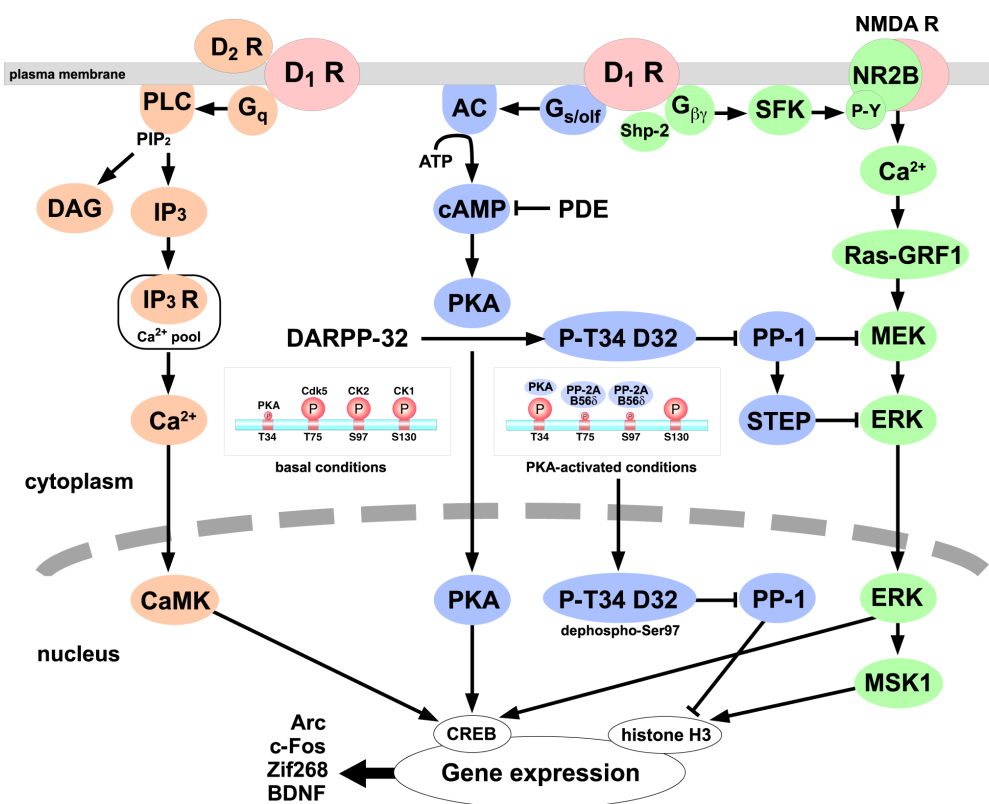


FIGURE 1 | The D₁ receptor signaling cascades in striatonigral/direct pathway neurons. D₁ receptors couple to at least three distinct signaling cascades: (1) G_{s/olf}/adenylyl cyclase (AC)/cAMP/PKA/DARPP-32/protein phosphatase-1 (PP-1) signaling (blue; Svenningsson et al., 2004; Stipanovich et al., 2008), (2) G_q/phospholipase C (PLC)/inositol 1,4,5-trisphosphate (IP₃)/IP₃ receptor/Ca²⁺ signaling (orange; Rashid et al., 2007a; Kuroiwa et al., 2008; Hasbi et al., 2009), (3) G_{βγ}/Src family kinase (SFK)/NMDA receptor NR2B subunit/Ca²⁺/Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1)/mitogen-activated protein kinase/ERK kinase (MEK)/ERK signaling (green; Girault et al., 2007; Pascoli et al., 2011). The phosphorylation levels of DARPP-32 are low at Thr34 and high at Thr75, Ser97, and Ser130 under basal conditions. Activation of PKA induces the phosphorylation of DARPP-32 at

Thr34 and the dephosphorylation of DARPP-32 at Thr75 and Ser97 by PP-2A/B56 δ complex, and phospho-Thr34/dephospho-Ser97 DARPP-32 accumulates in nucleus and inhibits PP-1, leading to the increase in histone H3 phosphorylation (Stipanovich et al., 2008). ERK, activated by two D₁ receptor pathways, induces mitogen- and stress-activated kinase 1 (MSK1) activation and histone H3 and cAMP-response element binding protein (CREB) phosphorylation in the nucleus (Girault et al., 2007; Pascoli et al., 2011). Thus, D₁ receptor-mediated activation of PKA, intracellular Ca²⁺, and ERK signaling induces the changes in downstream signaling cascades and the transcriptional activation of many genes. CaMK, Ca²⁺/calmodulin-dependent protein kinase; DAG, diacylglycerol; PDE, phosphodiesterase; STEP, striatal-enriched tyrosine phosphatase.

DARPP-32 is selectively deleted in D₁ receptor-enriched striatonigral or D₂ receptor-enriched striatopallidal neurons, revealed that DARPP-32 expressed in two types of medium spiny neurons differentially regulates striatal motor behaviors (Bateup et al., 2010). The loss of DARPP-32 in striatonigral neurons decreases basal and cocaine-induced locomotor activities and attenuates L-DOPA-induced dyskinesia in a 6-hydroxydopamine hemi-lesioned model of Parkinson's disease, whereas the loss of DARPP-32 in striatopallidal neurons increases basal and cocaine-induced locomotor activities and abolishes haloperidol-induced catalepsy. These findings support the idea that DARPP-32 enhances D₁ receptor functions in striatonigral neurons, but opposes D₂ receptor functions in striatopallidal neurons.

The phosphorylation state of DARPP-32 at Thr34 is regulated by the balance of phosphorylation by PKA and dephosphorylation by protein phosphatase 2B (calcineurin) and 2A (PP-2A). PKA signaling in direct pathway neurons is activated by β_1 -adrenoreceptors

(Hara et al., 2010) and 5-HT_{4/6} receptors (Svenningsson et al., 2002, 2007) as well as D₁ receptors (Bateup et al., 2008; Kuroiwa et al., 2008), and is inhibited by adenosine A₁ receptors (Yabuuchi et al., 2006), α_2 -adrenoreceptors (Hara et al., 2010), and μ -opioid receptors (Lindskog et al., 1999). Dephosphorylation of phospho-Thr34 DARPP-32 is mainly regulated by calcineurin (Nishi et al., 1999). Activation of NMDA or AMPA receptors increases intracellular Ca²⁺ and activates calcineurin, leading to the dephosphorylation of DARPP-32 at Thr34 (Halpain et al., 1990; Nishi et al., 1999). D₂ receptors are known to activate calcineurin via PLC/intracellular Ca²⁺ signaling (Hernandez-Lopez et al., 2000), leading to the dephosphorylation of DARPP-32 at Thr34 in striatal neurons where D₁ and D₂ receptors are co-expressed (Nishi et al., 1997). PP-2A also contributes to control the dephosphorylation process of DARPP-32 at Thr34 in a coordinated manner with calcineurin, as inhibition of PP-2A and calcineurin induces the synergistic and robust increase in DARPP-32 Thr34 phosphorylation in

striatal slices (Nishi et al., 1999). However, the role of PP-2A in the dephosphorylation of DARPP-32 at Thr34 is not fully understood under physiological conditions.

ROLE OF THE Cdk5 PHOSPHORYLATION-SITE AT Thr75 OF DARPP-32

In addition to Thr34, DARPP-32 is phosphorylated at multiple sites by several protein kinases. The major phosphorylation sites are at Thr75 for cyclin-dependent kinase 5 (Cdk5; Bibb et al., 1999), at Ser97 for CK2 (Girault et al., 1989), and at Ser130 for CK1 (Desdouits et al., 1995b), and these sites are highly phosphorylated in intact cells. DARPP-32 phosphorylated at Thr75 inhibits PKA activity and thereby reduces the efficacy of dopamine D₁ receptor signaling (Bibb et al., 1999). Dopamine, via dopamine D₁ receptors, activates PKA, which directly stimulates DARPP-32 Thr34 phosphorylation, and indirectly stimulates DARPP-32 Thr75 dephosphorylation by PP-2A (Nishi et al., 2000). PP-2A associated with B56 δ regulatory subunit is involved in the dephosphorylation of Thr75. B56 δ at Ser566 is phosphorylated by PKA, and the phosphorylation increases the activity of PP-2A/B56 δ complex to dephosphorylate DARPP-32 at Thr75 (Ahn et al., 2007a). The ability of activated PKA to reduce the phosphorylation state of DARPP-32 at Thr75 and thereby de-inhibit PKA is important as a positive feedback mechanism for enhancing PKA signaling (Nishi et al., 2000). Further activation of PKA and inhibition of PP-1 via DARPP-32-dependent mechanisms synergistically increase the phosphorylation of various substrates.

Glutamate acting on NMDA or AMPA receptors also stimulates the dephosphorylation of DARPP-32 at Thr75 by PP-2A (Nishi et al., 2002). PP-2A associated with B''/PR72 regulatory subunit mediates Ca²⁺-dependent dephosphorylation of Thr75 (Ahn et al., 2007b). PR72 contains two Ca²⁺-binding EF hands, EF1 and EF2 (Janssens et al., 2003). EF2 likely promotes the assembly of PP-2A/PR72 complex, and Ca²⁺ binding to EF1 activates PP-2A/PR72 activity for DARPP-32 at Thr75 in a substrate-specific manner (Janssens et al., 2003; Ahn et al., 2007b). By utilizing the Ca²⁺-dependent PP-2A/PR72 pathway, glutamate increases PKA activity by dephosphorylating Thr75 of DARPP-32, similarly to the D₁ receptor-activated PP-2A/B56 δ pathway.

The importance of DARPP-32 Thr75 phosphorylation is implicated in drug abuse. Chronic administration of psychostimulants such as cocaine induces the accumulation of a transcription factor, Δ FosB, resulting in the induction of a downstream target gene, Cdk5 (Bibb et al., 2001). The induced Cdk5 increases DARPP-32 Thr75 phosphorylation and therefore decreases D₁ receptor/PKA signaling. The attenuation of D₁ receptor/PKA signaling is considered as adaptive changes to cocaine addiction. The role of DARPP-32 at Thr75 is also demonstrated in stimulatory action of caffeine (Lindskog et al., 2002). Caffeine, by antagonizing adenosine A_{2A} receptors in striatopallidal neurons, attenuates A_{2A} receptor/PKA signaling and PP-2A activity and subsequently increases DARPP-32 Thr75 phosphorylation, which likely contribute to the stimulatory action of caffeine.

ROLE OF THE CK2 PHOSPHORYLATION-SITE AT Ser97 OF DARPP-32 AND THE CK1 PHOSPHORYLATION-SITE AT Ser130 OF DARPP-32

Phosphorylation of DARPP-32 at Ser97 (in mouse sequence; Ser102 in rat sequence) by CK2 is reported to increase the efficacy of DARPP-32 Thr34 phosphorylation by PKA (Girault et al.,

1989). In parallel, phosphorylation of DARPP-32 at Ser130 (in mouse sequence; Ser137 in rat sequence) by CK1 decreases the rate of dephosphorylation of Thr34 by calcineurin (Desdouits et al., 1995a). Thus, DARPP-32 phosphorylation by CK2 or CK1 results in the increase in the phosphorylation states of DARPP-32 at Thr34, suggesting the role of CK2 and CK1 to enhance D₁ receptor/PKA/DARPP-32/PP-1 signaling cascade. However, an opposing action of CK2 to inhibit D₁ receptor/PKA signaling is demonstrated (Rebholz et al., 2009). CK2 directly interacts with G_s/olf, and negatively controls the functions of D₁ receptors as well as A_{2A} receptors by enabling faster internalization.

Recently, the phosphorylation state of DARPP-32 at Ser97 is found to be a key regulator of nuclear export of DARPP-32 (Stipanovich et al., 2008). DARPP-32 has been thought as cytoplasmic protein because majority of DARPP-32 was fractionated in the soluble fraction (Walaas and Greengard, 1984), although the immunoreactivity of DARPP-32 was noticed in some nuclei of medium spiny neurons (Ouimet and Greengard, 1990). Importantly, activation of D₁ receptor/PKA signaling induces the nuclear accumulation of DARPP-32 (Stipanovich et al., 2008). The phosphorylation of DARPP-32 at Ser97 by CK2 functions as nuclear export signal of DARPP-32. Ser97 is highly phosphorylated under basal conditions (Girault et al., 1989), and only small fraction of DARPP-32 is located in the nucleus. When PKA is activated, Ser97 is dephosphorylated by PKA-activated PP-2A/B56 δ complex, resulting in the accumulation of DARPP-32 (phospho-Thr34/dephospho-Ser97 form of DARPP-32) in the nucleus. The inhibition of PP-1 by phospho-Thr34 DARPP-32 promotes histone H3 phosphorylation and regulates the nuclear function via mechanisms of chromatin remodeling. These findings provide mechanisms for D₁ receptor/PKA/DARPP-32 signaling to regulate gene expression, especially in conditions of drug addiction.

REGULATION OF D₁ RECEPTOR/PKA/DARPP-32 SIGNALING BY NEUROTRANSMITTERS

The D₁ receptor/PKA/DARPP-32 signaling cascade is modulated by various neurotransmitters including glutamate, GABA, acetylcholine, adenosine, serotonin, norepinephrine, nitric oxide, and neuropeptides (opioids, cholecystokinin, and neurotensin), in addition to dopamine (for the review, see Svenningsson et al., 2004). The release of dopamine is also regulated by neurotransmitters, therapeutic drugs, and drugs of abuse (Schmitz et al., 2003; Sulzer, 2011). We recently reported the role of glutamate (Nishi et al., 2005), PGE₂ (Kitaoka et al., 2007), nitric oxide (Tanda et al., 2009), and norepinephrine (Hara et al., 2010) in the regulation of D₁ receptor/PKA/DARPP-32 signaling. The role of DARPP-32 in integrating dopamine and other neurotransmitter signaling in D₁-type striatonigral neurons as well as D₂-type striatopallidal neurons has been proposed (Svenningsson et al., 2004; Fernandez et al., 2006). Furthermore, alterations of the D₁ receptor/PKA/DARPP-32 signaling cascade in drug addiction and L-DOPA-induced dyskinesia are characterized (for the review, see Svenningsson et al., 2005; Borgkvist and Fisone, 2007; Fisone et al., 2007; Shuto and Nishi, 2011).

Dopamine D₁ receptors are known to interact and form hetero-oligomers with other neurotransmitter receptors such as dopamine D₂ receptors (Lee et al., 2004; Rashid et al., 2007b),

dopamine D₃ receptors (Fiorentini et al., 2008; Marcellino et al., 2008), adenosine A₁ receptors (Gines et al., 2000; Toda et al., 2003), and NMDA receptors (Lee et al., 2002), resulting in alteration of D₁ receptor functions (**Figure 2**). The role of D₁ receptor hetero-oligomerization will be discussed in other reviews of this special topic.

D₁ RECEPTOR/G_q/PLC/IP₃ SIGNALING

In addition to the dopamine D₁ (D_{1A}) receptor that couples to G_{s/olf}/AC, the presence of a dopamine D₁-like receptor that couples to G_q/PLC has been proposed (Felder et al., 1989; Undie and Friedman, 1990; Wang et al., 1995; Beaulieu and Gainetdinov, 2011; **Figure 1**). D₁-like receptors are shown to couple to both G_s/AC and G_q/PLC signaling in the striatum and frontal cortex, but solely to G_q/PLC signaling in the hippocampus and amygdala (Undie and Friedman, 1990; Wang et al., 1995; Jin et al., 2001). It has been reported that G_q/PLC-coupled D₁-like receptors are coded in mRNA with different size from that of G_{s/olf}/AC-coupled D₁ receptors (Mahan et al., 1990), and are functional in dopamine receptor D_{1A} knockout mice (Friedman et al., 1997; Tomiyama et al., 2002), but not in dopamine D₅ receptor knockout mice (Sahu et al., 2009). However, there is a report showing the lack of G_q activation in the striatal membrane from D_{1A} receptor knockout mice (Rashid et al., 2007b). It still needs to be determined whether the cloned D_{1A} receptor (Drd1a), different types of D₁-like receptors such as the D₅ receptor, or both couple(s) to G_q/PLC signaling in the striatum.

It has been demonstrated that D₁ receptors form the hetero-oligomer with D₂ receptors, and that the D₁–D₂ receptor hetero-oligomer preferentially couples to G_q/PLC signaling (Rashid et al., 2007a,b). The expression of dopamine D₁ and D₂ receptors are largely segregated in direct and indirect pathway neurons in the dorsal striatum, respectively (Gerfen et al., 1990; Hersch et al., 1995; Heiman et al., 2008). However, some proportion of medium spiny neurons are known to express both D₁ and D₂ receptors (Hersch et al., 1995). Gene expression analysis using single cell RT-PCR technique estimated that 40% of medium spiny neurons express both D₁ and D₂ receptor mRNA (Surmeier et al., 1996). Recently, analysis using drd1a-EGFP and drd2-EGFP bacterial artificial chromosome (BAC) mice revealed that D₁ and D₂ receptors are co-expressed in medium spiny neurons at 5%

in the dorsal striatum, 6% in the NAc core, and 17% in the NAc shell (Bertran-Gonzalez et al., 2008). Therefore, D₁ receptors likely interact with D₂ receptors in some populations of medium spiny neurons, where both D₁ and D₂ receptors are co-expressed.

D₁ and D₂ receptors, co-expressed in transfected cells and striatal neurons, form the hetero-oligomer (Lee et al., 2004). The D₁ and D₂ receptor hetero-oligomer couples to G_q and activates PLC and intracellular Ca²⁺ signaling (Lee et al., 2004; Rashid et al., 2007b). For activation of G_q/PLC signaling, both D₁ and D₂ receptors are required, because either D₁ or D₂ antagonist and genetic deletion of either D₁ or D₂ receptors abolish the effect (Rashid et al., 2007b). It is likely that SKF83959, which selectively activate G_q/PLC signaling but not G_{s/olf}/PKA signaling, acts as a full agonist for D₁ receptors and a partial agonist for D₂ receptors in the form of hetero-oligomer (Rashid et al., 2007a,b). The functional role of the D₁ and D₂ receptor hetero-oligomer to activate Ca²⁺/calmodulin-dependent kinase II α , which may lead to the induction of brain-derived neurotrophic factor (BDNF) expression, has been suggested (Hasbi et al., 2009), and therefore the D₁ and D₂ receptor hetero-oligomer is implicated as a potential therapeutic target for schizophrenia and drug addiction (Hasbi et al., 2010).

The G_{s/olf}/AC-coupled and G_q/PLC-coupled D₁-like receptor signaling cascades likely play differential roles in dopaminergic signaling. G_q/PLC-coupled D₁-like receptors show different sensitivity to benzazepine dopamine D₁ receptor agonists from G_{s/olf}/AC-coupled D₁ receptors (Undie et al., 1994). Many D₁ receptor agonists such as SKF38393 and SKF81297 activate both G_{s/olf}/AC and G_q/PLC signaling (Undie et al., 1994). In contrast, SKF83959 preferentially stimulates G_q/PLC-coupled D₁-like receptors (Panchalingam and Undie, 2001; Jin et al., 2003), and SKF83822 selectively stimulates G_{s/olf}/AC-coupled D₁ receptors (Undie et al., 1994). By utilizing SKF83959 and SKF83822, we investigated the D₁-like receptor signaling cascades, which regulate DARPP-32 phosphorylation at Thr34 (the PKA-site) in mouse striatal slices (Kuroiwa et al., 2008). Dopamine D₁ receptor agonists activate at least three D₁-like receptor signaling cascades in striatonigral/direct pathway neurons: (i) SCH23390-sensitive G_{s/olf}/AC/PKA signaling, (ii) SCH23390-insensitive G_{s/olf}/AC/PKA signaling, and (iii) G_q/PLC signaling. Activation of SCH23390-sensitive D₁ receptors stimulates AC/cAMP/PKA signaling, leading to the phosphorylation of DARPP-32 at Thr34 in striatonigral neurons. Activation of G_q-coupled D₁ receptors stimulates PLC signaling, resulting in the suppression of DARPP-32 Thr34 phosphorylation.

Behavioral studies revealed that activation of D₁ receptor/G_{s/olf}/AC signaling by SKF83822 mediates sniffing, locomotion, rearing, stereotypy, and seizure (O'Sullivan et al., 2004), whereas activation of D₁-like receptor/G_q/PLC signaling by SKF83959 as well as SKF38393 mediates vacuous jaw movements and intense grooming (Deveney and Waddington, 1995; Undie et al., 2000). Molecular mechanisms by which G_q/PLC-coupled D₁-like receptors regulate the function of neostriatal neurons, and their interaction with the D₁ receptor/G_{s/olf}/AC/PKA signaling cascades need to be clarified.

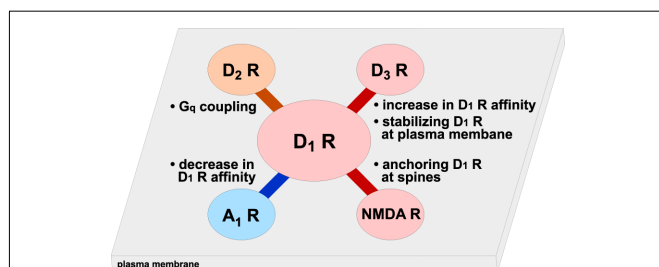


FIGURE 2 | D₁ receptors form hetero-oligomers with other receptors.

Formation of hetero-oligomers with dopamine D₁ receptors and other receptors such as D₂, D₃, A₁, and NMDA receptors is shown. Biding of these receptors induces the changes in D₁ receptor function and/or localization.

D₁ RECEPTORS COUPLED TO Src FAMILY KINASE SIGNALING

Activation of D₁ receptors with simultaneous activation of NMDA receptors by drugs of abuse such as cocaine and *d*-amphetamine induces extracellular-signal regulated kinase (ERK) activation selectively in striatonigral/direct pathway neurons (Valjent et al., 2005; Bertran-Gonzalez et al., 2008), leading to activation of transcription for genes critical for drug-induced plasticity (Girault et al., 2007; Bertran-Gonzalez et al., 2008; **Figure 1**). The role of D₁ receptor/PKA/DARPP-32 signaling is also implicated for ERK activation, since the inhibition of PP-1 by phospho-Thr34 DARPP-32 induces activation of mitogen-activated protein kinase/ERK kinase (MEK), which phosphorylates ERK, and inhibition of striatal-enriched tyrosine phosphatase (STEP), which dephosphorylates ERK (Valjent et al., 2005; Girault et al., 2007). In addition, the Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1), a neuronal specific activator of Ras/ERK signaling, is identified as an upstream of MEK/ERK signaling (Fasano et al., 2009). However, mechanisms for activation of NMDA receptors by drugs of abuse were not clearly understood, because the changes in extracellular glutamate levels induced by drugs of abuse were variable (Reid et al., 1997; Zhang et al., 2001). Recently, Pascoli et al. (2011) reported that D₁ receptors potentiate NMDA receptor function via phosphorylation of NR2B subunit (at Tyr1472) of NMDA receptors by SFK, which is responsible for activation of the Ras-GRF1/MEK/ERK signaling cascade by cocaine (Fasano et al., 2009; Pascoli et al., 2011). The activation of SFK by D₁ receptors is mediated through G_{βγ} subunit. The findings are in agreement with previous reports showing that D₁ receptor-mediated increase in NMDA receptor currents requires NR2B subunit and SFK activity (Wittmann et al., 2005), and that activation of D₁ receptors induces trafficking of NMDA receptors to the postsynaptic membrane mediated through NR2B phosphorylation by SFK, especially Fyn (Dunah and Standaert, 2001; Hallett et al., 2006). The novel D₁ receptor/G_{βγ}/SFK/NR2B signaling cascade, which results in activation of NMDA receptors/Ca²⁺/Ras-GRF1/MEK/ERK signaling, likely plays a critical role in pathological conditions such as drug addiction and L-DOPA-induced dyskinesia in the animal model of Parkinson's disease, in which NR2B tyrosine phosphorylation (Menegoz et al., 1995; Pascoli et al., 2011) and activation of ERK signaling by D₁ receptors (Gerfen et al., 2002; Girault et al., 2007; Santini et al., 2007) are enhanced.

MODULATION OF cAMP/PKA SIGNALING IN D₁-TYPE DIRECT PATHWAY NEURONS AS WELL AS D₂-TYPE INDIRECT PATHWAY NEURONS BY PHOSPHODIESTERASE (PDE) INHIBITION

Activation of G_s/olf-coupled dopamine D₁ receptors causes an increase in cAMP synthesis, while the subsequent hydrolysis of cAMP is mediated by PDEs. PDEs are subdivided into 11 families, encoded by 21 genes (Conti and Beavo, 2007). Multiple PDEs with different substrate specificities and subcellular localization are expressed in the striatum. PDE10A, PDE1B, and PDE7B are enriched in the striatum, and PDE4 (A, B, and D isoforms), PDE2A and PDE9A, which are widely distributed in the brain, are also expressed in the striatum (Menniti et al., 2006).

Several types of PDEs such as PDE10A, PDE4, and PDE1B are expressed in direct and indirect pathway neurons. The inhibition of PDEs can result in activation of cAMP/PKA signaling both in direct and indirect pathway neurons. If the function of the PDE (e.g., PDE1B) is predominant in direct pathway neurons, the inhibition of the PDE and activation of cAMP/PKA signaling results in activation of direct pathway neurons, leading to inhibition of GPi/SNpr inhibitory output neurons and activation of thalamocortical motor circuits. Conversely, if the function of the PDE (e.g., PDE10A and PDE4) is predominant in indirect pathway neurons, the inhibition of the PDE and activation of cAMP/PKA signaling results in activation of indirect pathway neurons, leading to activation of GPi/SNpr inhibitory output neurons and inhibition of thalamocortical motor circuits. Thus, PDE inhibitors that predominantly act in direct pathway neurons work like dopamine D₁ receptor agonists and activate motor function, whereas PDE inhibitors that predominantly act in indirect pathway neurons work like dopamine D₂ receptor antagonists and inhibit motor function (Nishi and Snyder, 2010). The balance of action of each PDE inhibitor in indirect and direct pathway neurons determines the behavioral effects (**Figure 3**).

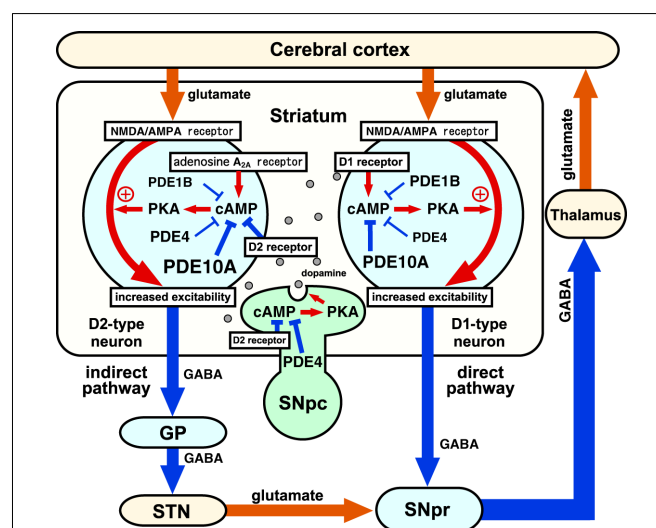


FIGURE 3 | Roles of phosphodiesterases (PDEs) in the control of basal ganglia-thalamocortical circuitry. Output neurons in the striatum are medium spiny neurons (MSNs), which consist of striatonigral/direct pathway and striatopallidal/indirect pathway neurons. Direct pathway neurons are GABAergic, and inhibit tonically active neurons in globus pallidus interna (GPi)/substantia nigra pars reticulata (SNpr). Indirect pathway neurons are also GABAergic, and activate neurons in GPi/SNpr via inhibition of globus pallidus externa (GPe) GABAergic neurons and activation of subthalamic nucleus (STN) glutamatergic neurons. Direct and indirect pathway neurons induce opposing effects on the output neurons in GPi/SNpr, resulting in disinhibition and pro-inhibition of output, respectively, to motor areas of the thalamus and cortex. The inhibition of PDEs increases cAMP/PKA signaling in both direct and indirect pathway neurons. PDE inhibitors that predominantly act in direct pathway neurons work like dopamine D₁ receptor agonists and activate motor function, whereas PDE inhibitors that predominantly act in indirect pathway neurons work like dopamine D₂ receptor antagonists and inhibit motor function. SNpc, substantia nigra pars compacta. Reproduced with permission from reference Nishi and Snyder (2010).

ROLE OF PDE10A IN DOPAMINE SIGNALING

PDE10A is a dual substrate PDE that hydrolyzes both cAMP and cGMP, and has a higher affinity for cAMP than for cGMP by ~20-fold (Fujishige et al., 1999; Bender and Beavo, 2006). In the striatum, PDE10A is expressed in two types of medium spiny neurons (direct and indirect pathway neurons), but not in interneurons (Xie et al., 2006; Nishi et al., 2008; Sano et al., 2008). Papaverine, an opium alkaloid primarily used for the treatment of visceral spasm and vasospasm, was found to selectively inhibit PDE10A with an IC₅₀ of 36 nM (Siuciak et al., 2006). Papaverine was used to explore the physiological role of PDE10A in the regulation of striatal function. Recently, the potent PDE10A inhibitors, TP-10 (IC₅₀ 0.3 nM) and MP-10 (IC₅₀ 0.18 nM), were developed (Schmidt et al., 2008). Using these PDE10A inhibitors, PDE10A was shown to hydrolyze both cAMP and cGMP in the striatum *in vivo* (Siuciak et al., 2006; Schmidt et al., 2008; Grauer et al., 2009). We examined the effect of papaverine on the phosphorylation of PKA substrates including DARPP-32 using neostriatal slices. Papaverine robustly increased the phosphorylation of DARPP-32 at Thr34 and GluR1 at Ser845 in striatal medium spiny neurons in slices as well as *in vivo* (Nishi et al., 2008). The effect of papaverine was mediated through the potentiation of cAMP/PKA signaling, but not cGMP/PKG signaling. Similarly to papaverine, inhibition of PDE10A by TP-10 and/or MP-10 in the striatum *in vivo* was demonstrated to induce the phosphorylation of DARPP-32, GluR1, and CREB at PKA-sites (Schmidt et al., 2008; Grauer et al., 2009).

PDE10A is abundantly expressed in direct and indirect pathway neurons, and the expression levels are similar in the two types of neurons (Xie et al., 2006; Nishi et al., 2008; Sano et al., 2008). In agreement, PDE10A regulates cAMP/PKA signaling (Nishi et al., 2008) as well as gene expression (Strick et al., 2010) in both direct and indirect pathway neurons. In direct pathway neurons, PDE10A inhibition by papaverine activates cAMP/PKA signaling, leading to the potentiation of dopamine D₁ receptor signaling. In indirect pathway neurons, PDE10A inhibition by papaverine also activates cAMP/PKA signaling by simultaneously potentiating adenosine A_{2A} receptor signaling and inhibiting dopamine D₂ receptor signaling. Since the balance of cAMP/PKA signaling between the direct and indirect pathways determines the output from the basal ganglia, neuronal type-specific regulation of DARPP-32 Thr34 phosphorylation was studied using neostriatal slices from D₁R-DARPP-32-Flag/D₂R-DARPP-32-Myc mice (Nishi et al., 2008), in which Flag-tagged DARPP-32 and Myc-tagged DARPP-32 are expressed selectively in direct and indirect pathway neurons under the control of D₁ and D₂ receptor promoters, respectively (Bateup et al., 2008). PDE10A inhibition by papaverine increases Myc-tagged DARPP-32 phosphorylation sixfold in indirect pathways, whereas Flag-tagged DARPP-32 phosphorylation only twofold in direct pathway neurons. Thus, PDE10A inhibitors activate cAMP/PKA signaling in indirect and direct pathway neurons, but the action of PDE10A inhibitors predominates in indirect pathway neurons. A recent electrophysiological study showing that PDE10A inhibition has greater facilitatory effect on corticostriatal synaptic activity in indirect pathway neurons supports the interpretation (Threlfell et al., 2009). The biochemical features of PDE10A inhibitors resemble those of antipsychotic drugs, which act primarily as D₂ receptor antagonists and increase DARPP-32

phosphorylation in indirect pathway neurons (Bateup et al., 2008). In agreement, PDE10A inhibition by papaverine, TP-10 and MP-10 and genetic deletion of PDE10A display behavioral phenotypes of antipsychotics with therapeutic potency for negative symptoms and cognitive deficits as well as positive symptoms in schizophrenics (Sano et al., 2008; Grauer et al., 2009; Nishi and Snyder, 2010).

ROLE OF PDE4 IN DOPAMINE SIGNALING

PDE4 is a cAMP-specific PDE with high affinity for cAMP (K_m 1–10 μM; Beavo, 1995; Bender and Beavo, 2006). The PDE4 family is encoded by four genes (PDE4A–PDE4D), and each isoform has multiple variants (Houslay and Adams, 2003; McCahill et al., 2008). In the CNS, PDE4A, PDE4B, and PDE4D are widely distributed, but the expression of PDE4C is restricted to the olfactory bulb in rodent brain (Cherry and Davis, 1999; Perez-Torres et al., 2000). Each PDE4 variant has a modular structure consisting of a variant-specific N-terminal domain, regulatory domains termed upstream conserved region 1 (UCR1) and UCR2, and a catalytic domain. The phosphorylation of UCR1 by PKA disrupts the inhibitory interaction of UCR2 with the catalytic domain and activates PDE4 activity (McCahill et al., 2008), leading to the downregulation of cAMP/PKA signaling. The N-terminal domain and UCR1/2 interact with PDE4 variant-specific binding proteins including A-kinase anchor proteins (AKAP) and disrupted in schizophrenia 1 (DISC1), leading to the compartmentalization of cAMP signaling in cells (McCahill et al., 2008; Houslay, 2010). The involvement of PDE4B in the molecular mechanisms of schizophrenia is supported by its interaction with DISC1 (Millar et al., 2005; Clapcote et al., 2007; Murdoch et al., 2007), which is a genetic susceptibility factor for schizophrenia (Chubb et al., 2008).

Function of PDE4 has been analyzed using a selective PDE4 inhibitor, rolipram (IC₅₀ 1 μM; Bender and Beavo, 2006). Inhibition of PDE4 by rolipram robustly increases tyrosine hydroxylase (TH) phosphorylation at Ser40 (PKA-site) at dopaminergic terminals in neostriatal slices and *in vivo*, leading to the enhancement of dopamine synthesis and metabolism (Nishi et al., 2008). In addition, the inhibition of PDE4 by rolipram weakly enhances cAMP/PKA signaling in striatal neurons, leading to the phosphorylation of DARPP-32 at Thr34 in neostriatal slices (Nishi et al., 2008). Rolipram treatment augments adenosine A_{2A} receptor-mediated phosphorylation of DARPP-32 at Thr34, but has no effect on dopamine D₁ receptor-mediated phosphorylation. However, in neostriatal slices from D₁R-Flag/D₂R-Myc DARPP-32 mice, rolipram induces the phosphorylation of both Flag- and Myc-tagged DARPP-32 in direct and indirect pathway neurons, respectively. Immunohistochemical analysis in D₁R-Flag/D₂R-Myc DARPP-32 mice revealed that PDE4B expression is higher in indirect pathway neurons than that in direct pathway neurons. These data suggest that PDE4 preferentially regulates cAMP/PKA signaling coupled to adenosine A_{2A} receptors in indirect pathway neurons compared to that coupled to dopamine D₁ receptors in direct pathway neurons. Activation of cAMP/PKA signaling in indirect pathway neurons elicited by the PDE4 inhibitor, rolipram, is expected to oppose dopamine D₂ receptor signaling. At the same time, rolipram stimulates dopamine synthesis, indicating that PDE4 inhibition raises dopaminergic tone in the striatum.

Therefore, rolipram mimics the biochemical effects of dopamine D₂ antagonists and to some extent D₁ agonists.

The pharmacological profile of the PDE4 inhibitor, including positive effects on mood and cognition, supports its possible efficacy for the treatment of negative symptoms and cognitive deficits in addition to positive symptoms in schizophrenics (Siuciak, 2008). However, the studies in PDE4B knockout mice generally fail to recapitulate the antipsychotic effects of rolipram, and the discrepancy might be explained by the lack of PDE4B selectivity of rolipram and chronic compensatory mechanisms for PDE4B gene deletion (Siuciak, 2008; Nishi and Snyder, 2010).

ROLE OF PDE1B IN DOPAMINE SIGNALING

PDE1B is a dual substrate PDE with a higher affinity for cGMP (K_m 2.4 μM) than for cAMP (K_m 24 μM; Bender and Beavo, 2006). PDE1B is activated by Ca²⁺ and calmodulin, providing a mechanism for crosstalk between Ca²⁺ and cyclic nucleotide signaling. PDE1B is abundantly expressed in the striatum (Polli and Kincaid, 1994), and striatal PDE1B is localized to all DARPP-32-positive medium spiny neurons, indicating the PDE1B expression in both direct and indirect pathway neurons (A. Nishi and M. Kuroiwa, unpublished observations). Biochemical studies in PDE1B knockout mice revealed that the function of dopamine D₁ receptors to stimulate the phosphorylation of DARPP-32 and GluR1 at PKA-sites is potentiated in striatal slices from PDE1B knockout mice (Reed et al., 2002). In behavioral analysis, PDE1B knockout mice exhibited the increase in spontaneous

locomotor activity and psychostimulant- and NMDA receptor antagonist-stimulated locomotor activity and the cognitive deficit in Morris water maze test (Reed et al., 2002; Ehrman et al., 2006; Siuciak et al., 2007). In spite of similar expression pattern of PDE1B with PDE10A in the striatum, the behavioral profiles of PDE1B knockout mice are pro-psychotic and completely opposite to those of PDE10A knockout mice. We hypothesize that PDE1B predominantly regulates cyclic nucleotide signaling in direct pathway neurons, whereas a predominant role of PDE10A and PDE4 in indirect pathway neurons.

CONCLUSION

D₁ receptor signaling in striatonigral/direct pathway neurons plays an essential role in motor functions (Gerfen and Surmeier, 2011) as well as reward and cognition (Bromberg-Martin et al., 2010; Cools, 2011). The alterations of D₁ receptor signaling are implicated in drug addiction (Shuto and Nishi, 2011) and L-DOPA-induced dyskinesia (Fisone et al., 2007). Besides the importance of D₁ receptor signaling under pathophysiological conditions, dopaminergic drugs are mainly acting on D₂ receptors, and therapeutic agents that selectively modulate D₁ receptor signaling are not currently utilized. Functional roles of the canonical and non-canonical D₁ receptor signaling cascades are implicated in the same category of diseases. Further understanding of each D₁ receptor signaling cascade and its regulation is required for the development of therapeutic agents targeting D₁ receptor signaling.

REFERENCES

- Ahn, J. H., McAvoy, T., Rakhilin, S. V., Nishi, A., Greengard, P., and Nairn, A. C. (2007a). Protein kinase A activates protein phosphatase 2A by phosphorylation of the B56delta subunit. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2979–2984.
- Ahn, J. H., Sung, J. Y., McAvoy, T., Nishi, A., Janssens, V., Goris, J., Greengard, P., and Nairn, A. C. (2007b). The B⁵⁶/PR72 subunit mediates Ca²⁺-dependent dephosphorylation of DARPP-32 by protein phosphatase 2A. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9876–9881.
- Bateup, H. S., Santini, E., Shen, W., Birnbaum, S., Valjent, E., Surmeier, D. J., Fisone, G., Nestler, E. J., and Greengard, P. (2010). Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14845–14850.
- Bateup, H. S., Svenningsson, P., Kuroiwa, M., Gong, S., Nishi, A., Heintz, N., and Greengard, P. (2008). Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. *Nat. Neurosci.* 11, 932–939.
- Beaulieu, J. M., and Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol. Rev.* 63, 182–217.
- Beavo, J. A. (1995). Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. *Physiol. Rev.* 75, 725–748.
- Bender, A. T., and Beavo, J. A. (2006). Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol. Rev.* 58, 488–520.
- Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamalas, M., Herve, D., Valjent, E., and Girault, J. A. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J. Neurosci.* 28, 5671–5685.
- Bertran-Gonzalez, J., Herve, D., Girault, J. A., and Valjent, E. (2010). What is the degree of segregation between striatonigral and striatopallidal projections? *Front. Neuroanat.* 4:136. doi: 10.3389/fnana.2010.00136
- Bibb, J. A., Chen, J., Taylor, J. R., Svenningsson, P., Nishi, A., Snyder, G. L., Yan, Z., Sagawa, Z. K., Ouimet, C. C., Nairn, A. C., Nestler, E. J., and Greengard, P. (2001). Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature* 410, 376–380.
- Bibb, J. A., Snyder, G. L., Nishi, A., Yan, Z., Meijer, L., Fienberg, A. A., Tsai, L. H., Kwon, Y. T., Girault, J. A., Czernik, A. J., Huganir, R. L., Hemmings, H. C. Jr., Nairn, A. C., and Greengard, P. (1999). Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature* 402, 669–671.
- Borgkvist, A., and Fisone, G. (2007). Psychoactive drugs and regulation of the cAMP/PKA/DARPP-32 cascade in striatal medium spiny neurons. *Neurosci. Biobehav. Rev.* 31, 79–88.
- Bromberg-Martin, E. S., Matsumoto, M., and Hikosaka, O. (2010). Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron* 68, 815–834.
- Cherry, J. A., and Davis, R. L. (1999). Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement, and affect. *J. Comp. Neurol.* 407, 287–301.
- Chubb, J. E., Bradshaw, N. J., Soares, D. C., Porteous, D. J., and Millar, J. K. (2008). The DISC locus in psychiatric illness. *Mol. Psychiatry* 13, 36–64.
- Clapcote, S. J., Lipina, T. V., Millar, J. K., Mackie, S., Christie, S., Ogawa, F., Lerch, J. P., Trimble, K., Uchiyama, M., Sakuraba, Y., Kaneda, H., Shi-roishi, T., Houslay, M. D., Henkelman, R. M., Sled, J. G., Gondo, Y., Porteous, D. J., and Roder, J. C. (2007). Behavioral phenotypes of Disc1 missense mutations in mice. *Neuron* 54, 387–402.
- Conti, M., and Beavo, J. (2007). Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu. Rev. Biochem.* 76, 481–511.
- Cools, R. (2011). Dopaminergic control of the striatum for high-level cognition. *Curr. Opin. Neurobiol.* 21, 402–407.
- Desdouits, F., Siciliano, J. C., Greengard, P., and Girault, J.-A. (1995a). Dopamine- and cAMP-regulated phosphoprotein DARPP-32: phosphorylation of Ser-137 by casein kinase I inhibits dephosphorylation of Thr-34 by calcineurin. *Proc. Natl. Acad. Sci. U.S.A.* 92, 2682–2685.
- Desdouits, F., Cohen, D., Nairn, A. C., Greengard, P., and Girault, J.-A. (1995b). Phosphorylation of DARPP-32, a dopamine- and cAMP-regulated phosphoprotein, by casein kinase I in vitro and in vivo. *J. Biol. Chem.* 270, 8772–8778.

- Deveney, A. M., and Waddington, J. L. (1995). Pharmacological characterization of behavioural responses to SKF 83959 in relation to "D1-like" dopamine receptors not linked to adenylyl cyclase. *Br. J. Pharmacol.* 116, 2120–2126.
- Dunah, A. W., and Standaert, D. G. (2001). Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. *J. Neurosci.* 21, 5546–5558.
- Ehrman, L. A., Williams, M. T., Schaefer, T. L., Gudelsky, G. A., Reed, T. M., Fienberg, A. A., Greengard, P., and Vorhees, C. V. (2006). Phosphodiesterase 1B differentially modulates the effects of methamphetamine on locomotor activity and spatial learning through DARPP32-dependent pathways: evidence from PDE1B-DARPP32 double-knockout mice. *Genes Brain Behav.* 5, 540–551.
- Fasano, S., D'Antoni, A., Orban, P. C., Valjent, E., Putignano, E., Vara, H., Pizzorusso, T., Giustetto, M., Yoon, B., Soloway, P., Maldonado, R., Caboche, J., and Brambilla, R. (2009). Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) controls activation of extracellular signal-regulated kinase (ERK) signaling in the striatum and long-term behavioral responses to cocaine. *Biol. Psychiatry* 66, 758–768.
- Felder, C. C., Jose, P. A., and Axelrod, J. (1989). The dopamine-1 agonist, SKF 82526, stimulates phospholipase-C activity independent of adenylyl cyclase. *J. Pharmacol. Exp. Ther.* 248, 171–175.
- Fernandez, E., Schiappa, R., Girault, J. A., and Le Novere, N. (2006). DARPP-32 is a robust integrator of dopamine and glutamate signals. *PLoS Comput. Biol.* 2, e176. doi: 10.1371/journal.pcbi.0020176
- Fienberg, A. A., and Greengard, P. (2000). The DARPP-32 knockout mouse. *Brain Res. Brain Res. Rev.* 31, 313–319.
- Fienberg, A. A., Hiroi, N., Mermelstein, P., Song, W.-J., Snyder, G. L., Nishi, A., Cheramy, A., O'Callaghan, J. P., Miller, D., Cole, D., Corbett, R., Haile, C., Cooper, D., Onn, S., Grace, A. A., Ouimet, C., White, F. J., Hyman, S. E., Surmeier, D. J., Girault, J. A., Nestler, E., and Greengard, P. (1998). DARPP-32, regulator of the efficacy of dopaminergic neurotransmission. *Science* 281, 838–842.
- Fiorentini, C., Busi, C., Gorruso, E., Gotti, C., Spano, P., and Missale, C. (2008). Reciprocal regulation of dopamine D1 and D3 receptor function and trafficking by heterodimerization. *Mol. Pharmacol.* 74, 59–69.
- Fisone, G., Hakansson, K., Borgkvist, A., and Santini, E. (2007). Signaling in the basal ganglia: postsynaptic and presynaptic mechanisms. *Physiol. Behav.* 92, 8–14.
- Friedman, E., Jin, L. Q., Cai, G. P., Hollon, T. R., Drago, J., Sibley, D. R., and Wang, H. Y. (1997). D1-like dopaminergic activation of phosphoinositide hydrolysis is independent of D1A dopamine receptors: evidence from D1A knockout mice. *Mol. Pharmacol.* 51, 6–11.
- Fujishige, K., Kotera, J., Michibata, H., Yuasa, K., Takebayashi, S., Okumura, K., and Omori, K. (1999). Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). *J. Biol. Chem.* 274, 18438–18445.
- Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. M., Monsma, F. J. Jr., and Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250, 1429–1432.
- Gerfen, C. R., Miyachi, S., Paletzki, R., and Brown, P. (2002). D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. *J. Neurosci.* 22, 5042–5054.
- Gerfen, C. R., and Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. *Annu. Rev. Neurosci.* 34, 441–466.
- Gines, S., Hillion, J., Torvinen, M., Le Crom, S., Casado, V., Canela, E. I., Rondin, S., Lew, J. Y., Watson, S., Zoli, M., Agnati, L. F., Verniera, P., Lluís, C., Ferre, S., Fuxe, K., and Franco, R. (2000). Dopamine D1 and adenosine A1 receptors form functionally interacting heteromeric complexes. *Proc. Natl. Acad. Sci. U.S.A.* 97, 8606–8611.
- Girault, J. A., Valjent, E., Caboche, J., and Herve, D. (2007). ERK2: a logical AND gate critical for drug-induced plasticity? *Curr. Opin. Pharmacol.* 7, 77–85.
- Girault, J.-A., Hemmings, H. C. Jr., Williams, K. R., Nairn, A. C., and Greengard, P. (1989). Phosphorylation of DARPP-32, a dopamine- and cAMP-regulated phosphoprotein, by casein kinase II. *J. Biol. Chem.* 264, 21748–21759.
- Grauer, S. M., Pulito, V. L., Navarra, R. L., Kelly, M., Kelley, C., Graf, R., Langen, B., Logue, S., Brennan, J., Jiang, L., Charych, E., Egerland, U., Liu, F., Marquis, K. L., Malamas, M., Hage, T., Comery, T. A., and Brandon, N. J. (2009). PDE10A inhibitor activity in preclinical models of the positive, cognitive and negative symptoms of schizophrenia. *J. Pharmacol. Exp. Ther.* 331, 574–590.
- Greengard, P., Allen, P. B., and Nairn, A. C. (1999). Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* 23, 435–447.
- Hallett, P. J., Spoelgen, R., Hyman, B. T., Standaert, D. G., and Dunah, A. W. (2006). Dopamine D1 activation potentiates striatal NMDA receptors by tyrosine phosphorylation-dependent subunit trafficking. *J. Neurosci.* 26, 4690–4700.
- Halpain, S., Girault, J.-A., and Greengard, P. (1990). Activation of NMDA receptors induced dephosphorylation of DARPP-32 in rat striatal slices. *Nature* 343, 369–372.
- Hara, M., Fukui, R., Hieda, E., Kuroiwa, M., Bateup, H. S., Kano, T., Greengard, P., and Nishi, A. (2010). Role of adrenoceptors in the regulation of dopamine/DARPP-32 signaling in neostriatal neurons. *J. Neurochem.* 113, 1046–1059.
- Hasbi, A., Fan, T., Alijanian, M., Nguyen, T., Perreault, M. L., O'Dowd, B. F., and George, S. R. (2009). Calcium signaling cascade links dopamine D1-D2 receptor heteromer to striatal BDNF production and neuronal growth. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21377–21382.
- Hasbi, A., O'Dowd, B. F., and George, S. R. (2010). Heteromerization of dopamine D2 receptors with dopamine D1 or D5 receptors generates intracellular calcium signaling by different mechanisms. *Curr. Opin. Pharmacol.* 10, 93–99.
- Heiman, M., Schaefer, A., Gong, S., Peterson, J. D., Day, M., Ramsey, K. E., Suarez-Farinas, M., Schwarz, C., Stephan, D. A., Surmeier, D. J., Greengard, P., and Heintz, N. (2008). A translational profiling approach for the molecular characterization of CNS cell types. *Cell* 135, 738–748.
- Hernandez-Lopez, S., Tkatch, T., Perez-Garci, E., Galarraaga, E., Bargas, J., Hamm, H., and Surmeier, D. J. (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca²⁺ currents and excitability via a novel PLC[β 1]-IP3-calcineurin-signaling cascade. *J. Neurosci.* 20, 8987–8995.
- Hersch, S. M., Ciliax, B. J., Gutekunst, C. A., Rees, H. D., Heilman, C. J., Yung, K. K., Bolam, J. P., Ince, E., Yi, H., and Levey, A. I. (1995). Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. *J. Neurosci.* 15, 5222–5237.
- Herve, D., Le Moine, C., Corvol, J. C., Belluscio, L., Ledent, C., Fienberg, A. A., Jaber, M., Studler, J. M., and Girault, J. A. (2001). Galpho(olf) levels are regulated by receptor usage and control dopamine and adenosine action in the striatum. *J. Neurosci.* 21, 4390–4399.
- Houslay, M. D. (2010). Underpinning compartmentalised cAMP signalling through targeted cAMP breakdown. *Trends Biochem. Sci.* 35, 91–100.
- Houslay, M. D., and Adams, D. R. (2003). PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. *Biochem. J.* 370, 1–18.
- Hyman, S. E., and Malenka, R. C. (2001). Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat. Rev. Neurosci.* 2, 695–703.
- Jackson, D. M., and Westlind-Danielsson, A. (1994). Dopamine receptors: molecular biology, biochemistry and behavioural aspects. *Pharmacol. Ther.* 64, 291–370.
- Janssens, V., Jordens, J., Stevens, I., Van Hoof, C., Martens, E., De Smedt, H., Engelborghs, Y., Waelkens, E., and Goris, J. (2003). Identification and functional analysis of two Ca²⁺-binding EF-hand motifs in the B¹PR72 subunit of protein phosphatase 2A. *J. Biol. Chem.* 278, 10697–10706.
- Jin, L. Q., Goswami, S., Cai, G., Zhen, X., and Friedman, E. (2003). SKF83959 selectively regulates phosphatidylinositol-linked D1 dopamine receptors in rat brain. *J. Neurochem.* 85, 378–386.
- Jin, L. Q., Wang, H. Y., and Friedman, E. (2001). Stimulated D(1) dopamine receptors couple to multiple Galpho proteins in different brain regions. *J. Neurochem.* 78, 981–990.
- Kebabian, J. W., and Calne, D. B. (1979). Multiple receptors for dopamine. *Nature* 277, 93–96.
- Kitaoka, S., Furuyashiki, T., Nishi, A., Shuto, T., Koyasu, S., Matsuoka, T., Miyasaka, M., Greengard, P., and Narumiya, S. (2007). Prostaglandin E2 acts on EP1 receptor and amplifies both dopamine D1 and D2 receptor signaling in the striatum. *J. Neurosci.* 27, 12900–12907.
- Kuroiwa, M., Bateup, H. S., Shuto, T., Higashi, H., Tanaka, M., and Nishi, A. (2008). Regulation of DARPP-32 phosphorylation by three distinct dopamine D1-like receptor signaling pathways in the neostriatum. *J. Neurochem.* 107, 1014–1026.

- Lee, F. J., Xue, S., Pei, L., Vukusic, B., Chery, N., Wang, Y., Wang, Y. T., Niznik, H. B., Yu, X. M., and Liu, F. (2002). Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. *Cell* 111, 219–230.
- Lee, S. P., So, C. H., Rashid, A. J., Varghese, G., Cheng, R., Lanca, A. J., O'Dowd, B. F., and George, S. R. (2004). Dopamine D1 and D2 receptor co-activation generates a novel phospholipase C-mediated calcium signal. *J. Biol. Chem.* 279, 35671–35678.
- Lindskog, M., Svenningsson, P., Fredholm, B., Greengard, P., and Fisone, G. (1999). Mu- and delta-opioid receptor agonists inhibit DARPP-32 phosphorylation in distinct populations of striatal projection neurons. *Eur. J. Neurosci.* 11, 2182–2186.
- Lindskog, M., Svenningsson, P., Pozzi, L., Kim, Y., Fienberg, A. A., Bibb, J. A., Fredholm, B. B., Nairn, A. C., Greengard, P., and Fisone, G. (2002). Involvement of DARPP-32 phosphorylation in the stimulant action of caffeine. *Nature* 418, 774–778.
- Mahan, L. C., Burch, R. M., Monsma, F. J. Jr., and Sibley, D. R. (1990). Expression of striatal D1 dopamine receptors coupled to inositol phosphate production and Ca²⁺ mobilization in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. U.S.A.* 87, 2196–2200.
- Marcellino, D., Ferre, S., Casado, V., Cortes, A., Le Foll, B., Mazzola, C., Drago, F., Saur, O., Stark, H., Soriano, A., Barnes, C., Goldberg, S. R., Luis, C., Fuxe, K., and Franco, R. (2008). Identification of dopamine D1–D3 receptor heteromers. Indications for a role of synergistic D1–D3 receptor interactions in the striatum. *J. Biol. Chem.* 283, 26016–26025.
- McCahill, A. C., Huston, E., Li, X., and Houslay, M. D. (2008). PDE4 associates with different scaffolding proteins: modulating interactions as treatment for certain diseases. *Handb. Exp. Pharmacol.* 186, 125–166.
- Menegoz, M., Lau, L. F., Herve, D., Huguier, R. L., and Girault, J. A. (1995). Tyrosine phosphorylation of NMDA receptor in rat striatum: effects of 6-OH-dopamine lesions. *Neuroreport* 7, 125–128.
- Menniti, F. S., Faraci, W. S., and Schmidt, C. J. (2006). Phosphodiesterases in the CNS: targets for drug development. *Nat. Rev. Drug Discov.* 5, 660–670.
- Millar, J. K., Pickard, B. S., Mackie, S., James, R., Christie, S., Buchanan, S. R., Malloy, M. P., Chubb, J. E., Huston, E., Baillie, G. S., Thomson, P. A., Hill, E. V., Brandon, N. J., Rain, J. C., Camargo, L. M., Whiting, P. J., Houslay, M. D., Blackwood, D. H., Muir, W. J., and Porteous, D. J. (2005). DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science* 310, 1187–1191.
- Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., and Caron, M. G. (1998). Dopamine receptors: from structure to function. *Physiol. Rev.* 78, 189–225.
- Murdoch, H., Mackie, S., Collins, D. M., Hill, E. V., Bolger, G. B., Klusmann, E., Porteous, D. J., Millar, J. K., and Houslay, M. D. (2007). Isoform-selective susceptibility of DISC1/phosphodiesterase-4 complexes to dissociation by elevated intracellular cAMP levels. *J. Neurosci.* 27, 9513–9524.
- Nishi, A., Bibb, J. A., Matsuyama, S., Hamada, M., Higashi, H., Nairn, A. C., and Greengard, P. (2002). Regulation of DARPP-32 dephosphorylation at PKA- and Cdk5-sites by NMDA and AMPA receptors: distinct roles of calcineurin and protein phosphatase-2A. *J. Neurochem.* 81, 832–841.
- Nishi, A., Bibb, J. A., Snyder, G. L., Higashi, H., Nairn, A. C., and Greengard, P. (2000). Amplification of dopaminergic signaling by a positive feedback loop. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12840–12845.
- Nishi, A., Kuroiwa, M., Miller, D. B., O'Callaghan, J. P., Bateup, H. S., Shuto, T., Sotogaku, N., Fukuda, T., Heintz, N., Greengard, P., and Snyder, G. L. (2008). Distinct roles of PDE4 and PDE10A in the regulation of cAMP/PKA signaling in the striatum. *J. Neurosci.* 28, 10460–10471.
- Nishi, A., and Snyder, G. L. (2010). Advanced research on dopamine signaling to develop drugs for the treatment of mental disorders: biochemical and behavioral profiles of phosphodiesterase inhibition in dopaminergic neurotransmission. *J. Pharmacol. Sci.* 114, 6–16.
- Nishi, A., Snyder, G. L., and Greengard, P. (1997). Bidirectional regulation of DARPP-32 phosphorylation by dopamine. *J. Neurosci.* 17, 8147–8155.
- Nishi, A., Snyder, G. L., Nairn, A. C., and Greengard, P. (1999). Role of calcineurin and protein phosphatase-2A in the regulation of DARPP-32 dephosphorylation in neostriatal neurons. *J. Neurochem.* 72, 2015–2021.
- Nishi, A., Watanabe, Y., Higashi, H., Tanaka, M., Nairn, A. C., and Greengard, P. (2005). Glutamate regulation of DARPP-32 phosphorylation in neostriatal neurons involves activation of multiple signaling cascades. *Proc. Natl. Acad. Sci. U.S.A.* 102, 1199–1204.
- O'Sullivan, G. J., Roth, B. L., Kinsella, A., and Waddington, J. L. (2004). SK&F 83822 distinguishes adenylyl cyclase from phospholipase C-coupled dopamine D1-like receptors: behavioural topography. *Eur. J. Pharmacol.* 486, 273–280.
- Ouimet, C. C., and Greengard, P. (1990). Distribution of DARPP-32 in the basal ganglia: an electron microscopic study. *J. Neurocytol.* 19, 39–52.
- Panchalingam, S., and Undie, A. S. (2001). SKF83959 exhibits biochemical agonism by stimulating [(35)S]GTP gamma S binding and phosphoinositide hydrolysis in rat and monkey brain. *Neuropharmacology* 40, 826–837.
- Pascoli, V., Besnard, A., Herve, D., Pages, C., Heck, N., Girault, J. A., Caboche, J., and Vanhoutte, P. (2011). Cyclic adenosine monophosphate-independent tyrosine phosphorylation of NR2B mediates cocaine-induced extracellular signal-regulated kinase activation. *Biol. Psychiatry* 69, 218–227.
- Perez-Torres, S., Miro, X., Palacios, J. M., Cortes, R., Puigdomenech, P., and Mengo, G. (2000). Phosphodiesterase type 4 isozymes expression in human brain examined by in situ hybridization histochemistry and [3H]rolipram binding autoradiography. Comparison with monkey and rat brain. *J. Chem. Neuroanat.* 20, 349–374.
- Polli, J. W., and Kincaid, R. L. (1994). Expression of calmodulin-dependent phosphodiesterase isoform (PDE1B1) correlates with brain regions having extensive dopaminergic innervation. *J. Neurosci.* 14, 1251–1261.
- Rashid, A. J., O'Dowd, B. F., Verma, V., and George, S. R. (2007a). Neuronal Gq/11-coupled dopamine receptors: an uncharted role for dopamine. *Trends Pharmacol. Sci.* 28, 551–555.
- Rashid, A. J., So, C. H., Kong, M. M., Furtak, T., El-Ghundi, M., Cheng, R., O'Dowd, B. F., and George, S. R. (2007b). D1–D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 104, 654–659.
- Rebholz, H., Nishi, A., Liebscher, S., Nairn, A. C., Flajolet, M., and Greengard, P. (2009). CK2 negatively regulates Galphas signaling. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14096–14101.
- Reed, T. M., Repaske, D. R., Snyder, G. L., Greengard, P., and Vorhees, C. V. (2002). Phosphodiesterase 1B knock-out mice exhibit exaggerated locomotor hyperactivity and DARPP-32 phosphorylation in response to dopamine agonists and display impaired spatial learning. *J. Neurosci.* 22, 5188–5197.
- Reid, M. S., Hsu, K. Jr., and Berger, S. P. (1997). Cocaine and amphetamine preferentially stimulate glutamate release in the limbic system: studies on the involvement of dopamine. *Synapse* 27, 95–105.
- Sahu, A., Tyeryar, K. R., Vongtau, H. O., Sibley, D. R., and Undie, A. S. (2009). D5 dopamine receptors are required for dopaminergic activation of phospholipase C. *Mol. Pharmacol.* 75, 447–453.
- Sano, H., Nagai, Y., Miyakawa, T., Shigemoto, R., and Yokoi, M. (2008). Increased social interaction in mice deficient of the striatal medium spiny neuron-specific phosphodiesterase 10A2. *J. Neurochem.* 105, 546–556.
- Santini, E., Valjent, E., Usiello, A., Carta, M., Borgkvist, A., Girault, J. A., Herve, D., Greengard, P., and Fisone, G. (2007). Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *J. Neurosci.* 27, 6995–7005.
- Schmidt, C. J., Chapin, D. S., Cianfrogna, J., Corman, M. L., Hajos, M., Harms, J. F., Hoffman, W. E., Lebel, L. A., McCarthy, S. A., Nelson, F. R., Proulx-LaFrance, C., Majchrzak, M. J., Ramirez, A. D., Schmidt, K., Seymour, P. A., Siuciak, J. A., Tingley, F. D. III, Williams, R. D., Verhoest, P. R., and Menniti, F. S. (2008). Preclinical characterization of selective phosphodiesterase 10A inhibitors: a new therapeutic approach to the treatment of schizophrenia. *J. Pharmacol. Exp. Ther.* 325, 681–690.
- Schmitz, Y., Benoit-Marand, M., Monon, F., and Sulzer, D. (2003). Presynaptic regulation of dopaminergic neurotransmission. *J. Neurochem.* 87, 273–289.
- Shuto, T., and Nishi, A. (2011). Treatment of the psychostimulant-sensitized animal model of schizophrenia. *CNS Neurosci. Ther.* 17, 133–139.
- Siuciak, J. A. (2008). The role of phosphodiesterases in schizophrenia: therapeutic implications. *CNS Drugs* 22, 983–993.
- Siuciak, J. A., Chapin, D. S., Harms, J. F., Lebel, L. A., McCarthy, S. A., Chambers, L., Shrikhande, A., Wong, S., Menniti, F. S., and Schmidt, C. J. (2006). Inhibition of the striatum-enriched phosphodiesterase PDE10A: a novel approach to the treatment of psychosis. *Neuropharmacology* 51, 386–396.

- Siuciak, J. A., McCarthy, S. A., Chapin, D. S., Reed, T. M., Vorhees, C. V., and Repaske, D. R. (2007). Behavioral and neurochemical characterization of mice deficient in the phosphodiesterase-1B (PDE1B) enzyme. *Neuropharmacology* 53, 113–124.
- Stipanovich, A., Valjent, E., Matamalas, M., Nishi, A., Ahn, J. H., Maroteaux, M., Bertran-Gonzalez, J., Brami-Cherrier, K., Enslen, H., Corbille, A. G., Filhol, O., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2008). A phosphatase cascade by which rewarding stimuli control nucleosomal response. *Nature* 453, 879–884.
- Strick, C. A., James, L. C., Fox, C. B., Seeger, T. F., Menniti, F. S., and Schmidt, C. J. (2010). Alterations in gene regulation following inhibition of the striatum-enriched phosphodiesterase, PDE10A. *Neuropharmacology* 58, 444–451.
- Sulzer, D. (2011). How addictive drugs disrupt presynaptic dopamine neurotransmission. *Neuron* 69, 628–649.
- Surmeier, D. J., Song, W. J., and Yan, Z. (1996). Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. *J. Neurosci.* 16, 6579–6591.
- Svenningsson, P., Nairn, A. C., and Greengard, P. (2005). DARPP-32 mediates the actions of multiple drugs of abuse. *AAPS J.* 7, E353–E360.
- Svenningsson, P., Nishi, A., Fisone, G., Girault, J. A., Nairn, A. C., and Greengard, P. (2004). DARPP-32: an integrator of neurotransmission. *Annu. Rev. Pharmacol. Toxicol.* 44, 269–296.
- Svenningsson, P., Tzavara, E. T., Liu, F., Fienberg, A. A., Nomikos, G. G., and Greengard, P. (2002). DARPP-32 mediates serotonergic neurotransmission in the forebrain. *Proc. Natl. Acad. Sci. U.S.A.* 99, 3188–3193.
- Svenningsson, P., Tzavara, E. T., Qi, H., Carruthers, R., Witkin, J. M., Nomikos, G. G., and Greengard, P. (2007). Biochemical and behavioral evidence for antidepressant-like effects of 5-HT₆ receptor stimulation. *J. Neurosci.* 27, 4201–4209.
- Tanda, K., Nishi, A., Matsuo, N., Nakanishi, K., Yamasaki, N., Sugimoto, T., Toyama, K., Takao, K., and Miyakawa, T. (2009). Abnormal social behavior, hyperactivity, impaired remote spatial memory, and increased D1-mediated dopaminergic signaling in neuronal nitric oxide synthase knockout mice. *Mol. Brain* 2, 19.
- Threlfell, S., Sammut, S., Menniti, F. S., Schmidt, C. J., and West, A. R. (2009). Inhibition of phosphodiesterase 10A increases the responsiveness of striatal projection neurons to cortical stimulation. *J. Pharmacol. Exp. Ther.* 328, 785–795.
- Toda, S., Alguacil, L. F., and Kalivas, P. W. (2003). Repeated cocaine administration changes the function and subcellular distribution of adenosine A1 receptor in the rat nucleus accumbens. *J. Neurochem.* 87, 1478–1484.
- Tomiyama, K., McNamara, F. N., Clifford, J. J., Kinsella, A., Drago, J., Tighe, O., Croke, D. T., Koshikawa, N., and Waddington, J. L. (2002). Phenotypic resolution of spontaneous and D1-like agonist-induced orofacial movement topographies in congenic dopamine D1A receptor “knockout” mice. *Neuropharmacology* 42, 644–652.
- Undie, A. S., Berki, A. C., and Beardsley, K. (2000). Dopaminergic behaviors and signal transduction mediated through adenylate cyclase and phospholipase C pathways. *Neuropharmacology* 39, 75–87.
- Undie, A. S., and Friedman, E. (1990). Stimulation of a dopamine D1 receptor enhances inositol phosphates formation in rat brain. *J. Pharmacol. Exp. Ther.* 253, 987–992.
- Undie, A. S., Weinstock, J., Sarau, H. M., and Friedman, E. (1994). Evidence for a distinct D1-like dopamine receptor that couples to activation of phosphoinositide metabolism in brain. *J. Neurochem.* 62, 2045–2048.
- Valjent, E., Bertran-Gonzalez, J., Herve, D., Fisone, G., and Girault, J. A. (2009). Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci.* 32, 538–547.
- Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., Stipanovich, A., Caboche, J., Lombroso, P. J., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 102, 491–496.
- Walaas, S. I., and Greengard, P. (1984). DARPP-32, a dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein enriched in dopamine-innervated brain regions. I. Regional and cellular distribution in rat brain. *J. Neurosci.* 4, 84–98.
- Wang, H. Y., Undie, A. S., and Friedman, E. (1995). Evidence for the coupling of Gq protein to D1-like dopamine sites in rat striatum: possible role in dopamine-mediated inositol phosphate formation. *Mol. Pharmacol.* 48, 988–994.
- Wittmann, M., Marino, M. J., Henze, D. A., Seabrook, G. R., and Conn, P. J. (2005). Clozapine potentiation of N-methyl-D-aspartate receptor currents in the nucleus accumbens: role of NR2B and protein kinase A/Src kinases. *J. Pharmacol. Exp. Ther.* 313, 594–603.
- Xie, Z., Adamowicz, W. O., Eldred, W. D., Jakowski, A. B., Kleiman, R. J., Morton, D. G., Stephenson, D. T., Strick, C. A., Williams, R. D., and Menniti, F. S. (2006). Cellular and subcellular localization of PDE10A, a striatum-enriched phosphodiesterase. *Neuroscience* 139, 597–607.
- Yabuuchi, K., Kuroiwa, M., Shuto, T., Sotogaku, N., Snyder, G. L., Higashi, H., Tanaka, M., Greengard, P., and Nishi, A. (2006). Role of adenosine A(1) receptors in the modulation of dopamine D(1) and adenosine A(2a) receptor signaling in the neostriatum. *Neuroscience* 141, 19–25.
- Zhang, Y., Loonam, T. M., Noailles, P. A., and Angulo, J. A. (2001). Comparison of cocaine- and methamphetamine-evoked dopamine and glutamate overflow in somatodendritic and terminal field regions of the rat brain during acute, chronic, and early withdrawal conditions. *Ann. N. Y. Acad. Sci.* 937, 93–120.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 May 2011; paper pending published: 06 June 2011; accepted: 07 July 2011; published online: 18 July 2011.
Citation: Nishi A, Kuroiwa M and Shuto T (2011) Mechanisms for the modulation of dopamine D₁ receptor signaling in striatal neurons. *Front. Neuroanat.* 5:43. doi: 10.3389/fnana.2011.00043
Copyright © 2011 Nishi, Kuroiwa and Shuto. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Identification of a specific assembly of the G protein Golf as a critical and regulated module of dopamine and adenosine-activated cAMP pathways in the striatum

Denis Hervé^{1,2,3*}

¹ INSERM UMR-S839, Paris, France

² Université Pierre et Marie Curie, Paris, France

³ Institut du Fer à Moulin, Paris, France

Edited by:

Emmanuel Valjent, Université Montpellier 1 & 2, France

Reviewed by:

Sergi Ferre, Karolinska Institute, Sweden

Serge N. Schiffmann, Université Libre de Bruxelles, Belgium

*Correspondence:

Denis Hervé, INSERM UMR-S839, Institut du Fer à Moulin, 17, rue du Fer à Moulin, 75005 Paris, France.
e-mail: denis.herve@inserm.fr

In the principal neurons of striatum (medium spiny neurons, MSNs), cAMP pathway is primarily activated through the stimulation of dopamine D1 and adenosine A_{2A} receptors, these receptors being mainly expressed in striatonigral and striatopallidal MSNs, respectively. Since cAMP signaling pathway could be altered in various physiological and pathological circumstances, including drug addiction and Parkinson's disease, it is of crucial importance to identify the molecular components involved in the activation of this pathway. In MSNs, cAMP pathway activation is not dependent on the classical Gs GTP-binding protein but requires a specific G protein subunit heterotrimer containing G α olf/ β 2/ γ 7 in particular association with adenylyl cyclase type 5. This assembly forms an authentic functional signaling unit since loss of one of its members leads to defects of cAMP pathway activation in response to D1 or A_{2A} receptor stimulation, inducing dramatic impairments of behavioral responses dependent on these receptors. Interestingly, D1 receptor (D1R)-dependent cAMP signaling is modulated by the neuronal levels of G α olf, indicating that G α olf represents the rate-limiting step in this signaling cascade and could constitute a critical element for regulation of D1R responses. In both Parkinsonian patients and several animal models of Parkinson's disease, the lesion of dopamine neurons produces a prolonged elevation of G α olf levels. This observation gives an explanation for the cAMP pathway hypersensitivity to D1R stimulation, occurring despite an unaltered D1R density. In conclusion, alterations in the highly specialized assembly of G α olf/ β 2/ γ 7 subunits can happen in pathological conditions, such as Parkinson's disease, and it could have important functional consequences in relation to changes in D1R signaling in the striatum.

Keywords: D1 receptor, A_{2A} receptor, heterotrimeric G protein, cAMP pathway, extracellular signal-regulated kinase, Gnal gene, Parkinson's disease, cocaine

INTRODUCTION

Dopamine, probably the best characterized neurotransmitter involved in slow synaptic neurotransmission, plays a prominent role in a variety of brain functions, including motor control, motivation, short-term memory, and reward (Schultz, 1998). Five genes encoding dopamine receptors have been cloned in mammals (see Sibley and Monsma, 1992 for review). All these receptors belong to the superfamily of G protein-coupled receptors with seven transmembrane domains and the comparison of their amino acid sequence, pharmacological profile, and biochemical properties has revealed two distinct categories, named respectively D1- and D2-type dopamine receptors. The D1-type receptors, comprising D1 and D5 subtypes, are positively coupled to cAMP production whereas the D2-type receptors, comprising D2, D3, and D4 subtypes, are able to inhibit cAMP production (see Missale et al., 1998 review). The D1 receptor (D1R) is the most abundantly expressed dopamine receptors and is present in virtually all the brain areas innervated by dopamine neurons (Boyson et al., 1986). Consistent with its dense dopamine innervation, the striatum contains the highest concentration of D1Rs in the brain. Different approaches

using *in situ* hybridization, immunocytochemistry, and transgenic mice indicate that D1R in the striatum is highly expressed in a subpopulation of GABAergic medium spiny neurons (MSNs) projecting to the substantia nigra and entopeduncular nucleus (direct pathway of basal ganglia) and containing substance P and dynorphin as co-neurotransmitters (Gerfen et al., 1990; Le Moine and Bloch, 1995; Yung et al., 1995; Drago et al., 1998b; Gong et al., 2003; Lee et al., 2006; Bertran-Gonzalez et al., 2008). By contrast, D2 receptors are essentially present in the MSNs projecting to the globus pallidus and containing enkephalins (indirect pathway of basal ganglia). These neurons express abundantly the adenosine A_{2A} receptors that are able to stimulate production of intracellular cAMP (Schiffmann et al., 2007). In a recent study using transgenic mouse lines, it was estimated that about 50% of GABA MSNs express exclusively D1Rs and 35–45% exclusively D2 receptors (Bertran-Gonzalez et al., 2008). The population of MSNs co-expressing both D1R and D2 receptor, is low in the dorsal striatum and core of nucleus accumbens (about 5%) but is slightly higher in the shell of nucleus accumbens (17%; Bertran-Gonzalez et al., 2008; Hasbi et al., 2009; Matamalas et al., 2009).

Pharmacological studies and investigations on D1R-deleted mice have shown the importance of D1R in mediating the effects of DA neurotransmission (Drago et al., 1998a; El-Ghundi et al., 2007). The actions of D1R require the participation of heterotrimeric guanine nucleotide binding proteins (G proteins) whose roles in diverse signaling pathways may be determined by their specific $\alpha\beta\gamma$ subunit combinations. These heterotrimeric G proteins are molecular switches in which the agonist-activated receptors catalyze the exchange of GDP for GTP on the α -subunit of heterotrimeric G proteins ($G\alpha$), which in turn engages conformational and/or dissociational events between the $G\alpha$ and dimeric $G\beta\gamma$ subunits (Bourne et al., 1991). In the case of D1R, it is well established that the GTP-bound $G\alpha$ subunit initiates the activation of adenylyl cyclase (AC) leading to the intracellular production of cAMP and stimulation of cAMP-dependent protein kinase (PKA) and others cAMP-dependent proteins (Kebabian and Calne, 1979; Hervé and Girault, 2005). An extensive body of evidence indicates that D1-type receptors also couple via $G\alpha_q$ subunits to phospholipase C (Mahan et al., 1990; Arnt et al., 1992; Wang et al., 1995; Lezcano and Bergson, 2002; Mannoury La Cour et al., 2007) but a debate exists to know if the receptors involved are *bona fide* D1Rs (Mannoury La Cour et al., 2007), heteromers of D1R and D2 receptor (Hasbi et al., 2009), or different receptors with D1-type pharmacological properties (Friedman et al., 1997). Here, we will review the present knowledge about the nature of the G proteins able to couple D1R to AC in the striatum and about the regulatory processes at the level of these G proteins that control the D1R-mediated signaling and its functional consequences.

$G\alpha_{OLF}$ ROLE IN THE COUPLING OF D1R AND A_{2A} RECEPTOR TO ADENYLYL CYCLASE IN THE STRIATUM

Since D1R is positively coupled to AC, a long-standing dogma stated that this action was mediated by the classical heterotrimeric stimulatory G protein containing the $G\alpha_s$ subunit. However, the $G\alpha_s$ expression is low in the striatum compared with that observed in many other brain areas (Figure 1A; Largent et al., 1988). In the striatum, $G\alpha_s$ is replaced by a high expression of $G\alpha_{OLF}$, an isoform of $G\alpha_s$ (Figure 1; Drinnan et al., 1991; Hervé et al., 1993) which was first discovered in the olfactory epithelium and found crucial for olfaction by mediating the coupling of olfactory receptors to AC (Jones and Reed, 1989; Belluscio et al., 1998).

In rodents $G\alpha_{OLF}$ is highly expressed in all the MSNs in the striatum including those bearing the D1R (Kull et al., 2000; Hervé et al., 2001). It is also expressed in cholinergic aspiny interneurons in the striatum. In human, high expression of $G\alpha_{OLF}$ was also detected in the striatum and its decrease in patients with Huntington's disease is a strong indication of its expression in the MSNs (Corvol et al., 2004). In the striatum of mice with a null targeted mutation of $G\alpha_{OLF}$ -encoding gene, AC activation in response to D1R stimulation is absent, which demonstrates clearly that the D1R acts through the $G\alpha_{OLF}$ protein to stimulate cAMP production (Zhuang et al., 2000; Corvol et al., 2001).

However, $G\alpha_{OLF}$ and D1R are not systematically associated in the various neuronal types. D1R is present in neurons that do not express high level of $G\alpha_{OLF}$, such as the neurons of prefrontal cortex. In these neurons, AC activation to D1 agonist is mediated through $G\alpha_s$ as indicated by the lack of alteration of this

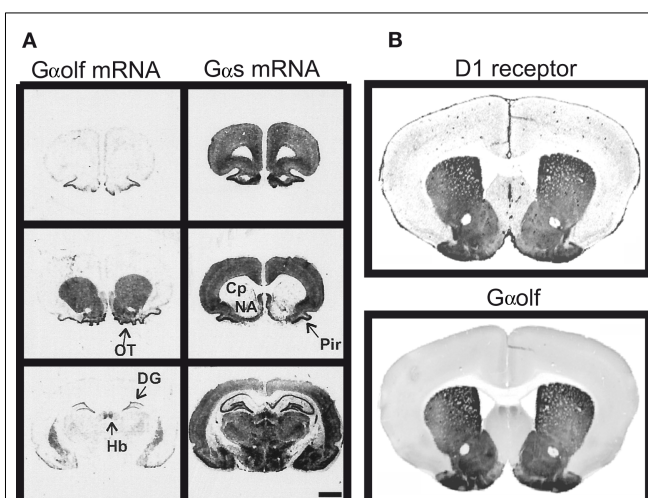


FIGURE 1 | Similar distribution of D1 receptor and $G\alpha_{OLF}$ protein in the striatum. (A) Distribution of $G\alpha_{OLF}$ and $G\alpha_s$ mRNAs in rat forebrain. $G\alpha_{OLF}$ mRNA (left) is highly expressed in striatal areas, including caudate putamen (Cp), nucleus accumbens (NA), and olfactory tubercle (OT). These areas show almost no expression of $G\alpha_s$, except for some sparse neurons (right). On the contrary, $G\alpha_s$ mRNA was highly expressed in many brain areas including all cortical areas, septum, most of the hypothalamic and thalamic nuclei, hippocampus, and amygdala, in which $G\alpha_{OLF}$ mRNA expression is very low. In few brain regions, substantial expression of both $G\alpha_{OLF}$ and $G\alpha_s$ mRNAs is observed: piriform cortex (Pyr), medial habenula (Hb), and dentate gyrus (DG). Positive of X-ray films exposed to rat brain sections hybridized with ^{35} S-labeled probes for $G\alpha_{OLF}$ (left) and $G\alpha_s$ mRNAs (right). Scale bar, 5 mm. Adapted with permission from the work of Hervé et al. (2001). **(B)** Mouse sections (30 μ m-thick) have been incubated with mouse antibodies against D1R (generous gift of R. Luedtke) and rabbit antibodies against $G\alpha_{OLF}$. The primary antibodies were detected with IRDye700 conjugated anti-mouse IgG and IRDye800 conjugated anti-rabbit IgG. The sections were scanned using a LI-COR Odyssey infrared fluorescent detection system. The figure shows the scans of one representative section with anti-D1R (upper panel) and anti- $G\alpha_{OLF}$ antibodies (lower panel). Adapted with permission from the work of E. Valjent.

response in $G\alpha_{OLF}$ -deficient mutant mice (Corvol et al., 2001). In the other hand, $G\alpha_{OLF}$ is present in high amount in the MSNs without D1R expression that project to the globus pallidus and contain D2 receptors. In these neurons, $G\alpha_{OLF}$ is involved in the positive coupling of adenosine A_{2A} receptors with AC since an A_{2A} receptor agonist dose dependently activates $G\alpha_{OLF}$ in striatal membranes (Kull et al., 2000) and the stimulatory effect of this agonist on AC activity is missing in the mutant mice deficient in $G\alpha_{OLF}$ (Corvol et al., 2001).

$G\alpha_{OLF}$ IS NECESSARY FOR DOPAMINE ACTION IN THE STRIATUM

Mice homozygous for null mutation in $G\alpha_{OLF}$ gene show a complete anosmia because of the crucial role played by $G\alpha_{OLF}$ in the transduction of the olfactory receptor at the level of primary olfactory neurons (Belluscio et al., 1998). This profound anosmia produces an important postnatal lethality (more than 80% of the mutant mice do not feed properly and die within 3 days after birth). The rare surviving homozygous animals exhibit reduction in body weight and they display a marked hyperactive

behavior, evoking a possible alteration of striatal functions (Beluscio et al., 1998). The psychostimulants, such as cocaine or D-amphetamine, produce in the striatum the activation of several D1R-dependent signaling events, including activation of PKA, extracellular signal-regulated kinase (ERK), or c-fos gene induction (Berretta et al., 1992; Valjent et al., 2000; Nairn et al., 2004). All these effects are absent when *G α olf* gene is deleted (Zhuang et al., 2000; Corvol et al., 2007), showing the crucial role played by *G α olf* in most of the known intracellular effects of D1R activation.

The D1 agonist-induced hyperlocomotor response is abolished in *G α olf* knockout mice, indicating that *G α olf* is necessary for behavior action of D1R stimulation (Zhuang et al., 2000). In addition, it is well established that the acute hyperlocomotion induced by cocaine is dependent on D1R stimulation (Drago et al., 1998a; Valjent et al., 2000). It is noteworthy that the acute locomotor response to cocaine is absent in *G α olf* knockout mice (Zhuang et al., 2000). Altogether, these observations demonstrate that acute responses to cocaine and probably other psychostimulants are highly dependent on *G α olf*-linked D1R signaling.

COMPARISON OF *G α OLF* AND *G α S*

G α olf shares 80% amino acid identity with *G α s* and the exon/intron structures of genes encoding *G α olf* and *G α s* (*Gnal* and *Gnas* respectively in mouse) are very similar, the main difference being the absence in *Gnal* of the alternatively spliced exon 3 of *Gnas* (Jones and Reed, 1989; Wadhawan et al., 2008). Both genes are characterized by the use of alternate upstream promoters and first exons giving rise to “extra-large” variants of the proteins (XL-*G α olf* and XL-*G α s*) in addition to the classically described proteins (Corradi et al., 2005). XL-*G α olf*, in which the N-terminal end of *G α olf* is replaced by a longer polypeptide, is able to couple D1R to AC in transfected cells because it retains all the functional domains of *G α olf*. In the human striatal areas, the expression of XL-*G α olf* mRNA is low, about 10-fold less than that of *G α olf* mRNA (Corradi et al., 2005). In striatal extracts of rodents, the protein is below the detection threshold in western blotting (personal observations). In the vicinity of the two alternatively used exons, both *Gnal* and *Gnas* locus contain CpG islands that could undergo differential methylation of DNA (Corradi et al., 2005; Wadhawan et al., 2008). DNA methylation most often results in repression of transcription and constitutes a hallmark of genomic imprinting. In the gene encoding *G α s*, differential methylation of CpG islands was reported in the alleles of maternal or paternal origins, and distinct transcripts are either biallelically expressed, maternally imprinted, or paternally imprinted (Weinstein et al., 2001). For instance, the XL-*G α s* mRNA is a transcript specific of the paternally derived allele (Plagge et al., 2008). It is possible that similar phenomenon exists for XL-*G α olf* mRNA but the specific imprinting that affects the maternal and paternal alleles of *G α olf* gene has not been precisely determined (Corradi et al., 2005).

The *Gnal* and *Gnas* genes are present in all the examined vertebrates, including mammals, amphibians, and fishes (Wadhawan et al., 2008). In contrast, studies in *Drosophila* indicate the existence only one *Gnas* ortholog (Wolfgang et al., 2001), suggesting that *Gnas* and *Gnal* result from a gene duplication after the divergence of vertebrates from invertebrates but before the divergence

of tetrapods from fishes. The time point of this event is estimated at –570 millions of years (Wadhawan et al., 2008).

G α olf displays some functional differences with *G α s*. Particularly, its affinity for GDP is lower and its deactivation after GTP-binding is more rapid (Liu et al., 2001). Because of these properties, *G α olf* has a higher constitutive activity than *G α s* *in vitro* (Liu et al., 2001), which may explain the decrease of basal AC activity in the *G α olf* knockout mice (Corvol et al., 2001). This relatively high constitutive activity of *G α olf* could result in a tonic AC activity *in vivo* leading to a constant activation of cAMP pathway in both striatonigral and striatopallidal MSNs.

Some evidence shows also that the percentage of AC activation is higher when receptor is coupled to *G α olf* than when it is coupled to *G α s* (Liu et al., 2001). Basically the signal-to-noise ratio for *G α olf*-coupled receptor appears considerably greater. In addition, because of its ability to deactivate more rapidly, *G α olf* could give rise to more transient activation of AC than *G α s*. It is conceivable that physiological functions of dopamine in the striatum require phasic AC activation and the fast deactivation of *G α olf* could contribute to rapidly restore responsiveness of MSNs between two dopamine stimuli. Adenosine signaling is generally regarded as a slow modulator regulating *A_{2A}* receptor-containing neurons in the striatum. However, evidence indicates that the formation of extracellular adenosine partly results from ATP released from nerve endings, which is dephosphorylated in adenosine by ecto-nucleotidases (Fredholm et al., 2005; Schiffmann et al., 2007). ATP is stored in synaptic vesicles together with most of neurotransmitters, including glutamate, and is co-released with the neurotransmitter upon nerve stimulation. Because *G α olf* provides high signal-to-noise ratio and rapidly deactivates, the *G α olf*-dependent signaling of *A_{2A}* receptor could mediate more time-limited actions than it is generally believed and could quickly adapt MSN functions to transient variations in synaptic input.

SPECIFIC ASSEMBLY OF *G α OLF*/β2/γ7 MEDIATES COUPLING OF D1R TO ADENYLYL CYCLASE

The regional expression of the γ7 subunit of G protein (Gγ7) in the brain was found to mirror that of D1R and *G α olf*, with a particularly high expression in MSNs (Watson et al., 1994), suggesting that Gγ7 subunit selectively associates with *G α olf* to couple D1R to AC. In agreement with this hypothesis, the deletion of Gγ7 gene in mutant mice causes an important reduction in the levels of *G α olf* and logically leads to drastic reduction of D1R or *A_{2A}* responses on the cAMP production (Schwindinger et al., 2003, 2010). In the Gγ7 mutant mice, reduction of β2 subunit of G protein (Gβ2) was also observed and quantitative measurements have indicated that the decrease in Gγ7, *G α olf*, and Gβ2 was very similar in term of molarity, strongly suggesting a specific assembly of *G α olf*/β2/γ7 heterotrimer enabling D1R coupling to AC in the striatal MSNs (Figure 2; Schwindinger et al., 2010).

Interestingly, in the mice with targeted deletion of *G α olf* gene, the levels of Gγ7 remain normal contrasting with the selective and coordinated reduction of *G α olf* and Gβ2 observed in the mutant mice lacking Gγ7 (Schwindinger et al., 2010). Because the corresponding mRNAs are not altered, the simplest explanation is to postulate that Gγ7 is required at a post-transcriptional level for the stabilization and/or trafficking of the *G α olf* and Gβ2 proteins

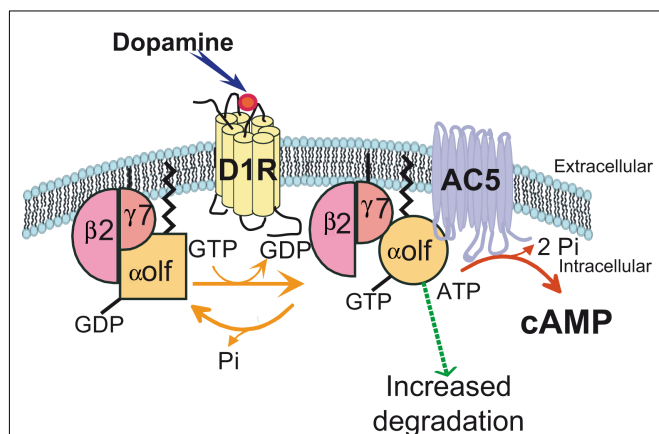


FIGURE 2 | A specific assembly of G α olf, G β 2, and G γ 7 subunits of G protein mediates the coupling of D1 receptor to adenylyl cyclase 5. The expression of G γ 7 subunit in striatal neurons recruits and stabilizes G α olf and G β 2 subunits. They form a specific heterotrimeric protein that provides the signaling complex necessary for the coupling of D1R receptor to the adenylyl cyclase 5 (AC5), an isoform particularly enriched in the striatum. The D1R stimulation by dopamine activates the G α olf/ β 2/ γ 7 heterotrimer by triggering substitution of GDP by GTP in the G α olf subunit and changes in the subunit conformation. The current data indicate that the G α activation does not necessarily cause its dissociation from G $\beta\gamma$ complex as it was thought previously (Bunemann et al., 2003). It has been proposed a “clamshell” model according to which activated receptor provokes movements in G $\alpha\beta\gamma$ complex that unmask previously buried interfaces and enable interaction of G α and G $\beta\gamma$ with specific effectors (Robishaw and Berlot, 2004). In this model, the G $\beta\gamma$ subunits are not shared among several α subunits but can remain associated with a specific pool of α subunit (Robishaw and Berlot, 2004). Such stable association may contribute to the specificity of G α olf interaction with G β 2 γ 7 complex. In addition, the activation of the heterotrimeric complex could increase its vulnerability to degradation processes. This effect could explain why the receptor usage reduces the G α olf levels in striatal neurons.

to the plasma membrane. These results indicate that in the MSNs, the formation of the G α olf/ β 2/ γ 7 heterotrimer is a hierarchical process that begins by the production of G γ 7 subunits and the later recruitment of G α olf and G β 2 subunits. These observations are surprising since MSNs express others types of γ subunits of G proteins, sometimes in higher abundance than G γ 7. The G γ 7 subunit appears to recruit selectively G α olf and G β 2 subunits to form a highly specialized heterotrimeric G protein in the MSNs, refuting the notion that G protein subunits are largely interchangeable (Schwindinger et al., 2010).

Medium spiny neurons are specially enriched in type 5 AC (AC5; Glatt and Snyder, 1993) that provides around 80% of basal AC activity in the striatum (Lee et al., 2002; Iwamoto et al., 2003). These observations suggest that this AC isoform is associated with the G α olf/ β 2/ γ 7 heterotrimeric G protein in the MSNs. In agreement with this idea, the lack of AC5 in the striatum of mice homozygous for a null mutation of AC5 gene produces drastic decrease of G α olf content in the striatum and AC activation in response to D1 agonist (Lee et al., 2002; Iwamoto et al., 2004). The mice deficient in AC5 display important deficit in appetitive pavlovian conditioning (Kheirbek et al., 2008) but surprisingly increased locomotor response to D1 agonist (Lee et al., 2002).

This paradoxical response is related to D1R stimulation since it is blocked by D1R antagonist, but its understanding remains unclear (Lee et al., 2002). It was hypothesized that the D1R-dependent behavior seen in AC5 knockout mice is related to non-AC effectors but the identification of these D1R-activated signaling pathways remains to be determined. The ERK pathway appears to be excluded since the AC5 knockout mice show a profound decoupling of D1R from downstream activation of ERK, similar to that observed on cAMP pathway (Kheirbek et al., 2008). It remains the possible implication of D1R coupled to phospholipase C in the striatum (Wang et al., 1995), or D1Rs independent from AC5 expressed in extrastriatal motor regions in the brain. Alternatively, the absence of AC5 in the striatum could produce profound alterations of other signaling pathways leading to an exacerbation of D1R-related responses despite the low D1R-related responses on cAMP production. Particularly, the behavioral responses linked to D2 receptors are completely eliminated in the mutant mice (Lee et al., 2002), possibly leading to an enhancement of responses produced by D1 agonist.

In conclusion, the signaling machinery enabling D1R to activate cAMP pathway is made up of a highly specialized assembly of G α olf/ β 2/ γ 7 heterotrimer and AC5. The consequences in term of functions, response dynamics, subcellular localization, or regulation remain largely unknown. Interestingly, the absence of G γ 7 or AC5 produces reduction of G α olf levels in the striatum, most probably by shortening its half-life. In activated state (GTP liganded), G α olf interacts with AC5 while in inactivated state, G α olf is associated with G β 2 γ 7 complex. G α olf stability appears thus to depend on the cellular availability of its two main interacting molecules, suggesting the tight and coordinated regulation of G α olf quantity in the neuron.

G α OLF LEVELS CONTROL THE EFFICACY OF THE ADENYLYL CYCLASE ACTIVATION BY D1R

The reductions of the levels of G α olf or D1R have contrasting consequences on various D1R signaling responses in the mouse striatum. These diminutions of G α olf or D1R can be obtained in mice heterozygous for targeted deletions of G α olf or D1R genes (Drago et al., 1994; Corvol et al., 2001), in which the striatal contents of corresponding proteins are decreased by about 50%. The reduction in G α olf levels induces a marked reduction of both basal and D1R-activated cAMP production in striatal membranes (Herve et al., 2001; Corvol et al., 2007). The AC activities in the presence of dopamine or in basal condition are reduced by approximately 50% and the D1R-related response (as estimated by the difference between the basal and stimulated activities) by about 35%. In contrast, the haplodeficiency in D1R leads to no significant change in D1 agonist response or in the basal and dopamine-stimulated activities (Corvol et al., 2007).

The levels of G α olf are not only determinant for *in vitro* AC responses, but also for *in vivo* responses. The increased cAMP levels resulting from D1R stimulation activate PKA in striatal neurons, leading to the phosphorylation of numerous PKA substrates including the GluR1 subunit of AMPA glutamate receptors (Valjent et al., 2005). Acute injection of psychostimulants like cocaine or D-amphetamine activates this pathway by increasing extracellular levels of dopamine in the brain. This response is highly

dependent on $G_{\alpha}olf$ in the striatum since it is strongly decreased when the $G_{\alpha}olf$ levels are reduced in the brain (mutant mice with heterozygous null mutation of $G_{\alpha}olf$ gene; Corvol et al., 2007). Reduction in D1R in mice heterozygous for null mutation of D1R gene did not alter significantly this response.

These results show that the levels of $G_{\alpha}olf$ protein, but not D1R, constitute a limiting factor determining the amplitude of cAMP pathway response upon D1R activation in the striatal neurons. This observation is consistent with the existence of “spare” D1Rs not coupled to AC in the striatum (Hess et al., 1987; Trovero et al., 1992). However, this is in apparent contradiction with the existence of a large excess of $G_{\alpha}olf/\beta 2/\gamma 7$ heterotrimers in comparison with D1Rs in term of number of molecules present in striatal membranes. Thorough measurements using quantitative immunoblots indicate that the concentration of G protein in striatal membrane is around 70–80 pmol/mg of membrane protein (Schwindinger et al., 2010). In contrast, the content in D1R (and A_{2A} receptors) would be almost two orders of magnitude lower (about 1 and 0.3 pmol/mg of membrane protein for D1R and A_{2A} receptor, respectively; Hess et al., 1987; Schwindinger et al., 2010). The mechanisms of activation of G proteins by receptors are still a matter of debate. Depending on receptor/G protein systems, two opposing models have been proposed (Lohse et al., 2008): (1) in the “collision coupling” model, the receptor/G protein interactions occur as a result of free lateral diffusion within the plasma membrane, wherein G proteins only interact with activated receptors; (2) the alternative model suggests that G proteins can interact with receptors before agonist binding, in a “precoupling” state. The second model is attractive because it could explain the specificity of coupling of D1R with precise G proteins. However, in this model, decreasing levels of receptor and G protein should lead to similar reductions of cAMP production. “Collision coupling” model explains probably better the mechanisms occurring in the striatal membranes even though the kinetics data are not enough precise to really settle this issue. In this model, the high excess of G proteins in the MSNs *in vivo* can result in amplification of signal, activated receptors being able to switch on multiple G proteins. This notion has been well established in the retina for the rhodopsin–transducin system (similar to the receptor–G protein couple) in which studies have given rates of 1300 transducin molecules activated per rhodopsin molecule per second (Heck and Hofmann, 2001). Probably the amplification factor is lower in the striatal cells, but it is well conceivable that despite the high excess in $G_{\alpha}olf/\beta 2/\gamma 7$ heterotrimers compared to D1R (or A_{2A} receptor), partial activation of D1R can saturate the G proteins present in the plasma membrane and thus the levels of the G proteins can represent a limiting factor controlling the D1R coupling with AC.

PARTIAL REDUCTION OF $G_{\alpha}OLF$ LEVELS DOES NOT ALTER ERK PATHWAY

Surprisingly, the haploinsufficiency of $G_{\alpha}olf$ gene does not affect D1R-dependent ERK pathway in the striatal neurons, contrary to what is observed for the cAMP pathway. Psychostimulants (cocaine or D-amphetamine) produce ERK activation specifically in D1R expressing striatal neurons (Valjent et al., 2000; Bertran-Gonzalez et al., 2008). This effect is dependent on D1R activation since it is prevented by pharmacological or genetic inactivation of

D1R (Valjent et al., 2000, 2005). Importantly this pathway appears critical for the long-lasting effects of cocaine or D-amphetamine, including conditioned place preference and locomotor sensitization (Valjent et al., 2000, 2006). In the heterozygous $G_{\alpha}olf$ mutant mice, psychostimulant-induced ERK activation is normal, similar to that observed in the wild type animals (Corvol et al., 2007).

Unexpectedly, this ERK response is impaired when the D1R levels are reduced by half in the mice heterozygous for null mutation of D1R gene (Pascoli et al., 2010). In the same mice cAMP/PKA response appears completely normal. In fact, the mechanisms of ERK activation following psychostimulants are complex since stimulation of D1R cannot activate ERK alone but potentiates the ERK activation initiated by calcium influx through glutamate-activated NMDA receptor (Pascoli et al., 2010). The mechanisms of this potentiation are probably multiple and combine PKA-dependent and independent processes. The cAMP/PKA-dependent potentiation of ERK pathway is mediated by the protein DARPP-32 via its ability to inhibit protein phosphatase 1 and striatal-enriched tyrosine phosphatase (STEP; Valjent et al., 2005). More recently, it has been uncovered an alternative pathway, by which D1R can stimulate glutamate-induced ERK activation by promoting D1R-dependent activation of Src family kinases and tyrosine phosphorylation of the NR2B subunit of NMDA receptor (Pascoli et al., 2010). This pathway is independent from the cAMP/PKA cascade and appears to be downregulated in mice heterozygous for null mutation of D1R gene (Pascoli et al., 2010). In these animals, the impairment of cocaine-induced ERK activation goes together with reduced activation of Src family kinase and phosphorylation of NR2B subunit.

In conclusion, D1R levels control the efficiency of the D1R-regulated ERK pathway whereas $G_{\alpha}olf$ levels controls that of the D1R-regulated cAMP/PKA pathway.

REDUCTION OF $G_{\alpha}OLF$ LEVELS HAS CONTRASTED BEHAVIORAL CONSEQUENCES

As previously mentioned, the acute responses to cocaine and D-amphetamine are highly dependent on D1R-linked signaling. The $G_{\alpha}olf$ heterozygous mice display a clear reduction in acute locomotor response to cocaine or D-amphetamine, in agreement with the decreased cAMP signaling responses *in vivo* (Herve et al., 2001; Corvol et al., 2007). By contrast a partial decrease in D1R amounts did not significantly affect the acute locomotor response to cocaine or D-amphetamine in D1R heterozygous mice (Corvol et al., 2007). This is consistent with the unaltered biochemical responses of the PKA pathway in these mice.

In contrast, the partial deficiency of $G_{\alpha}olf$ does not prevent the development of locomotor sensitization to cocaine or D-amphetamine in $G_{\alpha}olf$ heterozygous mice (Corvol et al., 2007). Moreover, because the acute locomotor response is very low in these mice, the sensitized response appears proportionally higher than in wild type animals. Similarly, conditioned place preference to D-amphetamine is not altered in $G_{\alpha}olf$ heterozygous mice. The contrast between altered responses to acute administrations of psychostimulants and normal responses to repeated treatments in these mice suggests that different signaling pathways may be limiting for the two types of effects. Several factors could account for the quasi-normal sensitizing and conditioning

properties of psychostimulants, including the possibility that these effects are partially independent from D1R activation (Salomon et al., 2006). One interesting possibility involves the ERK pathway which is normally activated by psychostimulants in G α olf heterozygous mice. ERK appears essential for long-lasting effects of drugs since its pharmacological inhibition blocks both locomotor sensitization and conditioned place preference with only minor effects on acute responses (Valjent et al., 2000, 2006). The normal psychostimulant-induced ERK activation could enable these responses in the G α olf heterozygous mutant mice. Some evidence argues in favor of this hypothesis. In particular, it has been found some alterations of the sensitization to cocaine in mice heterozygous for D1R gene, in which ERK activation, but not cAMP/PKA activation, is altered in response to cocaine (Valjent et al., 2010).

These studies indicate that variability in the levels of expression of specific genes involved in various aspects of D1R signaling can produce very different behavioral reactions in response to drugs. Depending on the element affected, genetic or environmental factors altering components of D1R signaling can have contrasted consequences leading to specific pathological or phenotypical traits.

REGULATION OF G α OLF LEVELS

Because G α olf levels constitute an important parameter controlling D1R-dependent cAMP/PKA pathway, they could represent an ideal target for regulation in physiological and pathological conditions. Such regulations have been well demonstrated following degeneration of dopamine neurons in the experimental context or human pathology of Parkinson's disease.

The dopamine denervation of the striatum produces an important hypersensitivity of the D1R signaling that could enable the therapeutic effects of L-DOPA in Parkinsonian patients but also promote adverse secondary effects, essentially the abnormal involuntary movements or dyskinesia that develop after 5–10 years of L-DOPA treatments (Bezard et al., 2001). This hypersensitivity affects both the cAMP/PKA and ERK pathways, since both are highly activated by D1R agonists in the denervated striatum (Geffen et al., 2002; Pavon et al., 2006; Santini et al., 2007; Westin et al., 2007). The denervation-induced hypersensitivity happens despite a lack of changes in the density of D1R in the striatum (Savasta et al., 1988; Hervé et al., 1989; Missale et al., 1989) or minimal alterations in the intracellular distribution of D1R (Berthet et al., 2009). The most plausible mechanism is an increase in the coupling of D1R with G protein, which has been demonstrated in both rodents and non-human primates after dopamine denervation of the striatum (Cai et al., 2002; Aubert et al., 2005). This higher coupling is essentially linked to an increase of G α olf levels in the denervated striatum. In the rat, 6-hydroxydopamine-induced lesions of dopamine neurons in adult or newborn animals lead to an increase by about 50% of G α olf levels in the following weeks (Hervé et al., 1993; Marcotte et al., 1994; Penit-Soria et al., 1997). Similar increase in G α olf levels has been observed in 6-hydroxydopamine-lesioned mice (Alcacer et al., unpublished data). Upregulation of G α olf was also observed in human putamen in Parkinsonian patients and, interestingly, this effect was associated with a parallel increase in G γ 7 levels (Corvol et al., 2004). In this study, there was a correlation between the increase

in G α olf levels and the duration of disease. In addition, the patients in whom the increase was the highest displayed intense L-DOPA-induced dyskinesia but there was no established causal relationship between the two effects.

In rat, the upregulation in the G α olf protein levels is not linked to a parallel increase in G α olf mRNA expression, showing that the regulation is post-transcriptional (Hervé et al., 1993). An attractive possibility is that changes in G α olf protein levels depend directly from its rate of activation. This hypothesis is supported by several studies on G α s which is a protein very close to G α olf. In cell culture, various long-lasting stimulations of G α s by receptors, cholera toxin, or mutation induce a downregulation of G α s at a post-translational level, which is independent of cAMP production and involves possibly an increased degradation rate of the protein (McKenzie and Milligan, 1990; Levis and Bourne, 1992; Milligan, 1993; Adie and Milligan, 1994). The chronic lack of D1R and G α olf stimulation in dopamine-denervated striatum could lower the G α olf degradation rate and lead to accumulation of the protein. In agreement with this hypothesis, total absence of D1R in mutant mice with targeted invalidation of D1R gene induces important increase of G α olf protein levels without any modification of G α olf mRNA expression (Hervé et al., 2001). Conversely, reduced levels of G α olf were observed in mutant mice devoid of dopamine transporter (Hervé et al., 2001), in which extracellular concentration of dopamine is strongly increased, leading thus to a chronic stimulation of D1R (Giros et al., 1996). Interestingly, the lack of A $_2$ A receptors in mutant mice produces also an upregulation of G α olf protein without any changes in the levels of G α olf transcripts (Hervé et al., 2001). Thus these results strongly suggest a homeostatic regulation of G α olf *in vivo*, in which the intensity of G α olf stimulation tends to reduce its levels. These variations are reminiscent, at the level of a G protein, of the classical “denervation hypersensitivity” and “agonist-induced desensitization,” well characterized at the level of receptors (Freedman and Lefkowitz, 1996; Bloch et al., 1999).

The mechanisms of elimination from membrane and degradation of G α olf or G α s are not known in detail. Upon stimulation of receptor, G α s was shown to internalize in a vesicle pool, corresponding probably to recycling endosomes with minimal overlap with vesicles containing receptors (Hynes et al., 2004). Recently, G α s was reported to be ubiquitinated and possibly degraded through proteasome (Nagai et al., 2010). Interestingly, Ric-8B, a protein highly expressed in the striatum (Von Dannecker et al., 2005), inhibits the G α s ubiquitination, and increases the G α s protein without affecting the G α s mRNA level (Nagai et al., 2010). Ric-8B plays the same essential role on G α olf and enhances the accumulation of G α olf at the cytoplasmic membrane (Von Dannecker et al., 2006). However further studies are needed to determine the precise mechanisms important for upregulation of G α olf following degeneration of dopamine neurons.

CONCLUSION

An assembly composed of G α olf, G β 2, and G γ 7 of G protein mediates the activation of AC5 by the D1R in the MSNs expressing this receptor while the same heterotrimer provides the coupling of adenosine A $_2$ A receptor to AC in the MSN population containing D2 receptors. The total absence of this assembly impairs all

the biochemical and behavioral responses involving D1R. These studies provide the proof, probably unique in the literature, that the receptor recognizes a specific assembly of $\alpha\beta\gamma$ subunits of G protein *in vivo*. The G α olf stability depends on the presence of the G γ 7 subunit and AC5 effector protein. The cellular concentration of G α olf appears thus to be regulated by the availability of its interacting proteins. By contrast, D1R receptor exerts a negative regulation on G α olf: more the receptor is stimulated more the cellular G α olf levels decrease. As important consequences of this regulation, it was observed an increase of striatal levels of G α olf following degeneration of dopamine neurons in both lesioned animals and Parkinsonian patients. G α olf upregulation is certainly a major factor explaining the hypersensitivity of D1R-linked cAMP signaling detected after dopamine lesion since the G α olf levels control *in vivo* the efficiency of D1Rs on AC activation. Alterations of levels of G α olf or its interacting proteins because of genetic or pathological factors could play an important role in the physiology of Parkinson's disease as well as in the individual responses to therapeutic drugs. In addition, because of the involvement of dopamine signaling in several mental diseases, such as schizophrenia or drug addiction, dysregulation of G α olf could contribute to physiopathology of these diseases. The gene encoding G α olf (GNAL) has been investigated as a candidate gene for several

mental diseases. To date, no coding variants of G α olf have been tested but a strong association with schizophrenia was reported for an intronic marker (Schwab et al., 1998). In contrast, other genetic studies on bipolar affective disorder and unipolar depression have yielded negative results (Tsiouris et al., 1996; Berrettini et al., 1998; Zill et al., 2002). More recently, two studies, one in human using intronic GNAL polymorphisms (Laurin et al., 2008) and the other using rat models (DasBanerjee et al., 2008), suggest a possible contribution of G α olf in the susceptibility to attention deficit/hyperactivity disorder (ADHD) in children. These studies suggest that alterations or quantitative modifications of the components of specific signaling machinery associated with D1R in the striatum have the potential to affect the various behavioral responses linked to dopamine functions in human.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Emmanuel Valjent and Dr. Catherine Le Moine for having provided immunofluorescence and *in situ* hybridization images as well as Jean-Antoine Girault and Lucile Marion-Poll for their support. The work was supported by INSERM and grants from the Fondation pour la Recherche Médicale and the Agence Nationale de la Recherche (ANR09-MNPS-014).

REFERENCES

- Adie, E. J., and Milligan, G. (1994). Agonist regulation of cellular Gs α -subunit levels in neuroblastoma x glioma hybrid NG108-15 cells transfected to express different levels of the human β 2 adrenoceptor. *Biochem. J.* 300(Pt 3), 709–715.
- Arnt, J., Hyttel, J., and Sanchez, C. (1992). Partial and full dopamine D1 receptor agonists in mice and rats: relation between behavioural effects and stimulation of adenylate cyclase activity *in vitro*. *Eur. J. Pharmacol.* 213, 259–267.
- Aubert, I., Guigoni, C., Hakansson, K., Li, Q., Dovero, S., Barthe, N., Bioulac, B. H., Gross, C. E., Fisone, G., Bloch, B., and Bezard, E. (2005). Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. *Ann. Neurol.* 57, 17–26.
- Belluscio, L., Gold, G. H., Nemes, A., and Axel, R. (1998). Mice deficient in G(olf) are anosmic. *Neuron* 20, 69–81.
- Berretta, S., Robertson, H. A., and Graybiel, A. M. (1992). Dopamine and glutamate agonists stimulate neuron-specific expression of Fos-like protein in the striatum. *J. Neurophysiol.* 68, 767–777.
- Berrettini, W. H., Vuoristo, J., Ferraro, T. N., Buono, R. J., Wildenauer, D., and Ala-Kokko, L. (1998). Human G(olf) gene polymorphisms and vulnerability to bipolar disorder. *Psychiatr. Genet.* 8, 235–238.
- Berthet, A., Porras, G., Doudnikoff, E., Stark, H., Cador, M., Bezard, E., and Bloch, B. (2009). Pharmacological analysis demonstrates dramatic alteration of D1 dopamine receptor neuronal distribution in the rat analog of L-DOPA-induced dyskinesia. *J. Neurosci.* 29, 4829–4835.
- Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamalas, M., Herve, D., Valjent, E., and Girault, J. A. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J. Neurosci.* 28, 5671–5685.
- Bezard, E., Brotchie, J. M., and Gross, C. E. (2001). Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. *Nat. Rev. Neurosci.* 2, 577–588.
- Bloch, B., Dumartin, B., and Bernard, V. (1999). *In vivo* regulation of intraneuronal trafficking of G protein-coupled receptors for neurotransmitters. *Trends Pharmacol. Sci.* 20, 315–319.
- Bourne, H. R., Sanders, D. A., and McCormick, F. (1991). The GTPase superfamily: conserved structure and molecular mechanism. *Nature* 349, 117–127.
- Boyson, S. J., McGonigle, P., and Molinoff, P. B. (1986). Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. *J. Neurosci.* 6, 3177–3188.
- Bunemann, M., Frank, M., and Lohse, M. J. (2003). Gi protein activation in intact cells involves subunit rearrangement rather than dissociation. *Proc. Natl. Acad. Sci. U.S.A.* 100, 16077–16082.
- Cai, G., Wang, H. Y., and Friedman, E. (2002). Increased dopamine receptor signaling and dopamine receptor-G protein coupling in denervated striatum. *J. Pharmacol. Exp. Ther.* 302, 1105–1112.
- Corradi, J. P., Ravyn, V., Robbins, A. K., Hagan, K. W., Peters, M. E., Bostwick, R., Buono, R. J., Berrettini, W. H., and Furlong, S. T. (2005). Alternative transcripts and evidence of imprinting of GNAL on 18p11.2. *Mol. Psychiatry* 10, 1017–1025.
- Corvol, J. C., Muriel, M. P., Valjent, E., Feger, J., Hanoun, N., Girault, J. A., Hirsch, E. C., and Herve, D. (2004). Persistent increase in olfactory type G-protein α subunit levels may underlie D1 receptor functional hypersensitivity in Parkinson disease. *J. Neurosci.* 24, 7007–7014.
- Corvol, J. C., Studler, J. M., Schonn, J. S., Girault, J. A., and Herve, D. (2001). Galpha(olf) is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. *J. Neurochem.* 76, 1585–1588.
- Corvol, J. C., Valjent, E., Pascoli, V., Robin, A., Stipanovich, A., Luedtke, R. R., Belluscio, L., Girault, J. A., and Herve, D. (2007). Quantitative changes in Galphaolf protein levels, but not D1 receptor, alter specifically acute responses to psychostimulants. *Neuropsychopharmacology* 32, 1109–1121.
- DasBanerjee, T., Middleton, F. A., Berger, D. F., Lombardo, J. P., Sagvolden, T., and Faraone, S. V. (2008). A comparison of molecular alterations in environmental and genetic rat models of ADHD: a pilot study. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 147B, 1554–1563.
- Drago, J., Gerfen, C. R., Lachowicz, J. E., Steiner, H., Hollon, T. R., Love, P. E., Ooi, G. T., Grinberg, A., Lee, E. J., Huang, S. P., Bartlett, P. F., Jose, P. A., Sibley, D. R., and Westphal, H. (1994). Altered striatal function in a mutant mouse lacking D1A dopamine receptors. *Proc. Natl. Acad. Sci. U.S.A.* 91, 12564–12568.
- Drago, J., Padungchaichot, P., Accili, D., and Fuchs, S. (1998a). Dopamine receptors and dopamine transporter in brain function and addictive behaviors: insights from targeted mouse mutants. *Dev. Neurosci.* 20, 188–203.
- Drago, J., Padungchaichot, P., Wong, J. Y., Lawrence, A. J., McManus, J. F., Sumarsono, S. H., Natoli, A. L., Lakso, M., Wreford, N., Westphal, H., Kola, I., and Finkelstein, D. I. (1998b). Targeted expression of a toxin gene to D1 dopamine receptor neurons by cre-mediated site-specific recombination. *J. Neurosci.* 18, 9845–9857.

- Drinnan, S. L., Hope, B. P., Snutch, T. P., and Vincent, S. R. (1991). Golf in the basal ganglia. *Mol. Cell. Neurosci.* 2, 66–70.
- El-Ghundi, M., O'Dowd, B. F., and George, S. R. (2007). Insights into the role of dopamine receptor systems in learning and memory. *Rev. Neurosci.* 18, 37–66.
- Fredholm, B. B., Chen, J. F., Masino, S. A., and Vaugeois, J. M. (2005). Actions of adenosine at its receptors in the CNS: insights from knockouts and drugs. *Annu. Rev. Pharmacol. Toxicol.* 45, 385–412.
- Freedman, N. J., and Lefkowitz, R. J. (1996). Desensitization of G protein-coupled receptors. *Recent Prog. Horm. Res.* 51, 319–351; discussion 352–313.
- Friedman, E., Jin, L. Q., Cai, G. P., Hollon, T. R., Drago, J., Sibley, D. R., and Wang, H. Y. (1997). D1-like dopaminergic activation of phosphoinositide hydrolysis is independent of D1A dopamine receptors: evidence from D1A knockout mice. *Mol. Pharmacol.* 51, 6–11.
- Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J. Jr., and Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250, 1429–1432.
- Gerfen, C. R., Miyachi, S., Paletzki, R., and Brown, P. (2002). D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. *J. Neurosci.* 22, 5042–5054.
- Giros, B., Jaber, M., Jones, S. R., Wightman, R. M., and Caron, M. G. (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* 379, 606–612.
- Glatt, C. E., and Snyder, S. H. (1993). Cloning and expression of an adenylyl cyclase localized to the corpus striatum. *Nature* 361, 536–538.
- Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E., and Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425, 917–925.
- Hasbi, A., Fan, T., Alijanian, M., Nguyen, T., Perreault, M. L., O'Dowd, B. F., and George, S. R. (2009). Calcium signaling cascade links dopamine D1-D2 receptor heteromer to striatal BDNF production and neuronal growth. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21377–21382.
- Heck, M., and Hofmann, K. P. (2001). Maximal rate and nucleotide dependence of rhodopsin-catalyzed transducin activation: initial rate analysis based on a double displacement mechanism. *J. Biol. Chem.* 276, 10000–10009.
- Hervé, D., and Girault, J. A. (2005). "Signal transduction of dopamine receptors," in *Handbook of Chemical Neuroanatomy*, 21; *Dopamine*, eds S. B. Dunnett, M. Bentivoglio, A. Björklund, and T. Hökfelt (Amsterdam: Elsevier Science), 109–151.
- Herve, D., Le Moine, C., Corvol, J. C., Belluscio, L., Ledent, C., Fienberg, A. A., Jaber, M., Studler, J. M., and Girault, J. A. (2001). G α (olf) levels are regulated by receptor usage and control dopamine and adenosine action in the striatum. *J. Neurosci.* 21, 4390–4399.
- Herve, D., Levi-Strauss, M., Marey-Semper, I., Verney, C., Tassin, J. P., Glowinski, J., and Girault, J. A. (1993). G(olf) and Gs in rat basal ganglia: possible involvement of G(olf) in the coupling of dopamine D1 receptor with adenylyl cyclase. *J. Neurosci.* 13, 2237–2248.
- Herve, D., Trovero, F., Blanc, G., Thierry, A. M., Glowinski, J., and Tassin, J. P. (1989). Nondopaminergic prefrontocortical efferent fibers modulate D1 receptor denervation supersensitivity in specific regions of the rat striatum. *J. Neurosci.* 9, 3699–3708.
- Hess, E. J., Battaglia, G., Norman, A. B., and Creese, I. (1987). Differential modification of striatal D1 dopamine receptors and effector moieties by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline in vivo and in vitro. *Mol. Pharmacol.* 31, 50–57.
- Hynes, T. R., Mervine, S. M., Yost, E. A., Sabo, J. L., and Berlot, C. H. (2004). Live cell imaging of Gs and the beta2-adrenergic receptor demonstrates that both alphas and beta1gamma7 internalize upon stimulation and exhibit similar trafficking patterns that differ from that of the beta2-adrenergic receptor. *J. Biol. Chem.* 279, 44101–44112.
- Iwamoto, T., Iwatsubo, K., Okumura, S., Hashimoto, Y., Tsunematsu, T., Toya, Y., Herve, D., Umemura, S., and Ishikawa, Y. (2004). Disruption of type 5 adenylyl cyclase negates the developmental increase in G α (olf) expression in the striatum. *FEBS Lett.* 564, 153–156.
- Iwamoto, T., Okumura, S., Iwatsubo, K., Kawabe, J., Ohtsu, K., Sakai, I., Hashimoto, Y., Izumitani, A., Sango, K., Ajiki, K., Toya, Y., Umemura, S., Goshima, Y., Arai, N., Vatter, S. F., and Ishikawa, Y. (2003). Motor dysfunction in type 5 adenylyl cyclase-null mice. *J. Biol. Chem.* 278, 16936–16940.
- Jones, D. T., and Reed, R. R. (1989). Golf: an olfactory neuron specific G protein involved in odorant signal transduction. *Science* 244, 790–795.
- Kebabian, J. W., and Calne, D. B. (1979). Multiple receptors for dopamine. *Nature* 277, 93–96.
- Kheirbek, M. A., Beeler, J. A., Ishikawa, Y., and Zhuang, X. (2008). A cAMP pathway underlying reward prediction in associative learning. *J. Neurosci.* 28, 11401–11408.
- Kull, B., Svenningsson, P., and Fredholm, B. B. (2000). Adenosine A(2A) receptors are colocalized with and activate g(olf) in rat striatum. *Mol. Pharmacol.* 58, 771–777.
- Largent, B. L., Jones, D. T., Reed, R. R., Pearson, R. C., and Snyder, S. H. (1988). G protein mRNA mapped in rat brain by in situ hybridization. *Proc. Natl. Acad. Sci. U.S.A.* 85, 2864–2868.
- Laurin, N., Ickowicz, A., Pathare, T., Malone, M., Tannock, R., Schachar, R., Kennedy, J. L., and Barr, C. L. (2008). Investigation of the G protein subunit G α (olf) gene (GNAL) in attention deficit/hyperactivity disorder. *J. Psychiatr. Res.* 42, 117–124.
- Le Moine, C., and Bloch, B. (1995). D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. *J. Comp. Neurol.* 355, 418–426.
- Lee, K. W., Hong, J. H., Choi, I. Y., Che, Y., Lee, J. K., Yang, S. D., Song, C. W., Kang, H. S., Lee, J. H., Noh, J. S., Shin, H. S., and Han, P. L. (2002). Impaired D2 dopamine receptor function in mice lacking type 5 adenylyl cyclase. *J. Neurosci.* 22, 7931–7940.
- Lee, K. W., Kim, Y., Kim, A. M., Helmin, K., Nairn, A. C., and Greengard, P. (2006). Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proc. Natl. Acad. Sci. U.S.A.* 103, 3399–3404.
- Levis, M. J., and Bourne, H. R. (1992). Activation of the alpha subunit of Gs in intact cells alters its abundance, rate of degradation, and membrane avidity. *J. Cell Biol.* 119, 1297–1307.
- Lezano, N., and Bergson, C. (2002). D1/D5 dopamine receptors stimulate intracellular calcium release in primary cultures of neocortical and hippocampal neurons. *J. Neurophysiol.* 87, 2167–2175.
- Liu, H. Y., Wenzel-Seifert, K., and Seifert, R. (2001). The olfactory G protein G α (olf) possesses a lower GDP-affinity and deactivates more rapidly than G α (saphashort): consequences for receptor-coupling and adenylyl cyclase activation. *J. Neurochem.* 78, 325–338.
- Lohse, M. J., Nikolaev, V. O., Hein, P., Hoffmann, C., Vilardaga, J. P., and Bunemann, M. (2008). Optical techniques to analyze real-time activation and signaling of G-protein-coupled receptors. *Trends Pharmacol. Sci.* 29, 159–165.
- Mahan, L. C., Burch, R. M., Monsma, F. J. Jr., and Sibley, D. R. (1990). Expression of striatal D1 dopamine receptors coupled to inositol phosphate production and Ca²⁺ mobilization in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. U.S.A.* 87, 2196–2200.
- Mannoury La Cour, C., Vidal, S., Pasteau, V., Cussac, D., and Millan, M. J. (2007). Dopamine D1 receptor coupling to Gs/olf and Gq in rat striatum and cortex: a scintillation proximity assay (SPA)/antibody-capture characterization of benzazepine agonists. *Neuropharmacology* 52, 1003–1014.
- Marcotte, E. R., Sullivan, R. M., and Mishra, R. K. (1994). Striatal G-proteins: effects of unilateral 6-hydroxydopamine lesions. *Neurosci. Lett.* 169, 195–198.
- Matamalos, M., Bertran-Gonzalez, J., Salomon, L., Degos, B., Deniau, J. M., Valjent, E., Herve, D., and Girault, J. A. (2009). Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. *PLoS ONE* 4, e4770. doi: 10.1371/journal.pone.0004770
- McKenzie, F. R., and Milligan, G. (1990). Prostaglandin E1-mediated, cyclic AMP-independent, down-regulation of Gs alpha in neuroblastoma x glioma hybrid cells. *J. Biol. Chem.* 265, 17084–17093.
- Milligan, G. (1993). Agonist regulation of cellular G protein levels and distribution: mechanisms and functional implications. *Trends Pharmacol. Sci.* 14, 413–418.
- Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., and Caron, M. G. (1998). Dopamine receptors: from structure to function. *Physiol. Rev.* 78, 189–225.
- Missale, C., Nisoli, E., Liberini, P., Rizzonelli, P., Memo, M., Buonamici, M., Rossi, A., and Spano, P. (1989). Repeated reserpine administration up-regulates the transduction mechanisms of D1 receptors without changing the density of [³H]SCH 23390 binding. *Brain Res.* 483, 117–122.

- Nagai, Y., Nishimura, A., Tago, K., Mizuno, N., and Itoh, H. (2010). Ric-8B stabilizes the alpha subunit of stimulatory G protein by inhibiting its ubiquitination. *J. Biol. Chem.* 285, 11114–11120.
- Nairn, A. C., Svenningsson, P., Nishi, A., Fisone, G., Girault, J. A., and Greengard, P. (2004). The role of DARPP-32 in the actions of drugs of abuse. *Neuropharmacology* 47(Suppl. 1), 14–23.
- Pascoli, V., Besnard, A., Herve, D., Pages, C., Heck, N., Girault, J. A., Caboche, J., and Vanhoutte, P. (2010). Cyclic adenosine monophosphate-independent tyrosine phosphorylation of NR2B mediates cocaine-induced extracellular signal-regulated kinase activation. *Biol. Psychiatry* 69, 218–227.
- Pavon, N., Martin, A. B., Mendialdua, A., and Moratalla, R. (2006). ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. *Biol. Psychiatry* 59, 64–74.
- Penit-Soria, J., Durand, C., Besson, M. J., and Herve, D. (1997). Levels of stimulatory G protein are increased in the rat striatum after neonatal lesion of dopamine neurons. *Neuroreport* 8, 829–833.
- Plagge, A., Kelsey, G., and Germain-Lee, E. L. (2008). Physiological functions of the imprinted Gnas locus and its protein variants Galpha(s) and XLalpha(s) in human and mouse. *J. Endocrinol.* 196, 193–214.
- Robishaw, J. D., and Berlot, C. H. (2004). Translating G protein subunit diversity into functional specificity. *Curr. Opin. Cell Biol.* 16, 206–209.
- Salomon, L., Lanteri, C., Glowinski, J., and Tassin, J. P. (2006). Behavioral sensitization to amphetamine results from an uncoupling between noradrenergic and serotonergic neurons. *Proc. Natl. Acad. Sci. U.S.A.* 103, 7476–7481.
- Santini, E., Valjent, E., Usiello, A., Carta, M., Borgkvist, A., Girault, J. A., Herve, D., Greengard, P., and Fisone, G. (2007). Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *J. Neurosci.* 27, 6995–7005.
- Savasta, M., Dubois, A., Benavides, J., and Scatton, B. (1988). Different plasticity changes in D1 and D2 receptors in rat striatal subregions following impairment of dopaminergic transmission. *Neurosci. Lett.* 85, 119–124.
- Schiffmann, S. N., Fisone, G., Moresco, R., Cunha, R. A., and Ferre, S. (2007). Adenosine A2A receptors and basal ganglia physiology. *Prog. Neurobiol.* 83, 277–292.
- Schultz, W. (1998). Predictive reward signal of dopamine neurons. *J. Neurophysiol.* 80, 1–27.
- Schwab, S. G., Hallmayer, J., Lerer, B., Albus, M., Borrmann, M., Honig, S., Strauss, M., Segman, R., Lichtermann, D., Knapp, M., Trixler, M., Maier, W., and Wildenauer, D. B. (1998). Support for a chromosome 18p locus conferring susceptibility to functional psychoses in families with schizophrenia, by association and linkage analysis. *Am. J. Hum. Genet.* 63, 1139–1152.
- Schwindinger, W. F., Betz, K. S., Giger, K. E., Sabol, A., Bronson, S. K., and Robishaw, J. D. (2003). Loss of G protein gamma 7 alters behavior and reduces striatal alpha(olf) level and cAMP production. *J. Biol. Chem.* 278, 6575–6579.
- Schwindinger, W. F., Mihalcik, L. J., Giger, K. E., Betz, K. S., Stauffer, A. M., Linden, J., Herve, D., and Robishaw, J. D. (2010). Adenosine A2A receptor signaling and golf assembly show a specific requirement for the gamma7 subtype in the striatum. *J. Biol. Chem.* 285, 29787–29796.
- Sibley, D. R., and Monsma, F. J. Jr. (1992). Molecular biology of dopamine receptors. *Trends Pharmacol. Sci.* 13, 61–69.
- Trovero, F., Herve, D., Blanc, G., Glowinski, J., and Tassin, J. P. (1992). In vivo partial inactivation of dopamine D1 receptors induces hypersensitivity of cortical dopamine-sensitive adenylate cyclase: permissive role of alpha 1-adrenergic receptors. *J. Neurochem.* 59, 331–337.
- Tsiouris, S. J., Breschel, T. S., Xu, J., Mcinnis, M. G., and McMahon, F. J. (1996). Linkage disequilibrium analysis of G-olf alpha (GNAL) in bipolar affective disorder. *Am. J. Med. Genet.* 67, 491–494.
- Valjent, E., Bertran-Gonzalez, J., Aubier, B., Greengard, P., Herve, D., and Girault, J. A. (2010). Mechanisms of locomotor sensitization to drugs of abuse in a two-injection protocol. *Neuropsychopharmacology* 35, 401–415.
- Valjent, E., Corvol, J. C., Pages, C., Besson, M. J., Maldonado, R., and Caboche, J. (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J. Neurosci.* 20, 8701–8709.
- Valjent, E., Corvol, J. C., Trzaskos, J. M., Girault, J. A., and Herve, D. (2006). Role of the ERK pathway in psychostimulant-induced locomotor sensitization. *BMC Neurosci.* 7, 20. doi: 10.1186/1471-2202-7-20
- Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., Stipanovich, A., Caboche, J., Lombroso, P. J., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 102, 491–496.
- Von Dannecker, L. E., Mercadante, A. E., and Malnic, B. (2005). Ric-8B, an olfactory putative GTP exchange factor, amplifies signal transduction through the olfactory-specific G-protein Galphalolf. *J. Neurosci.* 25, 3793–3800.
- Von Dannecker, L. E., Mercadante, A. E., and Malnic, B. (2006). Ric-8B promotes functional expression of odorant receptors. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9310–9314.
- Wadhawan, S., Dickens, B., and Nekrutenko, A. (2008). Wheels within wheels: clues to the evolution of the Gnas and Gnal loci. *Mol. Biol. Evol.* 25, 2745–2757.
- Wang, H. Y., Undie, A. S., and Friedman, E. (1995). Evidence for the coupling of Gq protein to D1-like dopamine sites in rat striatum: possible role in dopamine-mediated inositol phosphate formation. *Mol. Pharmacol.* 48, 988–994.
- Watson, J. B., Coulter, P. M. II, Margulies, J. E., De Lecea, L., Danielson, P. E., Erlander, M. G., and Sutcliffe, J. G. (1994). G-protein gamma 7 subunit is selectively expressed in medium-sized neurons and dendrites of the rat neostriatum. *J. Neurosci. Res.* 39, 108–116.
- Weinstein, L. S., Yu, S., Warner, D. R., and Liu, J. (2001). Endocrine manifestations of stimulatory G protein alpha-subunit mutations and the role of genomic imprinting. *Endocr. Rev.* 22, 675–705.
- Westin, J. E., Vercammen, L., Strome, E. M., Konradi, C., and Cenci, M. A. (2007). Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D1 receptors. *Biol. Psychiatry* 62, 800–810.
- Wolfgang, W. J., Hoskote, A., Roberts, I. J., Jackson, S., and Forte, M. (2001). Genetic analysis of the *Drosophila* Gs(alpha) gene. *Genetics* 158, 1189–1201.
- Yung, K. K., Bolam, J. P., Smith, A. D., Hersch, S. M., Ciliax, B. J., and Levey, A. I. (1995). Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. *Neuroscience* 65, 709–730.
- Zhuang, X., Belluscio, L., and Hen, R. (2000). G(olf)alpha mediates dopamine D1 receptor signaling. *J. Neurosci.* 20, RC91.
- Zill, P., Engel, R., Baghai, T. C., Zwanzger, P., Schule, C., Minov, C., Behrens, S., Rupprecht, R., Moller, H. J., and Bondy, B. (2002). Analysis of polymorphisms in the olfactory G-protein Golf in major depression. *Psychiatr. Genet.* 12, 17–22.

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 June 2011; paper pending published: 27 June 2011; accepted: 20 July 2011; published online: 05 August 2011.
Citation: Hervé D (2011) Identification of a specific assembly of the G protein Golf as a critical and regulated module of dopamine and adenosine-activated cAMP pathways in the striatum. *Front. Neuroanat.* 5:48. doi: 10.3389/fnana.2011.00048
Copyright © 2011 Hervé. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



The dopamine D1–D2 receptor heteromer in striatal medium spiny neurons: evidence for a third distinct neuronal pathway in basal ganglia

Melissa L. Perreault^{1,2}, Ahmed Hasbi^{1,2}, Brian F. O'Dowd^{1,2} and Susan R. George^{1,2,3}*

¹ Centre for Addiction and Mental Health, University of Toronto, Toronto, ON, Canada

² Department of Pharmacology, University of Toronto, Toronto, ON, Canada

³ Department of Medicine, University of Toronto, Toronto, ON, Canada

Edited by:

Emmanuel Valjent, Université Montpellier 1 and 2, France

Reviewed by:

Jean-Philippe R. Pin, Université de Montpellier, France

John Q. Wang, University of Missouri-Kansas City School of Medicine, USA

*Correspondence:

Susan R. George, Department of Pharmacology, Medical Sciences Bldg., Room 4358, University of Toronto, 1 King's College Circle, Toronto, ON, Canada M5S 1A8.
e-mail: s.george@utoronto.ca

Dopaminergic signaling within the basal ganglia has classically been thought to occur within two distinct neuronal pathways; the direct striatonigral pathway which contains the dopamine D1 receptor and the neuropeptides dynorphin (DYN) and substance P, and the indirect striatopallidal pathway which expresses the dopamine D2 receptor and enkephalin (ENK). A number of studies have also shown, however, that D1 and D2 receptors can co-exist within the same medium spiny neuron and emerging evidence indicates that these D1/D2-coexpressing neurons, which also express DYN and ENK, may comprise a third neuronal pathway, with representation in both the striatonigral and striatopallidal projections of the basal ganglia. Furthermore, within these coexpressing neurons it has been shown that the dopamine D1 and D2 receptor can form a novel and pharmacologically distinct receptor complex, the dopamine D1–D2 receptor heteromer, with unique signaling properties. This is indicative of a functionally unique role for these neurons in brain. The aim of this review is to discuss the evidence in support of a novel third pathway coexpressing the D1 and D2 receptor, to discuss the potential relevance of this pathway to basal ganglia signaling, and to address its potential value, and that of the dopamine D1–D2 receptor heteromer, in the search for new therapeutic strategies for disorders involving dopamine neurotransmission.

Keywords: dopamine D1–D2 receptor heteromer, dynorphin, enkephalin, substance P, basal ganglia, striatonigral, striatopallidal

The striatum, consisting of the ventrally located nucleus accumbens (NAc) and dorsal caudate putamen (CP), is the largest component of a group of nuclei that comprise the basal ganglia and associated mesolimbic system. The neuronal makeup of the striatum consists predominantly (>90%) of medium spiny neurons (MSNs), all of which express the inhibitory neurotransmitter GABA. However MSNs also exist as different subtypes, exhibiting discrete phenotypes that are characterized by their neuropeptide and dopamine receptor content, and which are dependent upon the neuronal pathways to which they are localized.

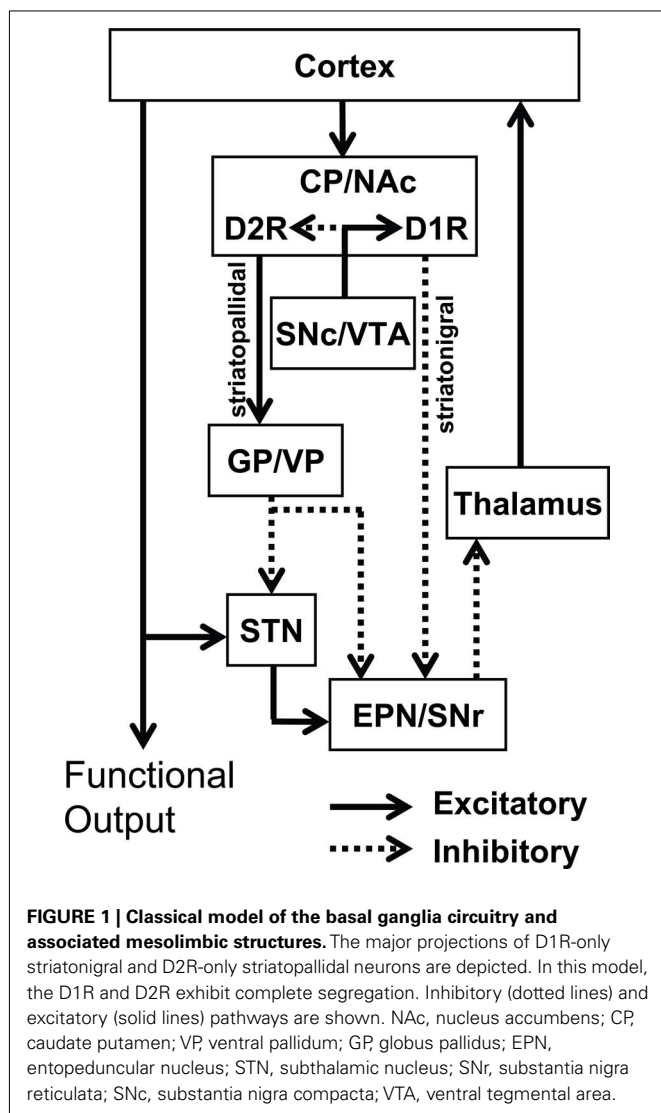
At least two major neuronal pathways in the basal ganglia have been identified (**Figure 1**). The MSNs of the striatonigral pathway, also called the “direct” pathway, project to substantia nigra reticulata (SNr) and the internal segment of the globus pallidus (GPi; analogous to rat entopeduncular nucleus, EPN). These neurons are characterized by the expression of the neuropeptides dynorphin (DYN) and substance P (SP), as well as the dopamine D1 receptor (D1R). In contrast, the neurons of the striatopallidal “indirect” pathway, projecting to the external segment of GP (GPe; analogous to rat GP), express the neuropeptide enkephalin (ENK) and the dopamine D2 receptor (D2R; Gerfen et al., 1990). These two pathways work in concert to regulate thalamic output.

Traditional thinking has depicted the D1R and D2R as being segregated to these discrete populations of striatal MSNs. However,

this idea has been becoming more difficult to reconcile as increasing evidence, both functional and neuroanatomical, indicates that D1R and D2R can be coexpressed in a subpopulation of striatal MSNs. Furthermore, the recent identification of a novel dopamine receptor complex in striatum, the dopamine D1–D2 receptor heteromer, also suggests that these coexpressing MSNs may have unique functional characteristics that are distinct from those neurons that exhibit dopamine receptor segregation. In this review we describe the evidence showing D1R/D2R coexpression in a subset of striatal MSNs and discuss their unique functional role in basal ganglia and mesolimbic neurotransmission. We put forth that these D1R/D2R-coexpressing MSNs represent a third functional striatal pathway with significant physiological relevance.

DOPAMINE D1R AND D2R ARE FUNCTIONALLY LINKED

Numerous reports have documented opposing effects of the Gs-coupled D1R and Gi/o-coupled D2R on biological processes such as adenylyl cyclase activity and cAMP formation (Hyttel, 1978; Onali et al., 1985) and striatal acetylcholine release (DeBoer and Abercrombie, 1996). However, it has also been demonstrated that the concomitant activation of both D1R and D2R is essential to achieve certain other behavioral, biochemical, and electrophysiological effects. For instance, past studies have shown



coactivation of the D1R and D2R as being necessary for maximal stereotyped and locomotor behavior (Walters et al., 1987; White et al., 1988; DeBoer and Abercrombie, 1996), and more recently it was reported that to evoke neural and behavioral phenotypes of cocaine sensitization, both D1R and D2R stimulation is required (Capper-Loup et al., 2002). Similarly, at a cellular level, combined administration of specific D1R and D2R agonists was shown to potentiate immediate early gene expression (LaHoste et al., 1993; Keefe and Gerfen, 1995). For example, while the systemic administration of a dopamine D1R agonist induced the expression of *c-fos* and *zif268* in the dopamine-depleted striatum, and a D2R agonist decreased *zif268* expression, both agonists together significantly enhanced gene expression compared to the D1R agonist alone (Keefe and Gerfen, 1995). Electrophysiological evidence has also demonstrated that simultaneous administration of D1R and D2R agonists could synergistically inhibit the neuronal activity of NAc neurons (White and Wang, 1986; Hu et al., 1990), and restoration of long-term depression of synaptic transmission following dopamine depletion occurred

by dopamine administration or coadministration of specific D1R and D2R agonists but not by either selective agonist alone (Calabresi et al., 1992).

Although the mechanisms for the D1R and D2R interaction have been suggested to occur by receptor activation on discrete neurons (Gerfen et al., 1995; LaHoste et al., 2000), or changes in D2R-mediated cholinergic interneuron activity with subsequent impact on D1R-expressing striatonigral neuronal transmission (Di Chiara et al., 1994), in many studies the mechanisms were not clearly understood. However, an interaction between the receptors occurring within the same cell may have been involved in some instances. For example, single MSN–MSN connections in neonatal striatal cultures have been reported to exhibit modulation by both D1R and D2R agonists (Geldwert et al., 2006) and the selective activation of D1R or D2R in single striatal neurons differentially modulated tetrodotoxin-sensitive sodium channels (Aizman et al., 2000). The synergistic potentiation of the arachidonic acid release by coadministration of D1R-selective and D2R-selective agonists has also been observed in cells expressing both receptors (Piomelli et al., 1991).

NEUROANATOMICAL EVIDENCE FOR D1R AND D2R COLOCALIZATION

Given the functional linkage between D1R and D2R, neuroanatomical studies began to address the question of dopamine receptor segregation and many of these studies have now shown, both at the mRNA level and the protein level, that D1R and D2R coexist in a fraction of striatal MSNs. In situ hybridization studies examining D1R and D2R mRNA in serial rat brain striatal sections have shown overlap of D1R and D2R transcript expression in the same neurons (Meador-Woodruff et al., 1991; Lester et al., 1993) and double in situ hybridization has been used to report colocalization of D1R and D2R mRNA in some striatal neurons in primate (Aubert et al., 2000). D1R/D2R mRNA coexistence was also reported using single cell reverse transcription PCR in neonatal neurons (Surmeier et al., 1992, 1996) and immunolabeling has been a widely used tool for visualizing striatal D1R/D2R coexpression at the protein level in both neonatal striatal cultures (Shetreat et al., 1996; Wong et al., 1999; Aizman et al., 2000; Hasbi et al., 2009) and in adult brain slices (Lee et al., 2004; Deng et al., 2006; Hasbi et al., 2009; Perreault et al., 2010).

It had been noted that while neurons expressing solely mRNA for the D1R or D2R were abundant in SP or ENK mRNA respectively, neurons containing mRNA for both D1R and D2R expressed both SP and ENK mRNAs (Surmeier et al., 1996). Similarly, in a recent study by Perreault et al. (2010), using highly validated antibodies, it was shown that the D1R and D2R were expressed exclusively in neurons containing both DYN and ENK. Although a small minority of DYN/ENK neurons did not express D1R or D2R, these findings indicated that SP/DYN–ENK coexpression could be a useful marker for identifying neurons coexpressing D1R and D2R. Interestingly, unlike the controversy surrounding the localization of D1R and D2R, SP/DYN–ENK coexpression in striatal neurons has been widely accepted, and overlap of SP/DYN and ENK mRNA or protein in a proportion of striatal projection neurons has been reported for a number of species (Penny et al., 1986; Gerfen and Young, 1988; Anderson and Reiner, 1990; Besson

et al., 1990; Chen et al., 1998; Stefani et al., 1998; Reiner et al., 1999; Vorobjev et al., 2000; Wang et al., 2006, 2007; Perreault et al., 2010).

Drd1a-EGFP AND Drd2-EGFP BAC TRANSGENIC MICE

In recent years, technological advances have provided novel approaches for assessing dopamine receptor colocalization. Enhanced green fluorescent protein (EGFP)-tagged promoter elements of D1R and D2R in bacterial artificial chromosome (BAC) transgenic mice is one such method, and has been used to quantify the proportion of striatal neurons expressing the receptors within the striatonigral and striatopallidal pathways (Valjent et al., 2009). In an elegant study by Bertran-Gonzalez et al. (2008) it was reported that while the majority of D1R and D2R were segregated to these discrete pathways, an estimated 17% of NAc shell MSNs exhibited receptor colocalization with ~6% D1R/D2R coexpression in CP. Although there has been doubt as to the reliability of BAC transgenic mice in providing an accurate representation of dopamine receptor expression due to unlabeled neurons (Shuen et al., 2008), another group was able to show that 100% of MSNs in these mice expressed D1R, D2R or both (Matamales et al., 2009) and that the proportion of neurons showing coexpression were similar to that observed in the Bertran-Gonzalez study.

Fluorescence activated cell sorting (FACS), another relatively novel method, is used for sorting a heterogeneous mixture of biological cells and is based upon the fluorescent characteristics of each cell. Essentially, this method employs the fluorescent properties of the neurons in the BAC transgenic *drd1a*-EGFP or *drd2*-EGFP mice to purify D1R-expressing and D2R-expressing MSNs, and is often used to assess specific characteristics of these two neuronal subtypes. The quality of the purification process can then be verified by assessing mRNA content of the each sample. Using this process, it was recently shown that while purified D1R-positive neurons from whole striatum displayed enrichment of D1R mRNA, D2R mRNA was also evident, albeit in small amounts (Lobo et al., 2010). Similarly, D2R-positive neurons also displayed evidence of minimal D1R mRNA being present. In addition, using BAC transgenic mice with Cre recombinase being driven by the D1R or D2R promoters, and using double immunofluorescence to stain for Cre and ENK, this group also showed that a fraction of striatal D1R-expressing neurons were also positive for ENK.

FUNCTIONAL RELEVANCE OF D1R/D2R-COEXPRESSING NEURONS

Although the existence of D1R- and D2R-coexpressing MSNs is now generally accepted, little is yet known about the functional relevance of these neurons, most likely as a result of the methodological difficulties attempting to isolate them. However, it has been shown that while all MSNs express mRNA for the GluA1 and GluA2 subunits of the AMPA receptor (Chen et al., 1998; Stefani et al., 1998; Vorobjev et al., 2000), only SP/ENK neurons preferentially express mRNA for GluA3 (Stefani et al., 1998) a finding which the authors posited as being suggestive of distinctive postsynaptic glutamatergic mechanisms in the neurons. A role in neurotransmission is supported by evidence documenting that striatal projection neurons coexpressing D1R and D2R or SP and ENK terminate in a number of regions including GP and EPN (GPI), as well as substantia nigra and ventral tegmental area

(Deng et al., 2006; Wang et al., 2006, 2007), two regions rich in dopamine cell bodies. In addition, a region-specific distribution of D1R/D2R-DYN/ENK neurons throughout the rat basal ganglia and mesolimbic pathway has been reported, being present in NAc (**Figure 2**) and CP, as well as GP and ventral pallidum and EPN (Perreault et al., 2010). Specifically, it was estimated that the fraction of D1R-expressing neurons also expressing the D2R in NAc core and shell was ~25% and ~35% respectively, whereas in CP the percentage was much lower being only ~7%. In GP and VP, while the total number of D1R neurons was quite low compared to other regions, a high percentage of these neurons (~60% and ~30%) also contained D2R. Similarly, in EPN the fraction of D1R containing neurons also expressing the D2R was relatively high (~50%).

In basal ganglia, the coexpression of D1R and D2R has been shown to occur both within neuronal cell bodies and selectively at presynaptic, but not postsynaptic terminals, as evidenced by D1R and D2R coexpression with the presynaptic marker synaptophysin, but not the postsynaptic marker PSD-95 (Perreault et al., 2010). Presynaptic colocalization of D1R and D2R on a fraction of varicosities has also been reported in neonatal striatal cultures (Wong et al., 1999; Geldwert et al., 2006; Mizuno et al., 2007) and electrophysiology studies have demonstrated that presynaptic dopamine receptors on MSN terminals could modulate GABAergic inhibitory postsynaptic currents (IPSCs; Delgado et al., 2000; Guzman et al., 2003; Geldwert et al., 2006). Interestingly, in one of these studies it was shown that while the D2R agonist quinpirole predominantly mediated inhibition of autaptic connections in MSNs, the D1R agonist SKF 38393 mediated either inhibition or facilitation (Geldwert et al., 2006). Although the authors did not directly address the seemingly discrepant effects of the D1R agonist on GABA transmission, it has been shown that SKF 38393 activates two biologically different signaling pathways, the cAMP pathway and phospholipase C (PLC)-phosphoinositide (PI) pathway (Undie and Friedman, 1990; Undie et al., 1994). Although it has been suggested that these two pathways are linked directly to D1R (Undie et al., 1994), it is now known that in MSNs that coexpress D1R and D2R, the receptors can form a heteromeric complex, the dopamine D1–D2 receptor heteromer, and it is this complex that directly activates the PLC-PI pathway (Lee et al., 2004; Rashid et al., 2007).

THE DOPAMINE D1–D2 RECEPTOR HETEROMER

Dopamine receptors exist as receptor homomers and can additionally form heteromeric receptor complexes that often exhibit pharmacological and cell signaling properties distinct from their constituent receptors (Lee et al., 2004; Rashid et al., 2007; Hasbi et al., 2009; So et al., 2009; Verma et al., 2010). A physical interaction between the D1R and D2R was first shown by coimmunoprecipitation from rat and human striatum (Lee et al., 2004), and was the first evidence of a dopamine heteromeric receptor complex in brain. These findings were later confirmed by fluorescence resonance energy transfer (FRET) a tool commonly used for the identification of receptor oligomers. Although first performed in cells (So et al., 2005; Dziedzicka-Wasylewska et al., 2006), quantitative FRET in situ has now been utilized to verify the presence of dopamine D1–D2 receptor heteromers in neonatal cultured rat

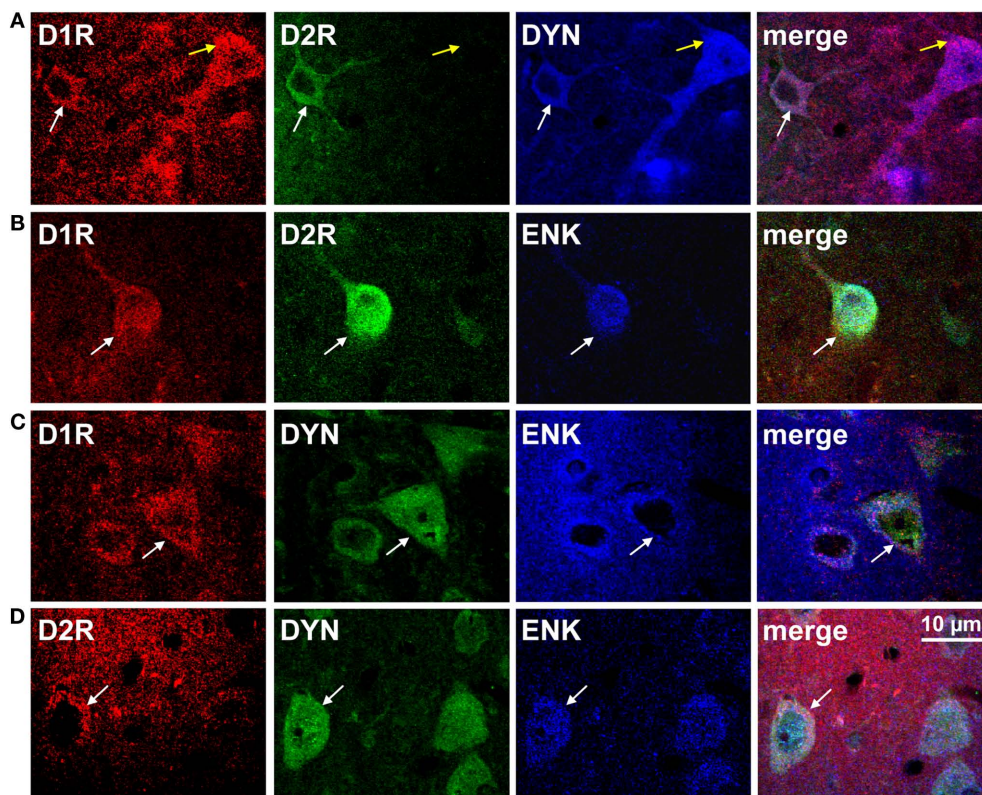


FIGURE 2 | The dopamine D1 and D2 receptor are colocalized with dynorphin and enkephalin in rat nucleus accumbens shell. (A,B) Confocal images showing D1R and D2R colocalization with dynorphin (DYN) or

enkephalin (ENK; white arrows). The D1R was also expressed individually with DYN (yellow arrows). **(C,D)** Neurons coexpressing DYN and ENK also expressed the D1R or the D2R (white arrows).

striatal neurons (Hasbi et al., 2009). Similarly, this method has now been employed to show, *in vivo*, that in adult rat NAc, CP (Hasbi et al., 2009; Perreault et al., 2010) and GP (Figure 3) the D1R and D2R existed in close $<50\text{--}100\text{ \AA}$ proximity indicative of D1–D2 receptor heteromerization. However, the propensity for striatal neurons to exhibit D1–D2 heteromers was region-dependent, with the majority ($>90\%$) of D1R/D2R-coexpressing neuronal cell bodies in NAc core and shell showing robust D1–D2 heteromer formation, but only $\sim 25\%$ of D1R/D2R neurons expressing the D1–D2 heteromer in CP (Perreault et al., 2010). This suggests that D1R and D2R can coexist as homomers in the same cell without forming heteromers. It was also shown in the neuropil of both NAc and CP the presence of D1–D2 heteromers at presynaptic terminals, a finding that suggests a possible involvement of synaptic D1–D2 receptor heteromer in GABA release in both regions.

The role of the D1–D2 heteromer *in vivo* is just beginning to be elucidated, however several lines of evidence have emerged implicating the complex as a potential therapeutic target in disorders involving elevated dopamine transmission, such as schizophrenia and drug addiction. Specifically, it has been postulated that abnormal regulation of calcium signaling may constitute the central dysfunction that is responsible for generating the psychopathology of schizophrenia (Lidow, 2003). Although the D1R and D2R have not been shown individually to be directly coupled to calcium

signaling, coactivation of both receptors within the dopamine D1–D2 receptor heteromer by concurrent administration of D1R and D2R agonists, or the selective D1–D2 heteromer agonist SKF 83959, resulted in a novel Gq-PLC-linked increase in intracellular calcium release (Lee et al., 2004; Rashid et al., 2007). In addition, dopamine D1–D2 heteromer activation by SKF 83959 also induced striatal calcium calmodulin kinase II α (CaMKII α) phosphorylation (Rashid et al., 2007; Ng et al., 2010) and brain-derived neurotrophic factor (BDNF) expression (Hasbi et al., 2009) two proteins that have been linked to schizophrenia (Weickert et al., 2003; Issa et al., 2010; Jindal et al., 2010; Novak and Seeman, 2010; Wong et al., 2010; Carlino et al., 2011). Finally, of particular relevance, an upregulation of striatal D1–D2 heteromeric activity was seen following repeated amphetamine treatment in rats and in human GP of patients with schizophrenia (Perreault et al., 2010). Given that amphetamine sensitization is often employed as an animal model for schizophrenia (Featherstone et al., 2007), but no common biochemical marker linking the two has previously been documented, it was inferred that the sensitized state of the D1–D2 heteromer may provide the first neuropharmacological correlate between increased dopamine neurotransmission and its functional consequence. As antipsychotics invariably also target the D1–D2 heteromer, these findings strongly suggest that further research examining the D1–D2 receptor heteromer as a potential drug target in schizophrenia is warranted.

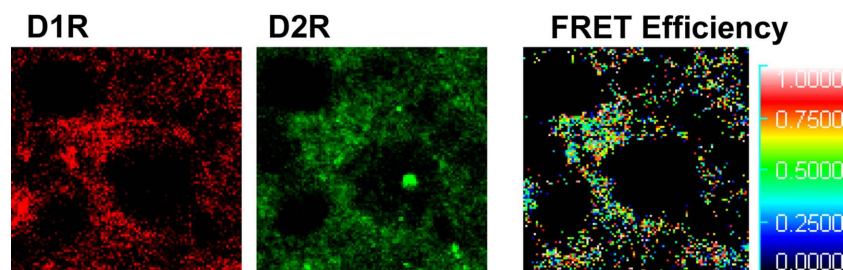


FIGURE 3 | Dopamine D1 and D2 receptors form D1–D2 receptor heteromers in rat globus pallidus. Fluorescence resonance energy transfer (FRET), using Alexa 488 and Alexa 350 was used to identify interactions between endogenous D1R and D2R in neurons of rat globus pallidus. An interaction between the D1R and D2R was evident,

with a relatively high mean FRET Efficiency (efficiency of energy transfer between the donor and acceptor fluorophores) of ~22%. The receptor antibody-linked fluorophores were calculated to be in close proximity with a relative distance of 5–7 nm (50–70 Å) indicative of D1–D2 receptor heteromer formation.

In addition to an involvement in schizophrenia, CaMKII and BDNF have also been shown to play a major role in cocaine addiction (Anderson et al., 2008; Lobo et al., 2010; McGinty et al., 2010; Wang et al., 2010). In one particularly compelling study, it was shown that cocaine reinstatement in rats depended upon an interaction between NAc dopamine and glutamate systems that was mediated by CaMKII (Anderson et al., 2008). More specifically, cocaine reinstatement increased activation of NAc shell CaMKII, which subsequently led to the phosphorylation of the GluA1 subunit of the AMPA receptor at Ser⁸³¹, and increased cell-surface expression of GluA1-containing AMPA receptors. Interestingly, in contrast to the effects of acute selective activation of the D1–D2 heteromer in NAc, more prolonged selective activation has been reported to reduce total CaMKII levels in this region and additionally reduce phosphorylation of GluA1 at Ser⁸³¹ (Perreault et al., 2010), a finding effectively linking the D1–D2 heteromer to the reward pathways of the brain.

A CAUTIONARY NOTE ON THE USE OF DOPAMINE RECEPTOR AGONISTS

It is now known that the agonist SKF 83959 directly activates calcium signaling via the dopamine D1–D2 receptor heteromer, but does not activate the D1R cAMP pathway or the Gi-coupled D2R (Rashid et al., 2007). Therefore findings from studies assessing the effects of SKF 83959 would not likely have been derived from functional interactions between D1R or D2R homomers within the same neuron, or between the D1R-expressing striatonigral and D2R-expressing striatopallidal populations of MSNs. However, for many years it was believed that the effects of the agonist SKF 83959 were mediated through its actions solely at the D1R. Reports had demonstrated that SKF 83959 had a substantial affinity for the D1R (Andringa et al., 1999; Neumeyer et al., 2003), however the physiological actions of SKF 83959 at the D1R were the subject of much debate as the drug exhibited antagonistic properties on adenylyl cyclase activity (Arnt et al., 1992; Andringa et al., 1999; Jin et al., 2003) yet mimicked certain behavioral characteristics of D1R agonism such as grooming or the induction of circling behavior (Deveney and Waddington, 1995; Gnanalingham et al., 1995; Waddington et al., 1995; Zhen et al., 2005; Perreault et al., 2010). However, SKF 83959 was also linked to PLC activation and PI hydrolysis in brain (Panchalingam and Undie, 2001; Jin et al.,

2003; Zhen et al., 2005; Rashid et al., 2007), a pathway that had also been observed to be induced with a number of other D1R agonists in brain tissue (Undie et al., 1994; Desai et al., 2005) but, interestingly, not in cells expressing only the D1R (Lin et al., 1995).

Given the ability of D1R agonists to directly induce PI hydrolysis in brain, but not in cells expressing solely the D1R, this suggests that D1R agonists that stimulate this pathway do so through agonist activity at the D1–D2 receptor heteromer. For example, the D1R agonists SKF 81297 and SKF 38393 are still often employed as “selective” D1R agonists, despite evidence demonstrating their ability to induce behavioral and neurochemical effects characteristic of dopamine D1–D2 heteromer activation, such as grooming and activation of PLC leading to intracellular calcium release (Molloy and Waddington, 1987; Undie and Friedman, 1990; Undie et al., 1994; Rashid et al., 2007). Furthermore, the D5 receptor has also been shown to induce a calcium signal through PLC activation or extracellular calcium influx (So et al., 2009), further confounding the pharmacological profile of these agonists. As such, the interpretation of both past and future results should take into account that activation of individual dopamine receptors by these compounds most likely occurs in tandem with D1–D2 receptor heteromer activation on the physiologically relevant subset neurons coexpressing both the D1R and D2R. The appropriate selection of dopaminergic drugs is therefore essential to effectively isolate the specific complex, or signaling pathway, under investigation.

CONCLUDING REMARKS

For the most part, studies agree that the D1R and D2R are predominantly segregated to discrete populations of MSNs in striatum and, additionally, that functional cross-talk between the D1R and D2R is critical in mediating some of the physiological effects of dopamine, such as the synergistic effects of D1R/D2R activation on immediate early gene expression. However, there is also an abundance of evidence showing that a proportion of MSNs in striatum coexpresses the D1R and D2R, and the physiological importance of these neurons is now beginning to become apparent. Specifically, unlike D1R-only and D2R-only striatal MSNs, D1R/D2R-coexpressing striatal projection neurons have been reported to terminate in regions within both the striatonigral and striatopallidal pathways, as well as regions containing dopamine

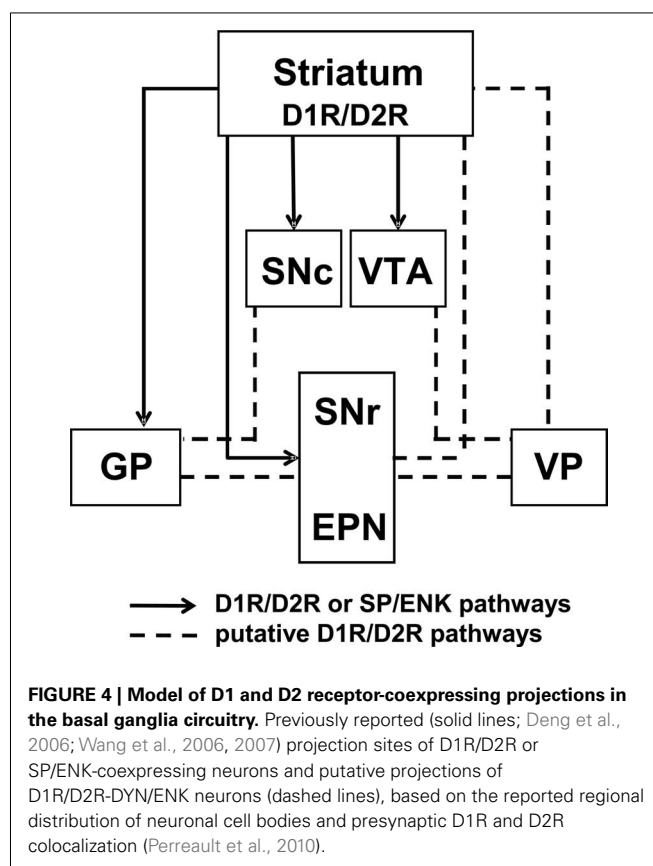
neuronal cell bodies. These findings, as well as the demonstration of D1R/D2R coexpression at presynaptic terminals, indicate that receptor coexpression may have a unique physiological function at a local level, via MSN–MSN synaptic connections, as well as distal effects through their efferent projections (Figure 4). This would likely contribute to the regulation of thalamic neurotransmission, perhaps with the purpose of maintaining homeostatic balance between the direct and indirect pathways. It is possible that this may occur, at least in part, through the regulation of post-synaptic glutamate transmission in the basal ganglia (Stefani et al., 1998), as these neurons exhibit a unique expression phenotype of the AMPA GluA subunits. In addition, these D1R/D2R MSNs also express the dopamine D1–D2 receptor heteromer, a novel receptor complex that links dopamine receptor activation directly to calcium signaling and BDNF production *in vivo*. Together, the evidence indicates that MSNs coexpressing the D1R and D2R in the basal ganglia embody a physiologically relevant, and functionally active, subset of neurons. We propose that D1R/D2R-coexpressing MSNs represent a third major dopamine receptor neuronal pathway, in addition to the striatonigral and striatopallidal pathways, with the potential to provide a novel approach to drug discovery in basal ganglia disorders.

ACKNOWLEDGMENTS

This work was supported by a grant (DA007223) from the National Institute on Drug Abuse (to Susan R. George and Brian F. O'Dowd), and a Canadian Institute of Health Research Fellowship (to Melissa L. Perreault). Susan R. George holds a Canada Research Chair in Molecular Neuroscience.

REFERENCES

- Aizman, O., Brismar, H., Uhlen, P., Zettergren, E., Levey, A. I., Forssberg, H., Greengard, P., and Aperia, A. (2000). Anatomical and physiological evidence for D1 and D2 dopamine receptor colocalization in neostriatal neurons. *Nat. Neurosci.* 3, 226–230.
- Anderson, K. D., and Reiner, A. (1990). Extensive co-occurrence of substance P and dynorphin in striatal projection neurons: an evolutionarily conserved feature of basal ganglia organization. *J. Comp. Neurol.* 295, 339–369.
- Anderson, S. M., Famous, K. R., Sadri-Vakili, G., Kumaresan, V., Schmidt, H. D., Bass, C. E., Terwilliger, E. F., Cha, J. H., and Pierce, R. C. (2008). CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. *Nat. Neurosci.* 11, 344–353.
- Andringa, G., Drukarch, B., Leysen, J. E., Cools, A. R., and Stoof, J. C. (1999). The alleged dopamine D1 receptor agonist SKF 83959 is a dopamine D1 receptor antagonist in primate cells and interacts with other receptors. *Eur. J. Pharmacol.* 364, 33–41.
- Arnt, J., Hyttel, J., and Sanchez, C. (1992). Partial and full dopamine D1 receptor agonists in mice and rats: relation between behavioural effects and stimulation of adenylate cyclase activity *in vitro*. *Eur. J. Pharmacol.* 213, 259–267.
- Aubert, I., Ghorayeb, I., Normand, E., and Bloch, B. (2000). Phenotypical characterization of the neurons expressing the D1 and D2 dopamine receptors in the monkey striatum. *J. Comp. Neurol.* 418, 22–32.
- Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamalas, M., Herve, D., Valjent, E., and Girault, J. A. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J. Neurosci.* 28, 5671–5685.
- Besson, M. J., Graybiel, A. M., and Quinn, B. (1990). Co-expression of neuropeptides in the cat's striatum: an immunohistochemical study of substance P, dynorphin B and enkephalin. *Neuroscience* 39, 33–58.
- Calabresi, P., Maj, R., Mercuri, N. B., and Bernardi, G. (1992). Coactivation of D1 and D2 dopamine receptors is required for long-term synaptic depression in the striatum. *Neurosci. Lett.* 142, 95–99.
- Capper-Loup, C., Canales, J. J., Kadaba, N., and Graybiel, A. M. (2002). Concurrent activation of dopamine D1 and D2 receptors is required to evoke neural and behavioral phenotypes of cocaine sensitization. *J. Neurosci.* 22, 6218–6227.
- Carlino, D., Leone, E., Di Cola, F., Baj, G., Marin, R., Dinelli, G., Tongiorgi, E., and De Vanna, M. (2011). Low serum truncated-BDNF isoform correlates with higher cognitive impairment in schizophrenia. *J. Psychiatr. Res.* 45, 273–279.
- Chen, Q., Veenman, L., Knopp, K., Yan, Z., Medina, L., Song, W. J., Surmeier, D. J., and Reiner, A. (1998). Evidence for the preferential localization of glutamate receptor-1 subunits of AMPA receptors to the dendritic spines of medium spiny neurons in rat striatum. *Neuroscience* 83, 749–761.
- DeBoer, P., and Abercrombie, E. D. (1996). Physiological release of striatal acetylcholine *in vivo*: modulation by D1 and D2 dopamine receptor subtypes. *J. Pharmacol. Exp. Ther.* 277, 775–783.
- Delgado, A., Sierra, A., Querejeta, E., Valdiosera, R. F., and Aceves, J. (2000). Inhibitory control of the GABAergic transmission in the rat neostriatum by D2 dopamine receptors. *Neuroscience* 95, 1043–1048.
- Deng, Y. P., Lei, W. L., and Reiner, A. (2006). Differential perikaryal localization in rats of D1 and D2 dopamine receptors on striatal projection neuron types identified by retrograde labeling. *J. Chem. Neuroanat.* 32, 101–116.
- Desai, R. I., Terry, P., and Katz, J. L. (2005). A comparison of the locomotor stimulant effects of D1-like receptor agonists in mice. *Pharmacol. Biochem. Behav.* 81, 843–848.
- Deveney, A. M., and Waddington, J. L. (1995). Pharmacological characterization of behavioural responses to SKF 83959 in relation to 'D1-like' dopamine receptors not linked to adenylyl cyclase. *Br. J. Pharmacol.* 116, 2120–2126.



- Di Chiara, G., Morelli, M., and Consolo, S. (1994). Modulatory functions of neurotransmitters in the striatum: ACh/dopamine/NMDA interactions. *Trends Neurosci.* 17, 228–233.
- Dziedzicka-Wasylewska, M., Faron-Gorecka, A., Andrecka, J., Polit, A., Kusmider, M., and Wasylewski, Z. (2006). Fluorescence studies reveal heterodimerization of dopamine D1 and D2 receptors in the plasma membrane. *Biochemistry* 45, 8751–8759.
- Featherstone, R. E., Kapur, S., and Fletcher, P. J. (2007). The amphetamine-induced sensitized state as a model of schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31, 1556–1571.
- Geldwert, D., Norris, J. M., Feldman, I. G., Schulman, J. J., Joyce, M. P., and Rayport, S. (2006). Dopamine presynaptically and heterogeneously modulates nucleus accumbens medium-spiny neuron GABA synapses in vitro. *BMC Neurosci.* 7, 53–69. doi: 10.1186/1471-2202-7-53
- Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J. Jr., and Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250, 1429–1432.
- Gerfen, C. R., Keefe, K. A., and Gauda, E. B. (1995). D1 and D2 dopamine receptor function in the striatum: coactivation of D1- and D2-dopamine receptors on separate populations of neurons results in potentiated immediate early gene response in D1-containing neurons. *J. Neurosci.* 15, 8167–8176.
- Gerfen, C. R., and Young, W. S. III. (1988). Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Res.* 460, 161–167.
- Gnanalingham, K. K., Hunter, A. J., Jenner, P., and Marsden, C. D. (1995). Stimulation of adenylate cyclase activity by benzazepine D-1 dopamine agonists with varying efficacies in the 6-hydroxydopamine lesioned rat – relationship to circling behaviour. *Biochem. Pharmacol.* 49, 1185–1193.
- Guzman, J. N., Hernandez, A., Galaraga, E., Tapia, D., Laville, A., Vergara, R., Aceves, J., and Vargas, J. (2003). Dopaminergic modulation of axon collaterals interconnecting spiny neurons of the rat striatum. *J. Neurosci.* 23, 8931–8940.
- Hasbi, A., Fan, T., Alijanian, M., Nguyen, T., Perreault, M. L., O'Dowd, B. F., and George, S. R. (2009). Calcium signaling cascade links dopamine D1-D2 receptor heteromer to striatal BDNF production and neuronal growth. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21377–21382.
- Hu, X. T., Wachtel, S. R., Galloway, M. P., and White, F. J. (1990). Lesions of the nigrostriatal dopamine projection increase the inhibitory effects of D1 and D2 dopamine agonists on caudate-putamen neurons and relieve D2 receptors from the necessity of D1 receptor stimulation. *J. Neurosci.* 10, 2318–2329.
- Hyttel, J. (1978). Effects of neuroleptics on 3H-haloperidol and 3H-cis-(Z)-flupenthixol binding and on adenylate cyclase activity in vitro. *Life Sci.* 23, 551–555.
- Issa, G., Wilson, C., Terry, A. V. Jr., and Pillai, A. (2010). An inverse relationship between cortisol and BDNF levels in schizophrenia: data from human postmortem and animal studies. *Neurobiol. Dis.* 39, 327–333.
- Jin, L. Q., Goswami, S., Cai, G., Zhen, X., and Friedman, E. (2003). SKF83959 selectively regulates phosphatidylinositol-linked D1 dopamine receptors in rat brain. *J. Neurochem.* 85, 378–386.
- Jindal, R. D., Pillai, A. K., Mahadik, S. P., Eklund, K., Montrose, D. M., and Keshavan, M. S. (2010). Decreased BDNF in patients with antipsychotic naive first episode schizophrenia. *Schizophr. Res.* 119, 47–51.
- Keefe, K. A., and Gerfen, C. R. (1995). D1-D2 dopamine receptor synergy in striatum: effects of intrastriatal infusions of dopamine agonists and antagonists on immediate early gene expression. *Neuroscience* 66, 903–913.
- LaHoste, G. J., Henry, B. L., and Marshall, J. F. (2000). Dopamine D1 receptors synergize with D2, but not D3 or D4, receptors in the striatum without the involvement of action potentials. *J. Neurosci.* 20, 6666–6671.
- LaHoste, G. J., Yu, J., and Marshall, J. F. (1993). Striatal Fos expression is indicative of dopamine D1/D2 synergism and receptor supersensitivity. *Proc. Natl. Acad. Sci. U.S.A.* 90, 7451–7455.
- Lee, S. P., So, C. H., Rashid, A. J., Varghese, G., Cheng, R., Lanza, A. J., O'Dowd, B. F., and George, S. R. (2004). Dopamine D1 and D2 receptor Co-activation generates a novel phospholipase C-mediated calcium signal. *J. Biol. Chem.* 279, 35671–35678.
- Lester, J., Fink, S., Aronin, N., and DiFiglia, M. (1993). Colocalization of D1 and D2 dopamine receptor mRNAs in striatal neurons. *Brain Res.* 621, 106–110.
- Lidow, M. S. (2003). Calcium signaling dysfunction in schizophrenia: a unifying approach. *Brain Res. Brain Res. Rev.* 43, 70–84.
- Lin, C. W., Miller, T. R., Witte, D. G., Bianchi, B. R., Stashko, M., Manelli, A. M., and Frail, D. E. (1995). Characterization of cloned human dopamine D1 receptor-mediated calcium release in 293 cells. *Mol. Pharmacol.* 47, 131–139.
- Lobo, M. K., Covington, H. E. III, Chaudhury, D., Friedman, A. K., Sun, H., Domez-Werno, D., Dietz, D. M., Zaman, S., Koo, J. W., Kennedy, P. J., Mouzon, E., Mogri, M., Neve, R. L., Deisseroth, K., Han, M. H., and Nestler, E. J. (2010). Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science* 330, 385–390.
- Matamalas, M., Bertran-Gonzalez, J., Salomon, L., Degos, B., Deniau, J. M., Valjent, E., Herve, D., and Girault, J. A. (2009). Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. *PLoS ONE* 4, e4770. doi: 10.1371/journal.pone.0004770
- McGinty, J. F., Whitfield, T. W. Jr., and Berglind, W. J. (2010). Brain-derived neurotrophic factor and cocaine addiction. *Brain Res.* 1314, 183–193.
- Meador-Woodruff, J. H., Mansour, A., Healy, D. J., Kuehn, R., Zhou, Q. Y., Bunzow, J. R., Akil, H., Civelli, O., and Watson, S. J. Jr. (1991). Comparison of the distributions of D1 and D2 dopamine receptor mRNAs in rat brain. *Neuropsychopharmacology* 5, 231–242.
- Mizuno, T., Schmauss, C., and Rayport, S. (2007). Distinct roles of presynaptic dopamine receptors in the differential modulation of the intrinsic synapses of medium-spiny neurons in the nucleus accumbens. *BMC Neurosci.* 8, 8–22. doi: 10.1186/1471-2202-8-8
- Molloy, A. G., and Waddington, J. L. (1987). Assessment of grooming and other behavioural responses to the D-1 dopamine receptor agonist SKF 38393 and its R- and S-enantiomers in the intact adult rat. *Psychopharmacology (Berl.)* 92, 164–168.
- Neumeyer, J. L., Kula, N. S., Bergman, J., and Baldessarini, R. J. (2003). Receptor affinities of dopamine D1 receptor-selective novel phenylbenzazepines. *Eur. J. Pharmacol.* 474, 137–140.
- Ng, J., Rashid, A. J., So, C. H., O'Dowd, B. F., and George, S. R. (2010). Activation of calcium/calmodulin-dependent protein kinase II α in the striatum by the heteromeric D1-D2 dopamine receptor complex. *Neuroscience* 165, 535–541.
- Novak, G., and Seeman, P. (2010). Hyperactive mice show elevated D2(High) receptors, a model for schizophrenia: calcium/calmodulin-dependent kinase II α knock-outs. *Synapse* 64, 794–800.
- Onali, P., Olanas, M. C., and Gessa, G. L. (1985). Characterization of dopamine receptors mediating inhibition of adenylate cyclase activity in rat striatum. *Mol. Pharmacol.* 28, 138–145.
- Panchalingam, S., and Undie, A. S. (2001). SKF83959 exhibits biochemical agonism by stimulating [(35S)]GTP gamma S binding and phosphoinositide hydrolysis in rat and monkey brain. *Neuropharmacology* 40, 826–837.
- Penny, G. R., Afsharpoor, S., and Kitai, S. T. (1986). The glutamate decarboxylase-, leucine enkephalin-, methionine enkephalin- and substance P-immunoreactive neurons in the neostriatum of the rat and cat: evidence for partial population overlap. *Neuroscience* 17, 1011–1045.
- Perreault, M. L., Hasbi, A., Alijanian, M., Fan, T., Varghese, G., Fletcher, P. J., Seeman, P., O'Dowd, B. F., and George, S. R. (2010). The dopamine D1-D2 receptor heteromer localizes in dynorphin/enkephalin neurons: increased high affinity state following amphetamine and in schizophrenia. *J. Biol. Chem.* 285, 36625–36634.
- Piomelli, D., Pilon, C., Giros, B., Sokoloff, P., Martres, M. P., and Schwartz, J. C. (1991). Dopamine activation of the arachidonic acid cascade as a basis for D1/D2 receptor synergism. *Nature* 353, 164–167.
- Rashid, A. J., So, C. H., Kong, M. M., Furtak, T., El-Ghundi, M., Cheng, R., O'Dowd, B. F., and George, S. R. (2007). D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 104, 654–659.

- Reiner, A., Medina, L., and Haber, S. N. (1999). The distribution of dynorphinergic terminals in striatal target regions in comparison to the distribution of substance P-containing and enkephalinergic terminals in monkeys and humans. *Neuroscience* 88, 775–793.
- Shetreat, M. E., Lin, L., Wong, A. C., and Rayport, S. (1996). Visualization of D1 dopamine receptors on living nucleus accumbens neurons and their colocalization with D2 receptors. *J. Neurochem.* 66, 1475–1482.
- Shuen, J. A., Chen, M., Gloss, B., and Calakos, N. (2008). Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. *J. Neurosci.* 28, 2681–2685.
- So, C. H., Varghese, G., Curley, K. J., Kong, M. M., Alijanian, M., Ji, X., Nguyen, T., O'Dowd, B. F., and George, S. R. (2005). D1 and D2 dopamine receptors form heterooligomers and cointernalize after selective activation of either receptor. *Mol. Pharmacol.* 68, 568–578.
- So, C. H., Verma, V., Alijanian, M., Cheng, R., Rashid, A. J., O'Dowd, B. F., and George, S. R. (2009). Calcium signaling by dopamine D5 receptor and D5-D2 receptor heterooligomers occurs by a mechanism distinct from that for dopamine D1-D2 receptor heterooligomers. *Mol. Pharmacol.* 75, 843–854.
- Stefani, A., Chen, Q., Flores-Hernandez, J., Jiao, Y., Reiner, A., and Surmeier, D. J. (1998). Physiological and molecular properties of AMPA/Kainate receptors expressed by striatal medium spiny neurons. *Dev. Neurosci.* 20, 242–252.
- Surmeier, D. J., Eberwine, J., Wilson, C. J., Cao, Y., Stefani, A., and Kitai, S. T. (1992). Dopamine receptor subtypes colocalize in rat striatonigral neurons. *Proc. Natl. Acad. Sci. U.S.A.* 89, 10178–10182.
- Surmeier, D. J., Song, W. J., and Yan, Z. (1996). Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. *J. Neurosci.* 16, 6579–6591.
- Undie, A. S., and Friedman, E. (1990). Stimulation of a dopamine D1 receptor enhances inositol phosphates formation in rat brain. *J. Pharmacol. Exp. Ther.* 253, 987–992.
- Undie, A. S., Weinstock, J., Sarau, H. M., and Friedman, E. (1994). Evidence for a distinct D1-like dopamine receptor that couples to activation of phosphoinositide metabolism in brain. *J. Neurochem.* 62, 2045–2048.
- Valjent, E., Bertran-Gonzalez, J., Herve, D., Fisone, G., and Girault, J. A. (2009). Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci.* 32, 538–547.
- Verma, V., Hasbi, A., O'Dowd, B. F., and George, S. R. (2010). Dopamine D1-D2 receptor Heteromer-mediated calcium release is desensitized by D1 receptor occupancy with or without signal activation: dual functional regulation by G protein-coupled receptor kinase 2. *J. Biol. Chem.* 285, 35092–35103.
- Vorobjev, V. S., Sharonova, I. N., Haas, H. L., and Sergeeva, O. A. (2000). Differential modulation of AMPA receptors by cyclothiazide in two types of striatal neurons. *Eur. J. Neurosci.* 12, 2871–2880.
- Waddington, J. L., Daly, S. A., Downes, R. P., Deveney, A. M., McCauley, P. G., and O'Boyle, K. M. (1995). Behavioural pharmacology of 'D1-like' dopamine receptors: further subtyping, new pharmacological probes and interactions with 'D2-like' receptors. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 19, 811–831.
- Walters, J. R., Bergstrom, D. A., Carlson, J. H., Chase, T. N., and Braun, A. R. (1987). D1 dopamine receptor activation required for postsynaptic expression of D2 agonist effects. *Science* 236, 719–722.
- Wang, H. B., Deng, Y. P., and Reiner, A. (2007). In situ hybridization histochemical and immunohistochemical evidence that striatal projection neurons co-containing substance P and enkephalin are overrepresented in the striosomal compartment of striatum in rats. *Neurosci. Lett.* 425, 195–199.
- Wang, H. B., Laverghetta, A. V., Foehring, R., Deng, Y. P., Sun, Z., Yamamoto, K., Lei, W. L., Jiao, Y., and Reiner, A. (2006). Single-cell RT-PCR, in situ hybridization histochemical, and immunohistochemical studies of substance P and enkephalin co-occurrence in striatal projection neurons in rats. *J. Chem. Neuroanat.* 31, 178–199.
- Wang, L., Lv, Z., Hu, Z., Sheng, J., Hui, B., Sun, J., and Ma, L. (2010). Chronic cocaine-induced H3 acetylation and transcriptional activation of CaMKIIalpha in the nucleus accumbens is critical for motivation for drug reinforcement. *Neuropsychopharmacology* 35, 913–928.
- Weickert, C. S., Hyde, T. M., Lipska, B. K., Herman, M. M., Weinberger, D. R., and Kleinman, J. E. (2003). Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia. *Mol. Psychiatry* 8, 592–610.
- White, F. J., Bednars, L. M., Wachtel, S. R., Hjorth, S., and Brooderson, R. J. (1988). Is stimulation of both D1 and D2 receptors necessary for the expression of dopamine-mediated behaviors? *Pharmacol. Biochem. Behav.* 30, 189–193.
- White, F. J., and Wang, R. Y. (1986). Electrophysiological evidence for the existence of both D-1 and D-2 dopamine receptors in the rat nucleus accumbens. *J. Neurosci.* 6, 274–280.
- Wong, A. C., Shetreat, M. E., Clarke, J. O., and Rayport, S. (1999). D1- and D2-like dopamine receptors are co-localized on the presynaptic varicosities of striatal and nucleus accumbens neurons in vitro. *Neuroscience* 89, 221–233.
- Wong, J., Hyde, T. M., Cassano, H. L., Deep-Soboslay, A., Kleinman, J. E., and Weickert, C. S. (2010). Promoter Specific Alterations of BDNF mRNA in Schizophrenia. *Neuroscience* 169, 1071–1084.
- Zhen, X., Goswami, S., and Friedman, E. (2005). The role of the phosphatidylinositol-linked D1 dopamine receptor in the pharmacology of SKF83959. *Pharmacol. Biochem. Behav.* 80, 597–601.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 April 2011; paper pending published: 11 May 2011; accepted: 16 May 2011; published online: 31 May 2011.

Citation: Perreault ML, Hasbi A, O'Dowd BF and George SR (2011) The dopamine D1–D2 receptor heteromer in striatal medium spiny neurons: evidence for a third distinct neuronal pathway in basal ganglia. *Front. Neuroanat.* 5:31. doi: 10.3389/fnana.2011.00031

Copyright © 2011 Perreault, Hasbi, O'Dowd and George. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Adenosine A_{2A} receptors and A_{2A} receptor heteromers as key players in striatal function

Sergi Ferré^{1*}, César Quiroz¹, Marco Orru¹, Xavier Guitart¹, Gemma Navarro^{2,3}, Antonio Cortés^{2,3}, Vicent Casadó^{2,3}, Enric I. Canela^{2,3}, Carme Lluís^{2,3} and Rafael Franco^{2,3,4}

¹ National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, U.S. Department of Health and Human Services, Baltimore, MD, USA

² Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas, University of Barcelona, Barcelona, Spain

³ Faculty of Biology, Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain

⁴ Centro de Investigación Médica Aplicada, Universidad de Navarra, Pamplona, Spain

Edited by:

Emmanuel Valjent, Université Montpellier 1 and 2, France

Reviewed by:

Jose L. Lanciego, University of Navarra, Spain

Julie Perroy, Centre National de la Recherche Scientifique, France

*Correspondence:

Sergi Ferré, National Institute on Drug Abuse, Intramural Research Program, 251 Bayview Boulevard, Baltimore, MD 21224, USA.
e-mail: sferre@intra.nida.nih.gov

A very significant density of adenosine A_{2A} receptors (A_{2A}Rs) is present in the striatum, where they are preferentially localized postsynaptically in striatopallidal medium spiny neurons (MSNs). In this localization A_{2A}Rs establish reciprocal antagonistic interactions with dopamine D₂ receptors (D₂Rs). In one type of interaction, A_{2A}R and D₂R are forming heteromers and, by means of an allosteric interaction, A_{2A}R counteracts D₂R-mediated inhibitory modulation of the effects of NMDA receptor stimulation in the striatopallidal neuron. This interaction is probably mostly responsible for the locomotor depressant and activating effects of A_{2A}R agonist and antagonists, respectively. The second type of interaction involves A_{2A}R and D₂R that do not form heteromers and takes place at the level of adenylyl cyclase (AC). Due to a strong tonic effect of endogenous dopamine on striatal D₂R, this interaction keeps A_{2A}R from signaling through AC. However, under conditions of dopamine depletion or with blockade of D₂R, A_{2A}R-mediated AC activation is unleashed with an increased gene expression and activity of the striatopallidal neuron and with a consequent motor depression. This interaction is probably the main mechanism responsible for the locomotor depression induced by D₂R antagonists. Finally, striatal A_{2A}Rs are also localized presynaptically, in cortico-striatal glutamatergic terminals that contact the striato-nigral MSN. These presynaptic A_{2A}Rs heteromerize with A₁ receptors (A₁Rs) and their activation facilitates glutamate release. These three different types of A_{2A}Rs can be pharmacologically dissected by their ability to bind ligands with different affinity and can therefore provide selective targets for drug development in different basal ganglia disorders.

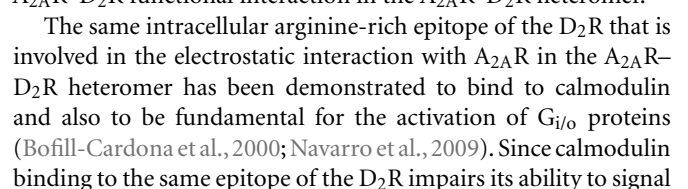
Keywords: adenosine A_{2A} receptor, striatum, receptor heteromers, dopamine receptors, cannabinoid receptors

POSTSYNAPTIC STRIATAL ADENOSINE A_{2A} RECEPTORS

A very significant density of adenosine A_{2A} receptors (A_{2A}Rs) is present in the striatum (Rosin et al., 1998; Hettinger et al., 1998; Schiffmann et al., 2007; Quiroz et al., 2009), where they are preferentially localized postsynaptically in the soma and dendrites of GABAergic striatopallidal. These neurons also show a high density of dopamine D₂ receptors (D₂Rs) and there is clear evidence for the existence of postsynaptic mechanisms in the control of glutamatergic neurotransmission to the enkephalinergic medium spiny neuron (MSN) by at least two reciprocal antagonistic interactions between A_{2A}Rs and D₂Rs (Ferré et al., 2008). In one type of interaction, stimulation of A_{2A}R counteracts the D₂R-mediated inhibitory modulation of NMDA receptor (NMDAR)-mediated effects, which include modulation of Ca²⁺ influx, transition to the up-state and neuronal firing (Azdad et al., 2009; Higley and Sabatini, 2010; Figure 1). This interaction seems to be mostly responsible for the locomotor depressant and activating effects of A_{2A}R agonists and antagonists, respectively (Ferré et al., 2008; Orru et al., 2011), which correlates with the results of behavioral experiments showing that A_{2A}R activation or blockade decreases or

increases, respectively, the motor effects elicited by D₂R activation (Ferré et al., 2008).

Initially, the main mechanism responsible for this A_{2A}R–D₂R interaction was attributed to what it was described as an “intramembrane interaction,” by which activation of A_{2A}R could decrease the affinity of an adjacent D₂R for agonists in striatal membrane preparations (Ferré et al., 1991). It was afterward hypothesized that this kind of intramembrane interaction was a biochemical property of receptor heteromers with important functional implications (Zoli et al., 1993). A receptor heteromer is now defined as a macromolecular complex composed of at least two (functional) receptor units with biochemical properties that are demonstrably different from those of its individual components (Ferré et al., 2009). The term “intramembrane interaction” is now known as “allosteric interaction in the receptor heteromer,” which is defined as an intermolecular interaction by which binding of a ligand to one of the receptor units in the receptor heteromer changes the binding properties of another receptor unit (Ferré et al., 2009). Another definition recently introduced in the field of receptor heteromers is “biochemical fingerprint,” which is a



through G_{i/o} proteins (Bofill-Cardona et al., 2000), it is likely that binding of the C terminus of the A_{2A}R to the same epitope reduces the capacity of the D₂R to bind calmodulin and to signal through G_{i/o} proteins. In fact, it has recently been shown that the binding of calmodulin to the A_{2A}R–D₂R heteromer is occurring within the proximal portion of the A_{2A}R but not with the D₂R (Navarro et al., 2009). It is possible that agonist binding to the A_{2A}R induces a conformational change in the A_{2A}R–D₂R heteromer that causes an even further impairment in the coupling of the D₂R to the G_{i/o} protein. Thus, it seems that, in the A_{2A}R–D₂R heteromer, D₂R does not signal through G_{i/o} proteins or that its main signaling is by a G-protein-independent mechanism. However, the recent study by Higley and Sabatini (2010) suggests that the D₂R-mediated inhibitory modulation of NMDAR-mediated Ca²⁺ signaling in the enkephalinergic MSN is mediated by PKA and, therefore, most probably related to the ability of D₂R to couple to G_{i/o} and to inhibit adenylyl cyclase (AC). Interestingly, in these experiments (and in agreement with the experiments by Azdad et al., 2009), an A_{2A}R agonist did not produce any significant effect on its own, but counteracted the effect of a D₂R agonist. Thus, although Higley and Sabatini (2010) suggested that this interaction between A_{2A}R and D₂R takes place at the AC level, it shows similar characteristics to the A_{2A}R–D₂R heteromer-dependent interaction. In summary, A_{2A}R–D₂R heteromers seem to play a key role in the modulation of NMDAR-mediated signaling in the enkephalinergic MSN, but the molecular mechanisms involved in these A_{2A}R–D₂R–NMDAR interactions are yet to be determined.

In addition to the antagonistic A_{2A}R–D₂R receptor interaction in the A_{2A}R–D₂R heteromer, D₂R stimulation impedes A_{2A}R to signal through AC (Kull et al., 1999; Chen et al., 2001; Hillion et al., 2002; Håkansson et al., 2006; **Figure 1**). This D₂R–A_{2A}R interaction takes place at the second messenger level, and stimulation of G_{i/o}-coupled D₂R counteracts the effects of G_{s/olf}-coupled A_{2A}R (Ferré et al., 2007, 2008). Due to a strong tonic effect of endogenous dopamine on striatal D₂R, this interaction keeps A_{2A}R from signaling through AC. However, under conditions of dopamine depletion or with pharmacological D₂R blockade, A_{2A}R-mediated signaling through the cAMP–PKA cascade may be unleashed. Antagonism of D₂R is biochemically associated with a significant increase in the phosphorylation of PKA-dependent substrates, which increases gene expression and the activity of the enkephalinergic MSN, producing locomotor depression (reviewed in Ferré et al., 2008). This appears to be the main mechanism responsible for the locomotor depression induced by D₂R antagonists. Thus the motor depressant and most biochemical effects induced by pharmacologic blockade of D₂R may be counteracted by pharmacological blockade of A_{2A}R (Chen et al., 2001; Håkansson et al., 2006).

The two reciprocal antagonistic interactions, A_{2A}R toward D₂R (A_{2A}R–D₂R) and D₂R toward A_{2A}R (D₂R–A_{2A}R), take place simultaneously in the same cell, which suggest that are most likely mediated by the existence of at least two different populations of postsynaptic striatal A_{2A}R in the enkephalinergic MSN (Ferré et al., 2008). One population would be forming heteromers with D₂R and would determine that A_{2A}R stimulation inhibits D₂R-mediated signaling (A_{2A}R–D₂R interaction), while another population would not be forming heteromers with D₂R and would

determine that D₂R stimulation inhibits A_{2A}R-mediated signaling (D₂R–A_{2A}R interaction). This second population of postsynaptic A_{2A}R would either not form heteromers or would form heteromers with other receptors, such as glutamate mGlu₅ receptors (mGlu₅Rs; Ferré et al., 2002; **Figure 1**). Importantly, heteromerization of A_{2A}R with mGlu₅R is associated with a synergistic effect upon A_{2A}R and mGlu₅R co-activation at the level of AC and MAPK, providing a physiological mechanism by which A_{2A}R can overcome the D₂R–A_{2A}R interaction (Ferré et al., 2002; Nishi et al., 2003). Co-stimulation of A_{2A}R and mGlu₅R *in vivo*, with the central administration of selective agonists, allowed A_{2A}R to get rid of the inhibitory effect of the D₂R and signal through the cAMP–PKA cascade (Ferré et al., 2002). Since this A_{2A}R–D₂R–mGlu₅R interaction could be demonstrated in animal models of Parkinson's disease (Popoli et al., 2001; Kachroo et al., 2005), it was postulated that co-administration of A_{2A}R and mGlu₅R antagonists could be useful as a therapeutic strategy in this disease (Popoli et al., 2001).

Still a third population of postsynaptic A_{2A}R would form heteromers with cannabinoid CB₁ receptors (CB₁Rs; Carriba et al., 2007; **Figure 1**). In this heteromer, activation of A_{2A}R is necessary to allow CB₁R-mediated signaling. Thus, in a human neuroblastoma cell line, CB₁R-mediated inhibition of AC activity was found to be completely dependent on A_{2A}R co-activation (Carriba et al., 2007). Similarly, several biochemical effects of CB₁R agonists in primary striatal cell cultures and striatal slices have been shown to depend on A_{2A}R co-activation (Yao et al., 2003; Andersson et al., 2005). Accordingly, Tebano et al. (2009) reported that the depression of synaptic transmission induced by a CB₁R agonist in cortico-striatal slices was prevented by A_{2A}R antagonists and also by the conditional genetic blockade of striatal postsynaptic A_{2A}R. The permissive effect of A_{2A}R toward CB₁R function did not seem to occur presynaptically, as the ability of the CB₁R agonist to increase the R2/R1 ratio under a protocol of paired-pulse stimulation was not modified by an A_{2A}R antagonist (Tebano et al., 2009). These results would predict that A_{2A}R antagonists should produce similar behavioral effects than CB₁R antagonists and, in fact, pharmacological or genetic inactivation of A_{2A}Rs reduce the motor depressant, cataleptic, and rewarding effects of CB₁R agonists (Soria et al., 2004; Andersson et al., 2005; Carriba et al., 2007; Justinova et al., 2011). Significantly, it has been recently reported that low doses of an A_{2A}R antagonist (MSX-3) reduce in squirrel monkeys self-administration of THC and anandamide, but not cocaine (Justinova et al., 2011).

Although the studies just mentioned indicate that the motor (depressant) effects of CB₁R agonists might depend on adenosine A_{2A} receptor signaling, a recent study by Lerner et al. (2010) suggested quite the opposite, that CB₁R signaling mediates the locomotor-activating effects of A_{2A}R antagonists. Thus, pharmacological or genetic inactivation of CB₁R reduced the locomotor activation induced by an A_{2A}R antagonist in mice habituated to the testing environment (Lerner et al., 2010). The mechanistic explanation of this interaction is related to the previously reported D₂R agonist-mediated endocannabinoid release by the enkephalinergic MSN, which by retrograde signaling would inhibit glutamate release by stimulating CB₁R localized in glutamatergic terminals. This would lead to a decreased stimulation of the striatopallidal MSN, which would produce locomotor activation (Kreitzer and

Malenka, 2007). In fact, Kreitzer and Malenka (2007) advocated that, instead of direct postsynaptic effects, such as the previously mentioned D₂R-mediated modulation of NMDAR-mediated signaling (Azdad et al., 2009; Higley and Sabatini, 2010), this indirect and endocannabinoid-mediated presynaptic effect is the main mechanism by which D₂R stimulation produces inhibition of the enkephalinergic MSN function. According to Lerner et al. (2010), an A_{2A}R antagonist would then produce locomotor activation by disinhibiting a tonic A_{2A}R-mediated inhibition of D₂R-mediated endocannabinoid release. However, this hypothesis would predict that CB₁R agonists and antagonists should produce locomotor activation and depression, respectively, and that CB₁R blockade should counteract the motor effects of D₂ receptor agonists. This is the opposite of what has been reported in previous studies (for a recent review, see Ferré et al., 2010). To reevaluate the findings by Lerner et al. (2010) we studied in detail the effects of pharmacological interactions between A_{2A}R antagonists and CB₁R antagonists on the locomotor activity in rats not habituated to the testing environment (Orri et al., submitted). Whereas we could indeed reproduce the results by Lerner et al. (2010) showing that a CB₁R antagonist significantly decreases the locomotor effects induced by an A_{2A}R antagonist, we found that the CB₁R antagonist also produces a comparable decrease in locomotion in vehicle-treated animals (statistical analysis indicated that the locomotor effects of A_{2A}R and CB₁R antagonists were not interrelated). It was therefore the use of habituated animals (which display very low locomotor activity in the testing environment) what masked the depressant effect of CB₁R antagonist in the vehicle-treated animals in the study by Lerner et al. (2010).

In addition to the three populations of postsynaptic striatal A_{2A}R so far reported, there is also experimental evidence for a potentially more complex picture, which includes the possibility of receptor heteromultimers. Thus, using a new biophysical/based technology, sequential resonance energy transfer (SRET), and bimolecular fluorescence complementation plus BRET, evidence for A_{2A}R–CB₁R–D₂R and A_{2A}R–D₂R–mGlu₅R heteromers in transfected cells has been recently obtained (Carriba et al., 2008; Cabello et al., 2009; Navarro et al., 2010). Mutation experiments indicated that the interactions of the intracellular domains of the CB₁R receptor with A_{2A}R and D₂R are fundamental for the correct formation of the quaternary structure needed for the function (MAPK signaling) of the A_{2A}R–CB₁R–D₂R heteromers. It should be noted that the analysis of MAPK signaling in striatal slices of CB₁R KO mice and wild-type littermates supports the existence of A_{2A}R–CB₁R–D₂R receptor heteromers in the brain (Navarro et al., 2010). Despite the stoichiometry of the different populations of postsynaptic striatal A_{2A}R heteromers (and homomers) is not known, taking into account the very high density of A_{2A}Rs and D₂Rs in the enkephalinergic MSM, we postulate that A_{2A}R and D₂R homomers and A_{2A}R–D₂R heteromers are the most common receptor populations, followed by combinations of those populations with CB₁R and mGlu₅R.

It is also of importance to mention that there is also evidence for the existence of A_{2A}R receptors, also co-localized with D₂Rs, in the somatodendritic and nerve terminal regions of the cholinergic striatal interneurons and that their interactions modulate acetylcholine release (James and Richardson, 1993; Jin et al., 1993;

Preston et al., 2000; Tozzi et al., 2011). The study by Jin et al. (1993) showed evidence for an antagonistic A_{2A}R–D₂R interaction in the modulation of striatal acetylcholine release. Thus, A_{2A}R stimulation counteracted the ability of D₂R activation to inhibit acetylcholine release. Similarly, a recent study showed that A_{2A}R blockade potentiates D₂R-mediated modulation of acetylcholine release (Tozzi et al., 2011), again indicating the existence of an antagonistic A_{2A}R–D₂R interaction and, probably, A_{2A}R–A_{2A}R heteromers in striatal cholinergic interneurons.

PRESYNAPTIC STRIATAL ADENOSINE A_{2A} RECEPTORS

Striatal A_{2A}Rs are not only localized postsynaptically but also presynaptically, in glutamatergic terminals, where they heteromerize with A₁ receptors (A₁Rs) and where they perform a fine-tuned modulation of glutamate release (Ciruela et al., 2006; Quiroz et al., 2009; **Figure 1**). Thus, A₁R–A_{2A}R heteromers seem to work as a concentration-dependent switch (Ferré et al., 2007), with adenosine acting primarily at A₁Rs at low concentrations, and at both A₁Rs and A_{2A}Rs at higher concentrations. Activation of the A₁R in the A₁R–A_{2A}R heteromer produces inhibition of glutamate release, while the additional activation of the A_{2A}R produces the opposite effect, on a mechanism that seems to involve an allosteric modulation in the receptor heteromer and interactions at the G protein level (Ciruela et al., 2006; Ferré et al., 2007). Interestingly, presynaptic A_{2A}Rs are preferentially localized in glutamatergic terminals of cortico-striatal afferents to the dynorphinergic MSN (Quiroz et al., 2009). Apart from morphological evidence provided by immunohistochemical and electron microscopy experiments, patch-clamp experiments in identified enkephalinergic and dynorphinergic MSNs provided a functional demonstration of the segregation of striatal presynaptic A_{2A}Rs. Thus, an A_{2A}R agonist and an A_{2A}R receptor antagonist significantly increased and decreased, respectively, the amplitude of excitatory postsynaptic currents induced by the intrastriatal stimulation of glutamatergic afferents measured in identified enkephalinergic, but not dynorphinergic MSNs. Mean-variance analysis indicated a presynaptic locus for the A_{2A}R-mediated modulation (Quiroz et al., 2009). Thus, there seems to be a selective A_{2A}R-mediated modulation of glutamate release to the dynorphinergic MSN, which is in disagreement with the recently proposed role of postsynaptic A_{2A}Rs in the modulation of glutamate release to the enkephalinergic MSN (Lerner et al., 2010).

The powerful modulatory role of presynaptic A_{2A}Rs on striatal glutamate release was first demonstrated with *in vivo* microdialysis experiments by Popoli et al. (1995), who showed that the striatal perfusion of an A_{2A}R agonist produced a very pronounced increase in the basal striatal extracellular concentrations of glutamate. Also intrastriatal perfusion of an A_{2A}R antagonist through a microdialysis probe could significantly counteract striatal glutamate release induced by cortical electrical stimulation in the orofacial premotor cortex (Quiroz et al., 2009). A striking unexpected finding was that the counteraction of glutamate release was also accompanied by a complete counteraction of the jaw movements induced by the cortical electrical stimulation, demonstrating the very important role of presynaptic A_{2A}Rs in the control of cortico-striatal glutamatergic neurotransmission. By combining cortical electrical stimulation and recording of EMG activity of the mastication

muscles, a power correlation coefficient (PCC) was established as a quantitative *in vivo* measure of cortico-striatal neurotransmission (Quiroz et al., 2009). PCC was shown to be significantly and dose dependently decreased by the systemic administration of an A_{2A}R receptor antagonist. PCC could therefore be used as a method to screen the presynaptic effect of A_{2A}R antagonists (see below).

According to the widely accepted functional basal circuitry model (Obeso et al., 2002; DeLong and Wichmann, 2007), blockade of postsynaptic striatal A_{2A}R in the A_{2A}R–D₂R heteromer, localized in the enkephalinergic MSN should potentiate spontaneous or psychostimulant-induced motor activation. On the other hand, according to the same model, blockade of presynaptic striatal A_{2A}R localized in the cortico-striatal glutamatergic terminals that make synaptic contact with the dynorphinergic MSN should decrease motor activity. The clear locomotor-activating effects of systemically administered A_{2A}R antagonists could be explained by the significantly higher density of postsynaptic versus presynaptic striatal A_{2A}R and to a stronger influence of a tonic adenosine and A_{2A}R-mediated modulation of the enkephalinergic versus dynorphinergic MSNs under basal conditions. The results by Shen et al. (2008) about the differential effects of A_{2A}R antagonists on psychostimulant-induced locomotor activation in WT versus conditional striatal postsynaptic A_{2A}R KO mice (potentiation versus counteraction, respectively) support this hypothesis. As previously suggested (Ferré et al., 2007), activation of presynaptic A_{2A}Rs seems to be highly dependent on the level of adenosine generated upon cortico-striatal glutamatergic input.

Striatal D₂Rs are also localized presynaptically, in dopaminergic and glutamatergic terminals (Higley and Sabatini, 2010), giving the frame for the existence of interactions with A_{2A}Rs at least in those terminals establishing contact with the dynorphinergic MSN. The experimental evidence suggest that there is also a presynaptic D₂R–A_{2A}R interaction by which D₂R activation tonically inhibits the ability of endogenous adenosine to produce an A_{2A}R-mediated increase in the basal extracellular levels of glutamate. Thus, dopamine denervation significantly potentiates A_{2A}R agonist-mediated stimulation of glutamate release (Tangianelli et al., 2004). This has the biochemical characteristics of an interaction between A_{2A}Rs and D₂Rs at the AC level and not forming A_{2A}R–D₂R heteromers. Furthermore, results Rodrigues et al. (2005) have also demonstrated the existence of mGlu₅Rs in striatal glutamatergic terminals co-localized with A_{2A}Rs and which facilitate glutamate release in a synergistic manner. The interplay between adenosine- and dopamine-mediated actions at the presynaptic level is therefore affected by the occurrence of mGlu₅Rs.

The presynaptic localization of CB₁Rs in striatal glutamatergic terminals is well established, and therefore they can be co-localized with A_{2A}R in terminals establishing contact with the dynorphinergic MSN (Ferré et al., 2010). The existence of A_{2A}R–CB₁R heteromers in striatal glutamatergic terminals which could mediate the reinforcing effects of cannabinoids has been recently postulated (Ferré et al., 2010; Justinova et al., 2011). However, a recent study by Martire et al. (2011) indicates that cannabinoid/adenosine functional interactions result from an interaction at the second messenger level. In the frame of heteromerization A_{2A}R activation should facilitate the G_{i/o}-mediated effect

of CB₁R activation measured, as inhibition of glutamate release. Nevertheless, Martire et al. (2011), by studying extracellular field potentials recordings in cortico-striatal slices and superfused striatal nerve terminals, very convincingly showed that, instead, A_{2A}R activation prevents CB₁R-mediated inhibition of glutamate release. These results indicate that regulation of glutamate release by cannabinoids is not dependent on presynaptic A_{2A}R–CB₁R heteromers.

In summary, a great amount of available data indicates that, presynaptically, A_{2A}Rs form heteromers mostly with A₁Rs. In addition, there seems to be a population of A_{2A}Rs not forming heteromers but establishing antagonistic interactions with D₂Rs and CB₁Rs and synergistic interactions with mGlu₅Rs. Apart from co-expression, at this moment we do not know the variables that determine the ability of A_{2A}Rs to bind to different receptors to form different pre and postsynaptic heteromers. Thus, D₂Rs are also localized presynaptically, but yet they do not seem to form heteromers with A_{2A}Rs. A_{2A}Rs could bind with more affinity to A₁Rs than to D₂Rs or particular scaffolding proteins could favor a particular A_{2A}R heteromer. All these are questions still need to be answered.

TARGETING STRIATAL PRE AND POSTSYNAPTIC A_{2A} RECEPTORS

A surprising yet fundamental finding of a recent study is that several A_{2A}R antagonists previously thought as being pharmacologically similar present different striatal pre and postsynaptic profiles (Orzu et al., 2011). Six compounds already known as selective A_{2A}R antagonists were first screened for their ability to block striatal pre and postsynaptic A_{2A}Rs with *in vivo* models. Locomotor activation was used to evaluate postsynaptic activity while PCC counteraction was used to determine presynaptic activity (see above). SCH-442416 and KW-6002, showed preferential pre and postsynaptic profiles, respectively, and four compounds, MSX-3, SCH-420814, SCH-58261, and ZM-241385, showed mixed pre–postsynaptic profiles. Combining *in vivo* microdialysis with cortical electrical stimulation was used as an additional *in vivo* evaluation of presynaptic activity of A_{2A}R antagonists. In agreement with its preferential presynaptic profile, SCH-442416 significantly counteracted striatal glutamate release induced by cortical stimulation at a dose that strongly counteracted PCC but did not induce locomotor activation. On the other hand, according to its preferential postsynaptic profile, KW-6002 did not modify striatal glutamate release induced by cortical stimulation at a dose that produced a pronounced locomotor activation but did not counteract PCC.

Another important finding of the study by Orzu et al. (2011) was that at least part of these pharmacological differences between A_{2A}R antagonists could be explained by the ability of pre and postsynaptic A_{2A}R to form different receptor heteromers, with A₁R and D₂R, respectively (see above). Radioligand-binding experiments were performed in cells stably expressing A_{2A}R, A_{2A}R–D₂R heteromers, or A₁R–A_{2A}R heteromers to determine possible differences in the affinity of these different A_{2A}Rs for A_{2A}R antagonists. Co-expression with A₁R did not significantly modify the affinity of A_{2A}Rs for the different ligands, but co-expression with D₂Rs decreased the affinity of all compounds, with the exception of

KW-6002 (Orru et al., 2011). The structural changes in the A_{2A}R induced by heteromerization with the D₂R could be detected not only by antagonists but also by agonist binding. Indeed, the affinity of the selective A_{2A}R agonist CGS-21680 was reduced in cells co-transfected with D₂Rs. When trying to explain the differential action of SCH-442416 observed *in vivo*, it is interesting to note that this compound in particular showed a much higher affinity for the A_{2A}R in a presynaptic-like than in a postsynaptic-like context. In fact, the affinity of A_{2A}R for SCH-442416 in cells expressing A_{2A}R–D₂R heteromers was markedly reduced (40 times higher B₅₀ values in competitive-inhibition experiments with [³H]ZM-241385 in cells expressing A_{2A}R–D₂R than A₁R–A_{2A}R heteromers).

The decrease in affinity upon co-expression with D₂Rs was much less pronounced for ZM-241385, SCH-58261, MSX2, or SCH-420814, for which the affinity was reduced from two to about ninefold (Orru et al., 2011). Taking into account that these A_{2A}R antagonists behaved qualitatively similar than the A_{2A}R agonist CGS-21680 in terms of binding to A₁R–A_{2A}R and A_{2A}R–D₂R heteromers, it was expected that these four compounds compete equally for the binding of the endogenous agonist at pre and at postsynaptic sites. This would fit with the *in vivo* data, which showed that these compounds have a non-preferred pre-postsynaptic profile. Yet, KW-6002 was the only antagonist whose affinity was not significantly different in cells expressing A_{2A}R, A₁R–A_{2A}R heteromers, or A_{2A}R–D₂R heteromers. Thus, KW-6002 showed the best relative affinity for A_{2A}R–D₂R heteromers of all compounds, which can at least partially explain its preferential postsynaptic profile. Experiments performed with the non-selective adenosine receptor antagonist caffeine also showed a correlation between the *in vivo* data and the *in vitro* preference for postsynaptic A_{2A}R-containing heteromers. It was previously reported that in transfected mammalian cells the affinity of A_{2A}R for the non-selective adenosine receptor antagonist caffeine did not change when co-transfected with D₂R, but it was significantly decreased (about 10 times) when co-transfected with A₁R (Ciruela et al., 2006). As predicted, caffeine did not significantly reduce PCC at doses that produce pronounced motor activation (Zanovelli et al., in preparation).

It must be pointed out that to say that SCH-442416 is a selective presynaptic A_{2A}R antagonist is an oversimplification. In fact, the *in vitro* data indicated that SCH-442416 binds equally well to the A_{2A}R not forming heteromers than to the A_{2A}R in the A₁R–A_{2A}R heteromer. Therefore, according to the previous description of the different populations of striatal A_{2A}Rs, SCH-442416 should also be effective at counteracting D₂R antagonist-induced motor depression. In fact, at doses that are not producing locomotor activation (but that reduce PCC), SCH-442416 significantly counteracts the locomotor depression induced by the D₂R antagonist raclopride (Orru et al., submitted). On the other hand, KW-6002 produced the same locomotor activation with or without co-administration with raclopride, in agreement with its ability to block the three populations of A_{2A}R studied so far *in vitro*, A_{2A}R, A_{2A}–D₂R, and A₁R–A_{2A}R. Importantly, KW-6002 also produced the same locomotor activation when co-administered with the A_{2A}R agonist CGS-21680, while SCH-442416, at the same dose that counteracted the depressant effect of raclopride, did not significantly counteract the depressant effect of CGS-21680. These

results, therefore agree with the hypothesis that the subpopulation of postsynaptic A_{2A}R forming heteromers with D₂R are mainly responsible for both the locomotor activation and depression induced by A_{2A}R antagonists and agonists, respectively. In summary, SCH-442416 can be considered as a compound that at relatively low doses not only binds preferentially to presynaptic A_{2A}Rs localized in cortico-striatal glutamatergic terminals (Orru et al., 2011), but also to a subpopulation of postsynaptic A_{2A}Rs most probably not forming heteromers with D₂Rs, but which function is tonically inhibited by D₂Rs activated by endogenous dopamine. Interestingly, [¹¹C]SCH-442416 has been used in rats, monkeys, and humans as a PET radioligand and shown to nicely label striatal A_{2A}Rs (Moresco et al., 2005; Schiffmann et al., 2007; Brooks et al., 2010). The low doses used in PET experiments indicate that [¹¹C]SCH-442416 is mostly labeling presynaptic A_{2A}Rs and postsynaptic A_{2A}Rs that do not form heteromers with D₂Rs. The use of [¹¹C]SCH-442416 and other less selective radioligand in combination with cold SCH-442416 could allow the identification of the different populations of A_{2A}Rs in the human brain. The picture is still incomplete, and a further evaluation of the affinity of A_{2A}R antagonists for A_{2A}R–mGlu₅R and A_{2A}R–CB₁ heteromers (and of heterotrimers) is needed. Nevertheless, the information so far available is very valuable to attempt the design of more efficient A_{2A}R antagonists to be used in basal ganglia disorders.

A_{2A} RECEPTOR HETEROMERS AS TARGETS FOR DRUG DEVELOPMENT

The results of the above mentioned studies support the notion that receptor heteromers may be used as selective targets for drug development. Main reasons are the very specific neuronal localization of receptor heteromers (even more specific than receptor subtypes themselves), and a differential ligand affinity of a receptor depending on its partner (or partners) in the receptor heteromer. Striatal A_{2A}R-containing heteromers become particularly interesting targets, eventually useful for a variety of neuropsychiatric disorders. Blocking postsynaptic A_{2A}Rs in the enkephalinergic MSN should be beneficial for Parkinson's disease because it would decrease the activity of the indirect striatal efferent pathway. On the one hand, one benefit would come from potentiating the effect of l-dopa or other dopamine receptor agonists on the D₂R-mediated signaling in the A_{2A}R–D₂R heteromer. On the other hand, blockade of A_{2A}Rs not forming heteromers with D₂Rs (but antagonistically interacting with D₂R at the AC level) should counteract the effects of the disinhibited A_{2A}R signaling. However, blocking presynaptic A_{2A}R in glutamatergic terminals contacting dynorphinergic MSN (either forming or not heteromers with A₁R) should decrease glutamatergic transmission through the direct striatal efferent pathway, thus decreasing motor activity and, therefore, decreasing the antiparkinsonian efficacy of A_{2A}R antagonists. The most convenient A_{2A}R antagonist to treat Parkinson's disease patients would have more affinity for postsynaptic than for presynaptic receptors. Additionally, a selective blockade of presynaptic A_{2A}Rs should be useful in dyskinetic disorders such as Huntington's disease and could also be useful in obsessive–compulsive disorders and drug addiction. Effective treatment of l-dopa-induced dyskinesia using “presynaptic” A_{2A}R antagonists would be a possibility to explore.

The results by Orru et al. (2011) give a mechanistic explanation to the already reported antiparkinsonian activity of KW-6002 and suggest that SCH-442416 could be useful for the treatment of dyskinetic disorders, obsessive-compulsive disorders and in drug addiction. Medicinal chemistry and *in silico* modeling should help in elucidating the molecular properties that determine the particular pharmacological profile of SCH-442416 and

KW-6002, which may be used as lead compounds to obtain, respectively, more effective antidyskinetic and antiparkinsonian compounds.

ACKNOWLEDGMENTS

Work supported with the intramural funds of the National Institute on Drug Abuse.

REFERENCES

- Albizu, L., Cottet, M., Kralikova, M., Stoev, S., Seyer, R., Brabet, I., Roux, T., Bazin, H., Bourrier, E., Lamarque, L., Breton, C., Rives, M. L., Newman, A., Javitch, J., Trinquet, E., Manning, M., Pin, J. P., Mouillac, B., and Durrux, T. (2010). Time-resolved FRET between GPCR ligands reveals oligomers in native tissues. *Nat. Chem. Biol.* 6, 587–594.
- Andersson, M., Usiello, A., Borgkvist, A., Pozzi, L., Dominguez, C., Fienberg, A. A., Svenningsson, P., Fredholm, B. B., Borrelli, E., Greengard, P., and Fisone, G. (2005). Cannabinoid action depends on phosphorylation of dopamine- and cAMP-regulated phosphoprotein of 32 kDa at the protein kinase A site in striatal projection neurons. *J. Neurosci.* 25, 8432–8438.
- Azad, K., Gall, D., Woods, A. S., Ledent, C., Ferré, S., and Schiffmann, S. N. (2009). Dopamine D2 and adenosine A_{2A} receptors regulate NMDA-excitation in accumbens neurons through A_{2A}-D2 receptor heteromerization. *Neuropsychopharmacology* 34, 972–986.
- Bofill-Cardona, E., Kudlacek, O., Yang, Q., Ahorn, H., Freissmuth, M., and Nanoff, C. (2000). Binding of calmodulin to the D₂-dopamine receptor reduces receptor signaling by arresting the G protein activation switch. *J. Biol. Chem.* 275, 32672–32680.
- Borrito-Escuela, D. O., Marcellino, D., Narvaez, M., Flajolet, M., Heintz, N., Agnati, L., Ciruela, F., and Fuxe, K. (2010a). A serine point mutation in the adenosine A_{2A}R C-terminal tail reduces receptor heteromerization and allosteric modulation of the dopamine D₂R. *Biochem. Biophys. Res. Commun.* 394, 222–227.
- Borrito-Escuela, D. O., Romero-Fernandez, W., Tarakanov, A. O., Gómez-Soler, M., Corrales, F., Marcellino, D., Narvaez, M., Frankowska, M., Flajolet, M., Heintz, N., Agnati, L. F., Ciruela, F., and Fuxe, K. (2010b). Characterization of the A_{2A}R-D₂R interface: focus on the role of the C-terminal tail and the transmembrane helices. *Biochem. Biophys. Res. Commun.* 402, 801–807.
- Bouvier, M. (2001). Oligomerization of G-protein-coupled transmitter receptors. *Nat. Rev. Neurosci.* 2, 274–286.
- Brooks, D. J., Papapetropoulos, S., Vandenhenne, F., Tomic, D., He, P., Coppel, A., and O'Neill, G. (2010). An open-label, positron emission tomography study to assess adenosine A_{2A} brain receptor occupancy of vipadenant (BIIB014) at steady-state levels in healthy male volunteers. *Clin. Neuropharmacol.* 33, 55–60.
- Cabello, N., Gandia, J., Bertarelli, D. C., Watanabe, M., Lluís, C., Franco, R., Ferré, S., Luján, R., and Ciruela, F. (2009). Metabotropic glutamate type 5, dopamine D₂ and adenosine A_{2A} receptors form higher-order oligomers in living cells. *J. Neurochem.* 109, 1497–1507.
- Carriba, P., Navarro, G., Ciruela, F., Ferré, S., Casado, V., Agnati, L. F., Cortes, A., Mallol, J., Fuxe, K., Canela, E. I., Lluís, C., and Franco, R. (2008). Detection of heteromerization of more than two proteins by sequential BRET-FRET. *Nat. Methods* 5, 727–733.
- Carriba, P., Ortiz, O., Patkar, K., Justinova, Z., Stroik, J., Themann, A., Müller, C., Woods, A. S., Hope, B. T., Ciruela, F., Casado, V., Canela, E. I., Lluís, C., Goldberg, S. R., Moratalla, R., Franco, R., and Ferré, S. (2007). Striatal adenosine A_{2A} and cannabinoid CB₁ receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. *Neuropsychopharmacology* 32, 2249–2259.
- Chen, J. F., Moratalla, R., Impagnatiello, F., Grandy, D. K., Cuellar, B., Rubinstein, M., Beilstein, M. A., Hackett, E., Fink, J. S., Low, M. J., Ongini, E., and Schwarzschild, M. A. (2001). The role of the D(2) dopamine receptor (D(2)R) in A(2A) adenosine receptor (A(2A)R)-mediated behavioral and cellular responses as revealed by A(2A) and D(2) receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1970–1975.
- Ciruela, F., Casado, V., Rodrigues, R. J., Luján, R., Burgueno, J., Canals, M., Borycz, J., Rebola, N., Goldberg, S. R., Mallol, J., Cortés, A., Canela, E. I., López-Giménez, J. F., Milligan, G., Lluís, C., Cunha, R. A., Ferré, S., and Franco, R. (2006). Presynaptic control of striatal glutamatergic neurotransmission by adenosine A₁-A_{2A} receptor heteromers. *J. Neurosci.* 26, 2080–2087.
- DeLong, M. R., and Wichmann, T. (2007). Circuits and circuit disorders of the basal ganglia. *Arch. Neurol.* 64, 20–24.
- Ferré, S., Baler, R., Bouvier, M., Caron, M. G., Devi, L. A., Durrux, T., Fuxe, K., George, S. R., Javitch, J. A., Lohse, M. J., Mackie, K., Milligan, G., Pflieger, K. D. G., Pin, J.-P., Volkow, N., Waldhoer, M., Woods, A. S., and Franco, R. (2009). Building a new conceptual framework for receptor heteromers. *Nat. Chem. Biol.* 5, 131–134.
- Ferré, S., Ciruela, F., Woods, A. S., Lluís, C., and Franco, R. (2007). Functional relevance of neurotransmitter receptor heteromers in the central nervous system. *Trends Neurosci.* 30, 440–446.
- Ferré, S., Karcz-Kubicha, M., Hope, B. T., Popoli, P., Burgueno, J., Casado, V., Fuxe, K., Lluís, C., Goldberg, S. R., Franco, R., and Ciruela, F. (2002). Synergistic interaction between adenosine A_{2A} and glutamate mGlu₅ receptors: implications for striatal neuronal function. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11940–11945.
- Ferré, S., Lluís, C., Justinova, Z., Quiroz, C., Orru, M., Navarro, G., Canela, E. I., Franco, R., and Goldberg, S. R. (2010). Adenosine-cannabinoid receptor interactions. Implications for striatal function. *Br. J. Pharmacol.* 160, 443–453.
- Ferré, S., Quiroz, C., Woods, A. S., Cunha, R., Popoli, P., Ciruela, F., Lluís, C., Franco, R., Azad, K., and Schiffmann, S. N. (2008). An update on adenosine A_{2A}-dopamine D₂ receptor interactions. Implications for the function of G protein-coupled receptors. *Curr. Pharm. Des.* 14, 1468–1474.
- Ferré, S., von Euler, G., Johansson, B., Fredholm, B. B., and Fuxe, K. (1991). Stimulation of high affinity adenosine A-2 receptors decreases the affinity of dopamine D-2 receptors in rat striatal membranes. *Proc. Natl. Acad. Sci. U.S.A.* 88, 7238–7241.
- Håkansson, K., Galdi, S., Hendrick, J., Snyder, G., Greengard, P., and Fisone, G. (2006). Regulation of phosphorylation of the GluR1 AMPA receptor by dopamine D₂ receptors. *J. Neurochem.* 96, 482–488.
- Hettinger, B. D., Lee, A., Linden, J., and Rosin, D. L. (1998). Ultrastructural localization of adenosine A_{2A} receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. *J. Comp. Neurol.* 431, 331–346.
- Higley, M. J., and Sabatini, B. L. (2010). Competitive regulation of synaptic Ca²⁺ influx by D₂ dopamine and A_{2A} adenosine receptors. *Nat. Neurosci.* 13, 958–966.
- Hillion, J., Canals, M., Torvinen, M., Casado, V., Scott, R., Terasmaa, A., Hansson, A., Watson, S., Olah, M. E., Mallol, J., Canela, E. I., Zoli, M., Agnati, L. F., Ibanez, C. F., Lluís, C., Franco, R., Ferré, S., and Fuxe, K. (2002). Coaggregation, cointernalization and codesensitization of adenosine A_{2A} receptors and dopamine D₂ receptors. *J. Biol. Chem.* 277, 18091–18097.
- James, S., and Richardson, P. J. (1993). The subcellular distribution of [3H]-CGS 21680 binding sites in the rat striatum: copurification with cholinergic nerve terminals. *Neurochem. Int.* 23, 115–122.
- Jin, S., Johansson, B., and Fredholm, B. B. (1993). Effects of adenosine A₁ and A₂ receptor activation on electrically evoked dopamine and acetylcholine release from rat striatal slices. *J. Pharmacol. Exp. Ther.* 267, 801–808.
- Justinova, Z., Ferré, S., Redhi, G. H., Mascia, P., Stroik, J., Quarta, D., Yasar, S., Muller, C. E., Franco, R., and Goldberg, S. R. (2011). Reinforcing and neurochemical effects of cannabinoid CB₁ receptor agonists, but not cocaine, are altered by an adenosine A_{2A} receptor antagonist. *Addict. Biol.* 16, 405–415.

- Kachroo, A., Orlando, L. R., Grandy, D. K., Chen, J. F., Young, A. B., and Schwarzschild, M. A. (2005). Interactions between metabotropic glutamate 5 and adenosine A_{2A} receptors in normal and parkinsonian mice. *J. Neurosci.* 25, 10414–10419.
- Kreitzer, A. C., and Malenka, R. C. (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature* 445, 643–647.
- Kull, B., Ferré, S., Arslan, G., Svenningsson, P., Fuxe, K., Owman, C., and Fredholm, B. B. (1999). Reciprocal interactions between adenosine A_{2A} and dopamine D₂ receptors in CHO cells co-transfected with the two receptors. *Biochem. Pharmacol.* 58, 1035–1045.
- Lerner, T. N., Horne, E. A., Stella, N., and Kreitzer, A. C. (2010). Endocannabinoid signaling mediates psychomotor activation by adenosine A_{2A} antagonists. *J. Neurosci.* 30, 2160–2164.
- Martire, A., Tebano, M. T., Chiodi, V., Ferreira, S. G., Cunha, R. A., Köfalvi, A., and Popoli, P. (2011). Presynaptic adenosine A_{2A} receptors control cannabinoid CB₁ receptor-mediated inhibition of striatal glutamatergic neurotransmission. *J. Neurochem.* 116, 273–280.
- Moresco, R. M., Todde, S., Belloli, S., Simonelli, P., Panzacchi, A., Rigamonti, M., Galli-Kienle, M., and Fazio, F. (2005). In vivo imaging of adenosine A_{2A} receptors in rat and primate brain using [¹¹C]SCH442416. *Eur. J. Nucl. Med. Mol. Imaging* 32, 405–413.
- Navarro, G., Aymerich, M. S., Marcellino, D., Cortes, A., Casado, V., Mallol, J., Canela, E. I., Agnati, L. F., Woods, A. S., Fuxe, K., Lluis, C., Lanciego, J. L., Ferré, S., and Franco, R. (2009). Interactions between calmodulin, adenosine A_{2A} and dopamine D₂ receptors. *J. Biol. Chem.* 284, 28058–28068.
- Navarro, G., Ferré, S., Cordomi, A., Moreno, E., Mallol, J., Casado, V., Cortés, A., Hoffmann, H., Ortiz, J., Canela, E. I., Lluis, C., Pardo, L., Franco, R., and Woods, A. S. (2010). Interactions between intracellular domains as key determinants of the quaternary structure and function of receptor heteromers. *J. Biol. Chem.* 285, 27346–27359.
- Nishi, A., Liu, F., Matsuyama, S., Hamada, M., Higashi, H., Nairn, A. C., and Greengard, P. (2003). Metabotropic mGlu5 receptors regulate adenosine A_{2A} receptor signaling. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1322–1327.
- Obeso, J. A., Rodríguez-Oroz, M. C., Rodríguez, M., Arbizu, J., and Giménez-Amaya, J. M. (2002). The basal ganglia and disorders of movement: pathophysiological mechanisms. *News Physiol. Sci.* 17, 51–55.
- Orru, M., Bakešová, J., Brugarolas, M., Quiroz, C., Beaumont, V., Goldberg, S. R., Lluis, C., Cortés, A., Franco, R., Casado, V., Canela, E. I., and Ferré, S. (2011). Striatal pre- and postsynaptic profile of adenosine A(2A) receptor antagonists. *PLoS ONE* 6, e16088. doi: 10.1371/journal.pone.0016088
- Popoli, P., Betto, P., Reggio, R., and Ricciarello, G. (1995). Adenosine A_{2A} receptor stimulation enhances striatal extracellular glutamate levels in rats. *Eur. J. Pharmacol.* 287, 215–217.
- Popoli, P., Pezzola, A., Torvinen, M., Reggio, R., Pintor, A., Scarchili, L., Fuxe, K., and Ferré, S. (2001). The selective mGlu5 receptor agonist CHPG inhibits quinpirole-induced turning in 6-hydroxydopamine-lesioned rats and modulates the binding characteristics of dopamine D₂ receptors in the rat striatum: interactions with adenosine A_{2A} receptors. *Neuropsychopharmacology* 25, 505–513.
- Preston, Z., Lee, K., Widdowson, L., Freeman, T. C., Dixon, A. K., and Richardson, P. J. (2000). Adenosine receptor expression and function in rat striatal cholinergic interneurons. *Br. J. Pharmacol.* 130, 886–890.
- Quiroz, C., Lujan, R., Uchigashima, M., Simoes, A. P., Lerner, T. N., Borycz, J., Kachroo, A., Canas, P. M., Orru, M., Schwarzschild, M. A., Rosin, D. L., Kreitzer, A. C., Cunha, R. A., Watanabe, M., and Ferré, S. (2009). Key modulatory role of presynaptic adenosine A_{2A} receptors in cortical neurotransmission to the striatal direct pathway. *ScientificWorldJournal* 9, 1321–1344.
- Rodrigues, R. J., Alfaro, T. M., Rebola, N., Oliveira, C. R., and Cunha R. A. (2005). Co-localization and functional interaction between adenosine A(2A) and metabotropic group 5 receptors in glutamatergic nerve terminals of the rat striatum. *J. Neurochem.* 92, 433–441.
- Rosin, D. L., Robeva, A., Woodard, R. L., Guyenet, P. G., and Linden, J. (1998). Immunohistochemical localization of adenosine A_{2A} receptors in the rat central nervous system. *J. Comp. Neurol.* 401, 163–186.
- Schiffmann, S. N., Fisone, G., Moresco, R., Cunha, R., and Ferré, S. (2007). Adenosine A_{2A} receptors and basal ganglia physiology. *Prog. Neurobiol.* 83, 277–292.
- Shen, H. Y., Coelho, J. E., Ohtsuka, N., Canas, P. M., Day, Y. J., Huang, Q. Y., Rebola, N., Yu, L., Boison, D., Cunha, R. A., Linden, J., Tsien, J. Z., and Chen, J. F. (2008). A critical role of the adenosine A_{2A} receptor in extrastriatal neurons in modulating psychomotor activity as revealed by opposite phenotypes of striatum and forebrain A_{2A} receptor knock-outs. *J. Neurosci.* 28, 2970–2975.
- Soria, G., Castañé, A., Ledent, C., Parmentier, M., Maldonado, R., and Valverde, O. (2004). Adenosine A_{2A} receptors are involved in physical dependence and place conditioning induced by THC. *Eur. J. Neurosci.* 20, 2203–2213.
- Tanganelli, S., Sandager-Nielsen, K., Ferraro, L., Antonelli, T., Kehr, J., Franco, R., Ferré, S., Agnati, L. F., Fuxe, K., and Scheel-Krüger, J. (2004). Striatal plasticity at the network level. Focus on adenosine A_{2A} and D₂ interactions in models of Parkinson's disease. *Parkinsonism Relat. Disord.* 10, 273–280.
- Tebano, M. T., Martire, A., Chiodi, V., Pepponi, R., Ferrante, A., Domenici, M. R., Frank, C., Chen, J. F., Ledent, C., and Popoli, P. (2009). Adenosine A_{2A} receptors enable the synaptic effects of cannabinoid CB₁ receptors in the rodent striatum. *J. Neurochem.* 110, 1921–1930.
- Tozzi, A., de Iure, A., Di Filippo, M., Tantucci, M., Costa, C., Borsini, F., Ghiglieri, V., Giampà, C., Fusco, F. R., Picconi, B., and Calabresi, P. (2011). The distinct role of medium spiny neurons and cholinergic interneurons in the D₂/A_{2A} receptor interaction in the striatum: implications for Parkinson's disease. *J. Neurosci.* 31, 1850–1862.
- Woods, A. S., and Ferré, S. (2005). The amazing stability of the arginine-phosphate electrostatic interaction. *J. Proteome Res.* 4, 1397–1402.
- Yao, L., Fan, P., Jiang, Z., Mailliard, W. S., Gordon, A. S., and Diamond, I. (2003). Addicting drugs utilize a synergistic molecular mechanism in common requiring adenosine and Gi-beta gamma dimers. *Proc. Natl. Acad. Sci. U.S.A.* 100, 14379–14384.
- Zoli, M., Agnati, L. F., Hedlund, P. B., Li, X.-M., Ferré, S., and Fuxe, K. (1993). Receptor-receptor interactions as an integrative mechanism in nerve cells. *Mol. Neurobiol.* 7, 293–334.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 02 May 2011; paper pending published: 17 May 2011; accepted: 08 June 2011; published online: 17 June 2011.

Citation: Ferré S, Quiroz C, Orru M, Guitart X, Navarro G, Cortés A, Casado V, Canela EI, Lluis C and Franco R (2011) Adenosine A_{2A} receptors and A_{2A} receptor heteromers as key players in striatal function. *Front. Neuroanat.* 5:36. doi: 10.3389/fnana.2011.00036

Copyright © 2011 Ferré, Quiroz, Orru, Guitart, Navarro, Cortés, Casado, Canela, Lluis and Franco. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Control of striatal signaling by G protein regulators

Keqiang Xie and Kirill A. Martemyanov*

The Scripps Research Institute, Jupiter, FL, USA

Edited by:

Emmanuel Valjent, Université
Montpellier 1 & 2, France

Reviewed by:

Jacqueline McGinty, University of
South Carolina, USA
Venetia Zachariou, University of Crete,
Greece

***Correspondence:**

Kirill A. Martemyanov, Department of
Neuroscience, The Scripps Research
Institute, 130 Scripps Way, #3C2,
Jupiter, FL 33458, USA.
e-mail: kirill@scripps.edu

Signaling via heterotrimeric G proteins plays a crucial role in modulating the responses of striatal neurons that ultimately shape core behaviors mediated by the basal ganglia circuitry, such as reward valuation, habit formation, and movement coordination. Activation of G protein-coupled receptors (GPCRs) by extracellular signals activates heterotrimeric G proteins by promoting the binding of GTP to their α subunits. G proteins exert their effects by influencing the activity of key effector proteins in this region, including ion channels, second messenger enzymes, and protein kinases. Striatal neurons express a staggering number of GPCRs whose activation results in the engagement of downstream signaling pathways and cellular responses with unique profiles but common molecular mechanisms. Studies over the last decade have revealed that the extent and duration of GPCR signaling are controlled by a conserved protein family named regulator of G protein signaling (RGS). RGS proteins accelerate GTP hydrolysis by the α subunits of G proteins, thus promoting deactivation of GPCR signaling. In this review, we discuss the progress made in understanding the roles of RGS proteins in controlling striatal G protein signaling and providing integration and selectivity of signal transmission. We review evidence on the formation of a macromolecular complex between RGS proteins and other components of striatal signaling pathways, their molecular regulatory mechanisms and impacts on GPCR signaling in the striatum obtained from biochemical studies and experiments involving genetic mouse models. Special emphasis is placed on RGS9-2, a member of the RGS family that is highly enriched in the striatum and plays critical roles in drug addiction and motor control.

Keywords: G proteins, GPCR, RGS proteins, striatum

STRIATAL G PROTEIN SIGNALING: AN OVERVIEW

Striatum is a large part of the subcortical basal ganglia system consisting of caudate and putamen nuclei in the dorsal side that receives dopaminergic input from substantia nigra and nucleus accumbens on the ventral side that receives dopaminergic input from the ventral tegmental area. In addition, both ventral and dorsal striatum receive major glutamatergic input from cortical areas as well as inputs from other nuclei of basal ganglia such as subthalamic nucleus and the external globus pallidus (Graybiel, 2000).

Striatal neurons play critical roles in initiating and maintaining movement, mood control, reward behavior, and drug addiction (Graybiel, 2000; Kreitzer and Malenka, 2008). Dysfunctions in the neurocircuitry of the striatum have been implicated in the development of a wide range of disorders, including Parkinson's disease (Picconi et al., 2005), Huntington's disease (Cepeda et al., 2007), depression (Nestler and Carlezon, 2006), and drug addiction (Everitt and Robbins, 2005). The striatum contains a relatively heterogeneous neuronal population. Medium spiny neurons (MSNs) are the most abundant type, accounting for 90–95% of neurons in this region (Graveland and DiFiglia, 1985). Despite their morphological similarities, MSNs are categorized into two different subtypes based on differences in their gene expression and axonal projections (Gerfen et al., 1990; Surmeier et al., 2007; Heiman et al., 2008). The first group, striatonigral MSNs, selectively expresses dynorphin and dopamine D1 receptors and sends

projections directly to basal ganglia output nuclei. The second group, striatopallidal MSNs, selectively expresses enkephalin and dopamine D2 receptors and sends axons to the external globus pallidus. In addition to MSNs, the striatum contains small populations of giant cholinergic interneurons, easily distinguished by their large cell bodies and tonic activities (Zhou et al., 2002), as well as parvalbumin and calretinin interneurons (Cicchetti et al., 2000).

One of the striking features of the striatum is the wide variety of neurotransmitter inputs that converge onto striatal neurons. The first major excitatory input is provided by glutamatergic afferents, most notably from the cerebral and prefrontal cortexes but also from several other brain regions, such as the thalamus, amygdala, and hippocampus (McGeorge and Faull, 1989; Bolam et al., 2000). The second major input to the striatum is provided by neurons from the substantia nigra and ventral tegmental area that release dopamine (Smith and Kiehl, 2000). The glutamate and dopamine systems intimately overlap, and their interaction is thought to be essential for striatal signaling (for reviews see Graybiel, 1990; Chase and Oh, 2000; David et al., 2005). In addition, the striatum receives several other neurotransmitter inputs that play important modulatory roles. Despite their scarcity, striatal cholinergic interneurons densely innervate both the ventral and dorsal striatum; they form an extensive local axon collateral system, making contacts with MSNs and other interneurons (Koos and Tepper, 2002; Zhou et al., 2002). Changes in the amount

of acetylcholine (ACh) released by these neurons are crucial for determining the activity of the striatal output neurons (Calabresi et al., 2000). Striatal neurons also receive substantial projections from noradrenergic (Nestler et al., 1999; Sara, 2009) and serotonergic neurons (Azmitia and Segal, 1978; Moukhles et al., 1997) located in the locus coeruleus and the dorsal raphe nuclei, respectively. Several locally synthesized and released neuromodulators also play important roles in striatal signaling. These neuromodulators include endogenous opioids, such as endorphins, enkephalins, dynorphins, and orphanin FG, which are found in abundance in the nucleus accumbens and the surrounding ventral striatum and are known to be involved in motivational aspects of behavior (Van Ree et al., 2000; Kelley et al., 2002), as well as endocannabinoids, which act in a retrograde manner to inhibit neurotransmitter release (Bisogno et al., 2005; Basavarajappa, 2007). Adenosine, a ubiquitous homeostatic substance released from most cells, including neurons and glia (Cunha, 2001; Dunwiddie and Masino, 2001), has important neuromodulatory effects on dopamine and glutamate transmission in the striatum. Presynaptically, adenosine inhibits or facilitates transmitter release while causing hyperpolarization or depolarization in postsynaptic neurons (Dunwiddie and Masino, 2001; Ribeiro et al., 2002). Finally, MSNs signal by releasing the inhibitory neurotransmitter GABA (Bolam et al., 2000; Graybiel, 2000). While their projections target a range of effector nuclei in the mesolimbic system, they also form extensive local contacts with neighboring neurons, making GABA important for regulating the spiking timing of spiny outputs (Tepper et al., 2004).

Thus, multiple neurotransmitter systems act simultaneously in the striatum both pre and postsynaptically to shape neuronal activity. This creates an environment where individual neurons integrate multiple inputs that often act cooperatively to affect excitability and the long-term adaptive effects that underlie their physiological function. This organization places a significant emphasis on the principles and mechanisms of intracellular signaling pathways that interpret, coordinate, and integrate signals carried by multiple neurotransmitters.

One of the central signaling systems that mediate the effects of neurotransmitters is the seven-transmembrane G protein-coupled receptors (GPCRs). In the canonical pathway, upon binding to neurotransmitter molecules, GPCRs undergo conformational changes that activate their ability to catalyze the exchange of GDP with GTP (Gilman, 1987; Pierce et al., 2002). From this perspective, GPCRs act as GDP/GTP guanine nucleotide exchange factors (GEFs) for G proteins. G proteins comprise three subunits: α , β and γ . GTP binding to the α subunit causes it to dissociate from the $\beta\gamma$ complex. Both the active (GTP-bound) form of $G\alpha$ and free $G\beta\gamma$ interact with a range of downstream signaling molecules ("effectors") and modulate their activity to generate cellular responses. Recent studies have also revealed an additional G protein-independent signaling mechanism of GPCRs: neurotransmitter binding induces phosphorylation of GPCRs, followed by the binding of an adaptor protein, β -arrestin, that recruits a host of signaling factors to transmit the signal (Lefkowitz and Shenoy, 2005; Lefkowitz et al., 2006; Premont and Gainetdinov, 2007). While the importance of this non-canonical GPCR signaling pathway for striatal signaling is emerging, the scope of

our review is limited to the traditional G protein-based signaling mechanisms.

Mammalian genomes contain more than 800 genes that encode GPCRs (Lander et al., 2001; Venter et al., 2001), and many different types of GPCRs are expressed in striatal neurons (see Versatility of the GPCR Repertoire in the Striatum). Despite such diversity, all GPCRs transduce signals via a limited set of G proteins. There are four subfamilies of $G\alpha$ subunits ($G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q}$, and $G_{\alpha 12}$) that have selective preferences for the activation of different GPCR classes (Oldham and Hamm, 2008). However, many GPCRs can couple to more than one $G\alpha$ protein. $G\alpha$ subunits also show selectivity in their regulation of downstream effectors (Gilman, 1987). Canonically, $G_{\alpha s}$ and $G_{\alpha i}$ proteins regulate adenylyl cyclase, $G_{\alpha q}$ family proteins activate phospholipase $C\beta$ (PLC β), and $G_{\alpha 12}$ protein control members of the RhoGEF superfamily (Pierce et al., 2002; Oldham and Hamm, 2008). Four signal-transducing beta subunits and 13 gamma subunits form constitutive complexes with poorly understood selectivity and, when released from the heterotrimer, can also regulate a range of effectors, most notably phospholipases (PLC $\beta 2$), and ion channels (Dupre et al., 2009). Signaling is terminated when the neurotransmitter dissociates from the receptor; the receptor's GEF activity is quenched, and the GTP on the $G\alpha$ subunit is hydrolyzed to allow reformation of the inactive $G\alpha\beta\gamma$ heterotrimer and dissociates from the effectors.

G protein-coupled receptor signaling pathways are organized in a way that allows tremendous integration of signaling events at the G protein level. The balance between reactions that lead to activation or deactivation plays a central role in dictating the extent of the effectors' activity regulation and is ultimately responsible for shaping neuronal responses. Intracellular factors that regulate G protein activity status are, thus, expected to play essential roles in controlling the responses of striatal neurons to neurotransmitters.

VERSATILITY OF THE GPCR REPERTOIRE IN THE STRIATUM

Consistent with multiple neurotransmitter inputs, striatal neurons utilize many GPCRs, which are abundantly expressed and play essential roles in striatal physiology. It is extremely difficult to provide a comprehensive overview about all of the GPCR systems involved in the physiology of the striatal neurons. Several orphan GPCRs for as yet unknown ligands are also present in the region (Mizushima et al., 2000; Marazziti et al., 2007; Logue et al., 2009). Our account below provides an overview of the GPCR systems that significantly contribute to G protein activation and have firmly established physiological roles (Table 1).

Dopamine receptors are perhaps the most studied GPCRs in the striatum and have firmly established physiological roles (Jaber et al., 1996; Missale et al., 1998; Glickstein and Schmauss, 2001; El-Ghundi et al., 2007). Although all five dopamine receptors are present in the striatum, the roles of D1R and D2R receptors have received the greatest attention. Both D1R and D2R are abundantly expressed in striatal neurons; however, they show remarkable segregation among cell types. D1R is expressed in the striatonigral MSNs, constituting the direct pathway, whereas D2R is expressed in the striatopallidal or indirect pathway (Gerfen et al., 1990; Graybiel, 2000; Shuen et al., 2008; Matamales et al., 2009). D2Rs are also

Table 1 | Major GPCRs and their downstream effectors in the striatum.

	Receptor type	G protein coupling	Signaling effector(s)	Reference
<i>Dopamine</i>	D1, D5	G α s(olf)	cAMP/PKA, L/P/Q-type Ca ²⁺ channels	Surmeier et al. (1995), Lee et al. (2002), Iwamoto et al. (2003), Kim et al. (2006)
	D2, D3, D4	G α i/o	cAMP/PKA, L-type Ca ²⁺ channel	Hernandez-Lopez et al. (2000), Lee et al. (2002), Iwamoto et al. (2003)
	D1/D2 dimer	G α q	PLC β /PKC	Lee et al. (2004), Hasbi et al. (2009)
<i>Opioid</i>	μ , δ , κ	G α i/o	cAMP/PKA, N-type Ca ²⁺ channel	Dhawan et al. (1996), Law et al. (2000), Spadoni et al. (2004)
<i>Muscarinic</i>	M1, M3, M5	G α q	PLC β /PKC	Caulfield and Birdsall (1998), Salah-Uddin et al. (2008)
	M2, M4	G α i/o	cAMP/PKA, K ⁺ channel	Akins et al. (1990), Gabel and Nisenbaum (1999), Wess (2004), Ishii and Kurachi (2006), Sanchez et al. (2009)
<i>Metabotropic glutamate</i>	mGluR 1, 5	G α q	PLC β /PKC	Conn and Pin (1997), Ferre et al. (2002), Jong et al. (2009)
<i>Adenosine</i>	mGluR 2, 3, 4, 7, 8	G α i/o	cAMP/PKA	Prezeau et al. (1994), Conn and Pin (1997)
	A1, A3	G α i/o	cAMP/PKA, N/P/Q-type Ca ²⁺ channels	Ambrosio et al. (1996), Song et al. (2000), Fredholm et al. (2001)
<i>Cannabinoid</i>	A2	G α s(olf)	cAMP/PKA	Lee et al. (2002)
	CB1, CB2	G α i/o	cAMP/PKA, voltage-gated Ca ²⁺ channel, K ⁺ channel	Pertwee (1997), Felder and Glass (1998), Piomelli (2003)
<i>Serotonin</i>	5-HT1, 5	G α i/o	cAMP/PKA, K ⁺ channel	Barnes and Sharp (1999), Di Matteo et al. (2008), Nichols and Nichols (2008)
	5-HT2	G α q	PLC β /PKC, K ⁺ channel	Barnes and Sharp (1999), Di Matteo et al. (2008), Nichols and Nichols (2008)
	5-HT4, 6, 7	G α s(olf)	cAMP/PKA	Barnes and Sharp (1999), Di Matteo et al. (2008), Nichols and Nichols (2008)

located presynaptically on dopaminergic terminals and participate in the autoregulation of dopamine release (Jaber et al., 1996). A small population of MSNs (~5%) co-expresses both D1R and D2R (Falk et al., 2006; Shuen et al., 2008), which have been shown to form heterodimers (Lee et al., 2004). Remarkably, D1R–D2R dimers can activate G α q, creating an additional signaling modality (Lee et al., 2004; Hasbi et al., 2009).

Our knowledge regarding the involvement of D3R, D4R, and D5R is much more limited, in part due to their relatively low abundance. D5R is highly expressed in cholinergic neurons in the striatum and is involved in the induction of long-term potentiation (LTP; Suzuki et al., 2001). Although present at low levels in the striatum, the D3R receptor has approximately 200-fold higher affinity for dopamine than does D2R and is thought to be primarily involved in regulating dopamine release at lower dopamine concentrations (Joseph et al., 2002) by acting as an autoreceptor. While little information about D4R is available, it is known to play an important role in the regulation of striatal function because genetic ablation impairs locomotor sensitization to cocaine and amphetamine (Rubinstein et al., 1997; Kruzich et al., 2004; Thanos et al., 2010).

Opioid receptors account for the actions of both endogenous opioid peptides and exogenous opiates and are thought to be one of the central molecular substrates that modulate reward signaling in the striatum. Opioid receptors are involved in the modulation of dopaminergic transmission in the striatum. Blockage of opioid

receptors, especially μ and δ , attenuates psychostimulant-induced behavior sensitization (Heidbreder et al., 1993; Schad et al., 1996; Balcells-Olivero and Vezina, 1997; Diaz-Otanez et al., 1997). μ opioid receptors are specifically enriched in striosomes and have been shown to inhibit corticostriatal EPSCs (Jiang and North, 1992), and IPSCs (Miura et al., 2007), indicating that they play a critical role in modulation of corticostriatal excitatory and inhibitory synaptic transmission. μ opioid receptors have also been recently found to be expressed in a subset of cholinergic neurons in the dorsal striatum, and activation of μ opioid receptors inhibits ACh release (Jabourian et al., 2005; Perez et al., 2007). κ and δ opioid receptors in striatum were also shown to modulate dopamine (Spanagel et al., 1992) and glutamate (Rawls and McGinty, 2000) release and subsequently regulate stimulant-induced behavior (Gray et al., 1999; Gonzalez-Nicolini et al., 2003).

Muscarinic receptors (mAChR) are expressed in the striatum in a complex, overlapping manner where they mediate the slow-acting response to ACh (Weiner et al., 1990; Levey et al., 1991; Bernard et al., 1992; Hersch et al., 1994). The M1, M2, and M4 receptors are the predominant muscarinic receptors in the striatum (Levey et al., 1991; Abrams et al., 2006). When activated, muscarinic receptors modulate the excitability of striatal MSNs via the enhancement of NMDA receptor-mediated currents (Calabresi et al., 1998a) or the inhibition of voltage-activated N-, P-, and L-type Ca²⁺ currents (Howe and Surmeier, 1995). Muscarinic receptors also modulate the functions of striatal cholinergic interneurons; activation of M2

and M4 receptors has been shown to inhibit both N- and P-type Ca^{2+} currents (Yan and Surmeier, 1996). The inhibition of Ca^{2+} channels located at presynaptic sites ultimately leads to the inhibition of ACh or glutamate release, which plays a crucial role in coordinated changes in striatal neuronal activity (Calabresi et al., 1998b; Rawls and McGinty, 1998). In addition, most mAChRs are expressed in the terminals of dopaminergic neurons, where they bidirectionally regulate dopamine release (Xu et al., 1989; De Klippel et al., 1993; Smolders et al., 1997; Zhang et al., 2002).

Metabotropic glutamate receptors (mGluRs) modulate relatively slow glutamate transmission through second messenger systems (Nakanishi et al., 1998). mGluR1/5 are abundantly expressed postsynaptically in the medium spiny projection neurons of the striatum (Testa et al., 1995; Paquet and Smith, 2003). The activation of group I mGluRs in MSNs induces synaptic plasticity by modulating NMDA receptor activity (Pisani et al., 1997, 2001; Gubellini et al., 2001, 2003; Swanson et al., 2001). Group II and group III mGluRs are mostly located at presynaptic glutamatergic terminals (Bradley et al., 1999; Kosinski et al., 1999; Tamaru et al., 2001; Corti et al., 2002) and serve as autoreceptor decreasing glutamate release (Cartmell and Schoepp, 2000; Schoepp, 2001).

In addition to these major GPCR players, many other receptors expressed in this region are recognized to impact striatal signaling. Two *cannabinoid receptors* (CB1 and CB2) have been characterized to date. In the striatum, presynaptically expressed CB1 receptors control both glutamate and GABA release in a retrograde manner (Huang et al., 2001; Fernandez-Ruiz et al., 2002; Basavarajappa, 2007). Several G protein coupled serotonin (5-HT) receptors are also expressed in the striatum and mediate diverse physiological functions including modulation of stress response, depression, and anxiety (Di Matteo et al., 2008; Nichols and Nichols, 2008). Adenosine A2a receptors are highly enriched in the striatal neurons, especially in the GABAergic striatopallidal neurons, where adenosine A2a and dopamine D2 are colocalized (Fink et al., 1992; Augood and Emson, 1994). The physiological function of the adenosine A2a receptor is characterized by its antagonistic effect on the dopamine D2 receptor, indicating a critical fine-tuning effect on indirect signaling pathway. There are several excellent reviews of the basal ganglia functions of the adenosine A2a receptor (Fuxe et al., 2007; Schiffmann et al., 2007).

In summary, the presence of multiple neurotransmitter receptors in striatal neurons creates an environment where many GPCRs signal to several effector molecules that uniquely control various aspects of striatal function. At the same time, a tremendous integration of the signaling occurs downstream from the GPCRs as many receptors share limited number of the effector molecules ultimately responsible for generating responses in the striatum.

REGULATOR OF G PROTEIN SIGNALING PROTEINS: KEY FOCAL POINTS OF THE REGULATORY INFLUENCE

The convergent organization of the G protein pathways suggests that the factors that exert regulatory influence downstream from the GPCR and upstream of the effector molecules provide universal control over the extent and duration of signaling and are key in shaping cellular responses. Recent research has uncovered a number of proteins capable of altering the kinetics and dynamics of G protein signal transduction (Ross and Wilkie, 2000; Siderovski

and Willard, 2005). Among these molecules, there has been substantial interest in proteins that facilitate termination of signaling, providing a major opposing force to GPCR-driven activation.

Once activated, G proteins transmit the signal until GTP hydrolysis occurs. G protein α subunits are inefficient GTPases, and their intrinsic inactivation takes up to several minutes. Nevertheless, signaling termination in many well-studied G protein pathways have been known to occur at substantially more rapid timescales (Zerangue and Jan, 1998). While some effectors can stimulate GTPase activity (Biddlecome et al., 1996; Paulssen et al., 1996; Hart et al., 1998; Kozasa et al., 1998) and serve as GTPase activating proteins (GAPs), these examples cannot account for the entire range of signaling situations. Over the last decade, it has become clear that the GAP function of heterotrimeric G proteins is driven by a conserved family of proteins called regulators of G protein signaling (RGS). RGS proteins associate with the GTP-bound form of the α subunits and stabilize the transition state, dramatically accelerating GTP hydrolysis (Ross and Wilkie, 2000). There are more than 30 members in the mammalian RGS family. They all share a conserved “catalytic” RGS domain (~130 amino acid residues) and are categorized into six subfamilies (R4, RZ, R7, R12, RA, and RL) based on RGS domain homology (Ross and Wilkie, 2000; Hollinger and Hepler, 2002). It should be noted that not all RGS domains are capable of accelerating GTP hydrolysis on G proteins. Moreover, for many RGS proteins, the relative efficiency of their GAP function varies, depending on the identity of the α subunit substrate.

In addition to the RGS domain, many RGS proteins possess other, “non-catalytic” domains. Several functions of these non-catalytic domains have been uncovered. First, they serve as regulatory elements for other signaling pathways (e.g., small GTPases), endowing RGS proteins with multifunctionality in the regulation of cellular signaling (Hart et al., 1998; De Vries and Gist Farquhar, 1999; Siderovski et al., 1999; Schiff et al., 2000; Willard et al., 2007; Shu et al., 2010). Non-catalytic domains can also modify the functions of the constituent catalytic domains of RGS proteins by recruiting additional cellular factors and regulating their GAP activity (Chen and Lin, 1998; Martemyanov et al., 2003a), G protein selectivity (He et al., 2000; Martemyanov and Arshavsky, 2002), and localization (Srinivasa et al., 1998; De Vries et al., 2000b; Martemyanov et al., 2003b).

During the last decade, the physiological functions of RGS proteins in controlling a multitude of cellular reactions have been extensively studied in a number of animal models by eliminating RGS protein function (Chen et al., 2000; Rahman et al., 2003; Martin-McCaffrey et al., 2004; Sun et al., 2005; Cho et al., 2008; Cifelli et al., 2008; Xie et al., 2008, 2010; Posokhova et al., 2010). Phenotypic analysis varies, depending on the expression profile of the targeted RGS protein, and allows elucidation of the contributions of RGS proteins to the regulation of cellular processes and specific G protein pathways. One of the earliest examples is RGS9-1, a short splice isoform of the RGS9 gene exclusively expressed in photoreceptors of the retina (Martemyanov and Arshavsky, 2009). In the absence of RGS9-1, inactivation of the G protein transducin was shown to be severely delayed, causing dramatic delays in the deactivation of cellular responses to light (Chen

et al., 2000). Another example is RGS2, a small, relatively ubiquitous RGS protein that shows high selectivity for G α q subunits (Heximer, 2004). Given the importance of G α q-coupled GPCRs in the regulation of smooth muscle tone, an RGS2 knockout model has been extensively evaluated in vascular systems and linked to the development of hypertension (Heximer et al., 2003).

An alternative strategy to investigate the physiological functions of endogenous RGS proteins is to create strains of mice carrying RGS-insensitive (RGSi) G proteins (Lan et al., 1998; Jeong and Ikeda, 2000; Huang et al., 2006; Talbot et al., 2010). This approach is based on the introduction of a point mutation in the G α region that mediates RGS binding, rendering them insensitive to the GAP actions of RGS proteins without affecting their interactions with GPCRs and effectors (DiBello et al., 1998; Lan et al., 1998). Genomic knock-in of RGSi G184S G α i2 resulted in severe dysfunctions in multiple organ systems, including the heart and myeloid and central nervous systems (Huang et al., 2006; Talbot et al., 2010).

RGS9-2, A KEY STRIATAL RGS PROTEIN WITH EFFECTS ON DOPAMINE AND OPIOID SIGNALING

Perhaps one of the most studied RGS proteins from the perspective of striatal function is the long splice isoform of RGS9 (RGS9-2). It first attracted attention due to its selective enrichment in the striatum (Gold et al., 1997; Thomas et al., 1998; Zhang et al., 1999). This was followed by extensive behavioral studies in mice lacking RGS9 that firmly established RGS9-2 as the key player in many critical aspects of striatal function. Specifically, RGS9 knockout mice display increased sensitivity to the reward and motor stimulatory properties of addictive drugs, including cocaine and amphetamine (Rahman et al., 2003; Zachariou et al., 2003). Elimination of RGS9 exaggerates physical dependence on morphine; mice lacking RGS9 showed heightened antagonist-precipitated withdrawal following chronic morphine administration (Zachariou et al., 2003). Furthermore, knockout of RGS9 delayed the development of analgesic tolerance to morphine (Zachariou et al., 2003), suggesting that RGS9-2 also controls long-term adaptation responses that adjust GPCR responsiveness following persistent stimulation and may contribute to the molecular changes underlying addiction. The changes in adaptive responses to drug administration in this model do not seem to be limited to morphine. RGS9 knockouts also display enhanced sensitization to repeated cocaine injections (Rahman et al., 2003). In contrast, overexpression of RGS9 in the striatum has the opposite effect and blunts responsiveness to cocaine (Rahman et al., 2003).

Consistent with the importance of the striatum in motor control, the role of RGS9 in this process was also examined. RGS9-2 elimination was found to be detrimental to the coordination of gross movements and led to delayed acquisition of motor learning in the rotarod test (Blundell et al., 2008; Anderson et al., 2010). RGS9-2 also appears to play a critical role in controlling the development of dyskinesia, a disorder in which patients involuntarily perform complex movements and which is often triggered by chronic treatment with drugs targeting dopamine signaling pathways (Rascol and Fabre, 2001; Jenner, 2008). RGS9 knockouts develop abnormal involuntary movements in response to chronic treatment with either antipsychotics (Kovoor et al.,

2005) or L-DOPA in 6-OHDA model of Parkinson's disease (Gold et al., 2007) much more rapidly than wild type animals. In contrast, virus-mediated overexpression of RGS9-2 in the striatum of 6-OHDA-treated rats and monkeys reduced the symptoms of L-DOPA-induced dyskinesia (Gold et al., 2007). Because drug-induced dyskinesia is a common debilitating side effect associated with many current anti-Parkinson's and antipsychotic therapies, RGS9-2 is an attractive candidate for the development of pharmaceuticals aimed at ameliorating this condition.

Observations from mouse models are consistent with the involvement of RGS9-2 in regulating motor and reward behaviors. These processes are controlled by dopamine and opioid neurotransmitter systems (Hyman et al., 2006; Arbuthnott and Wickens, 2007; Koob and Le Moal, 2008). Several groups have investigated the role of RGS9-2 in regulating responses mediated by dopamine and opioid receptors at the molecular and cellular levels. Knockdown of RGS9-2 *in vivo* enhances the potency and duration of antinociception mediated by μ -opioid, but not the other opioid receptors (Sanchez-Blazquez et al., 2003). Similarly, RGS9 knockout mice develop prominent involuntary movements in response to D2R- but not D1R-selective agonists (Kovoor et al., 2005). Further supporting the involvement of RGS9-2 in D2R and μ -opioid actions, agonist-induced internalization of these receptors is strongly inhibited by co-expression with RGS9-2 in transfected cells (Psifogeorgou et al., 2007; Celver et al., 2010). This regulation appears to be specific to RGS9-2 as RGS4 does not have any appreciable effects on the internalization of D2R receptors (Celver et al., 2010). This selectivity could be explained by the formation of a macromolecular complex between RGS9-2 and the receptors it regulates. Indeed, RGS9-2 coprecipitates with μ -opioid from striatal lysates (Garzon et al., 2005a; Charlton et al., 2008) and is recruited to the plasma membrane by co-expression with D2R in transfected cells (Kovoor et al., 2005).

These findings imply that RGS9-2 is a negative regulator that acts downstream of the D2R and μ -opioid in striatal neurons. Both of these GPCRs are coupled to the inhibitory G α i/o G proteins. Consistent with this notion, biochemical studies confirm that RGS9-2 is a potent and selective GAP, acting primarily on G α o and, to a lesser extent, G α i (Hooks et al., 2003; Martemyanov et al., 2003a). In this context, it is worth noting that several other G α i/o-coupled GPCRs are expressed in striatal neurons and play critical roles in striatal functions. It is possible that RGS9-2 is also involved in regulating the responses of other G α i/o GPCRs in this region, acting as a general G protein signaling inhibitor. However, the specificity of RGS9-2 action in the striatum is virtually unexplored and will need to be addressed in future studies.

The effectors regulated by RGS9-2 downstream from striatal GPCRs have not been fully identified. Existing evidence points to the involvement of RGS9-2 in the regulation of at least two ion channels in the striatum: Cav2.2 and NMDAR. The introduction of RGS9-2 into striatal cholinergic neurons reduced D2R receptor-mediated modulation of Cav2.2 channels (Cabrera-Vera et al., 2004). In striatal MSNs, knockout of RGS9 resulted in augmentation of D2R-mediated suppression of NMDA currents (Kovoor et al., 2005). Interestingly, RGS9-2 can physically bind to NMDAR by association with the adaptor protein α -actinin-2 to regulate Ca²⁺-dependent NMDAR inactivation (Bouhamdan et al.,

2006). Because both μ -opioid and D2R are known to control a variety of effector systems, it is possible that the role of RGS9-2 is not limited to the regulation of Cav2.2 and NMDAR channels. Indeed, RGS9-2 expression in transfected cells inhibits ERK phosphorylation in response to μ -opioid activation (Psifogeorgou et al., 2007). However, the full range of effector systems regulated by RGS9-2 in striatal neurons remains to be established.

The regulatory influence exerted by RGS9-2 in striatal neurons adjusts in response to changes in striatal signaling. Intriguingly, the expression of RGS9-2 changes in response to activation of signaling pathways that are normally regulated by RGS9-2. As discussed above, RGS9-2 regulates the extent of the effects produced by cocaine and morphine. On the other hand, administration of both cocaine and morphine leads to changes in RGS9-2 levels in the striatum. Acute morphine treatment increases, whereas chronic exposure decreases, the RGS9-2 protein content in the mouse ventral striatum (Zachariou et al., 2003; Psifogeorgou et al., 2007). Chronic cocaine exposure increases RGS9-2 levels in the same region (Rahman et al., 2003). RGS9-2 levels are also altered in response to several other stimuli and some neuropathological conditions. For example, repeated estradiol administration reduces both RGS9-2 mRNA and protein levels in the shell of the nucleus accumbens (Silverman and Koenig, 2007). RGS9-2 mRNA levels were significantly decreased in the striatum of a rat schizophrenia model and in the brains of schizophrenia patients (Seeman et al., 2007). RGS9-2 protein levels were elevated in the striata of Parkinson's disease patients (Tekumalla et al., 2001). Finally, ischemia and neuronal depolarization modeled in acutely cultured slices also result in rapid downregulation of RGS9-2 (Anderson et al., 2009a). Changes in RGS9-2 levels in response to RGS9-2-regulated pathways may provide a mechanism for adaptive physiological feedback, where the extent of regulation is adjusted based on the volume of signaling.

At the molecular level, the regulation of RGS9 function is achieved by controlling its association with auxiliary proteins. RGS9-2 contains multiple domains (Anderson et al., 2009b; Martemyanov and Arshavsky, 2009): the N-terminal DEP (Disheveled, Egl-10, Pleckstrin) and DHEX (DEP helical extension) domains are followed by the GGL (G protein γ -like) and C-terminal RGS catalytic domains. The GGL domain binds to the type 5 G β subunit (G β 5). The DEP/DHEX module mediates the interaction of R7 RGS with the anchor protein R7BP (R7 family binding protein). Both G β 5 and R7BP simultaneously interact with RGS9-2 and are considered bona fide subunits within the complex. The knockout of G β 5 in mice results in severe downregulation of RGS9-2 in the striatum (Chen et al., 2003). Similarly, levels of striatal RGS9-2 are dramatically reduced upon R7BP elimination (Anderson et al., 2007b). Conversely, R7BP overexpression in the striatum leads to the upregulation of RGS9-2 (Anderson et al., 2007a). In addition to ensuring proteolytic stability of the RGS9-2 complex, R7BP also controls the localization of RGS9-2 in the plasma membrane and targets the postsynaptic dendrites of striatal neurons (Anderson et al., 2007a).

Consistent with the roles of RGS9-2 in striatal functions and R7BP in maintaining a high expression level of RGS9-2, elimination of R7BP in mice results in a marked elevation in the sensitivity to the motor-stimulation actions of opioids and deficits

in motor coordination and learning that are similar to those reported for RGS9-2 knockouts (Anderson et al., 2010). Interestingly, the sensitivity of dopamine receptors to stimulation remained unchanged in R7BP knockouts. These mice showed normal behavioral responses to cocaine, suggesting the existence of selective compensatory mechanisms (Anderson et al., 2010) that may involve other RGS proteins binding to R7BP. The behavioral consequences and effects on striatal signaling due to the loss of G β 5 are largely unknown. G β 5 knockout in mice alters the timing of GABA(B)-mediated synaptic transmission in CA1 hippocampal synapses (Xie et al., 2010), and similar deficits may occur in striatal neurons.

OTHER RGS PROTEINS IN THE STRIATUM

While severely affecting the levels of RGS9-2, the elimination of R7BP does not reproduce the behavior of RGS9-2 knockouts, raising the possibility that other RGS proteins in the striatum can bind to R7BP and be involved in controlling striatal signaling in parallel with RGS9-2. Indeed, both R7BP and G β 5 have been shown to bind to RGS7. RGS7 belongs to the same R7 RGS family as RGS9-2 and shares the same domain organization (Anderson et al., 2009a, 2010). Although critical for RGS9-2 stability, R7BP is not necessary for RGS7 expression, as RGS7 protein levels are unchanged in R7BP knockout mice (Anderson et al., 2007a, 2009a). Moreover, RGS9-2 couples to R7BP with an affinity that is an order of magnitude greater than that of RGS7, resulting in a higher prevalence of RGS9-2/R7BP complexes when compared to RGS7/R7BP, despite equal concentrations of both RGS9-2 and RGS7 in striatal neurons (Anderson et al., 2009a). Elimination of RGS9-2 changes this balance and results in marked upregulation of R7BP/RGS7 complexes and their recruitment to postsynaptic densities (Anderson et al., 2009a). Therefore, the higher sensitivity to cocaine observed in RGS9 knockout mice might involve the alteration of RGS7 function, concurrent with the alteration of signaling directly controlled by RGS9-2. Indeed, viral knockdown of RGS7 in the striatum enhances behavioral sensitivity to cocaine (Anderson et al., 2010).

Several other RGS family members, including RGS2, RGS4, RGS10, and RGS17, are expressed in the striatum and play roles in signal processing in this region. The interest in these members is primarily driven by the observation that their expression levels change in response to different stimuli, most prominently psychostimulants, suggesting that they are involved in neuronal plasticity in this region. Specifically, RGS2 is rapidly induced in the striatum in response to maximal electroconvulsive seizure stimulation that evokes neuronal plasticity (Ingi et al., 1998). RGS2 is also upregulated upon administration of amphetamine (Burchett et al., 1998, 1999; Ingi et al., 1998; Robinet et al., 2001). Amphetamine-induced upregulation of RGS2 can be mimicked by D1 agonist administration and blocked by the D1 receptor antagonist, SCH 23390 (Taymans et al., 2003). In contrast, a D2 antagonist, haloperidol, upregulates RGS2 levels, whereas a D2 agonist, quinpirole, inhibits its expression (Ingi et al., 1998; Burchett et al., 1999; Taymans et al., 2003). Thus, D1 and D2 dopamine receptors play an antagonistic role in modulating the abundance of RGS2. RGS4 is another protein that belongs to the same R4 subfamily and shows prominent signal-dependent regulation of expression in the striatum.

Its levels are generally downregulated by the excess dopamine produced by amphetamine (Gonzalez-Nicolini and McGinty, 2002; Schwendt et al., 2006) or cocaine (Zhang et al., 2005a; Schwendt et al., 2007) and upregulated when dopamine is depleted (e.g., in a Parkinson's model; Geurts et al., 2002, 2003; Taymans et al., 2003; Zhang et al., 2005b; Ding et al., 2006). Interestingly, modulation of RGS4 expression levels has been linked to behavioral sensitization in response to repeated psychostimulant administration (Schwendt and McGinty, 2007) and upregulation of acetylcholine release with Parkinson's due to decreased autoinhibition of the striatal cholinergic interneurons (Ding et al., 2006). The molecular details of the RGS2 and RGS4 actions are beginning to emerge. Both RGS proteins are potent negative modulators of $G_{\alpha q}$ signaling and affect signaling downstream from mGluR5 receptors, influencing synaptic plasticity (Schwendt and McGinty, 2007). In addition, RGS4 and, to a lesser extent, RGS2 are capable of controlling G protein signaling downstream from $G_{\alpha i/o}$ -coupled receptors. By this action, RGS4 serves as an inhibitor of M4 autoreceptors in striatal cholinergic neurons and μ -opioid receptors in the nucleus accumbens (Ding et al., 2006; Han et al., 2010). RGS2 was also shown to modulate synaptic vesicle release by controlling $G_{\alpha i/o}$ -mediated presynaptic Ca^{2+} channel inhibition (Han et al., 2006). RGS2 has additional GAP-independent effects that directly inhibit AC activity (Sinnarajah et al., 2001). However, the role of this striatal signaling mechanism remains unexplored.

Among other striatal RGS proteins, RGS17 (Stanwood et al., 2006) is upregulated in the nucleus accumbens by ontogenetic treatment with the D2R agonist, quinpirole (Maple et al., 2007), and has been shown to regulate three different neurotransmitter systems, including muscarinic M2, μ -opioid, and dopamine D2 receptors (Mao et al., 2004; Garzon et al., 2005b). Finally, RGS10 is enriched in microglia where it regulates the expression of inflammation-related genes (Lee et al., 2008). By regulating the survival of dopaminergic neurons in the substantia nigra, RGS10 plays a critical role in setting the dopaminergic tone in the striatum, the central target nucleus innervated by dopaminergic neurons (Lee et al., 2008).

NOVEL PROTEINS INFLUENCING G PROTEIN CYCLE AND THEIR EMERGING ROLES IN THE STRIATUM

Regulator of G protein signaling proteins could be viewed as general inhibitors of G protein signaling because they negatively regulate both G_{α} and $G\beta\gamma$ subunit signaling. In recent years, there

has been an explosion of research on protein factors that affect G protein signaling at the same universal focal points as RGS proteins but exert differential effects on the activation of G protein subunits. Overall, these proteins can be separated into two groups. The first group is represented by molecules with guanine dissociation inhibitor (GDI) activity that stabilize G_{α} in the inactive GDP-bound form, preventing spontaneous and GPCR-dependent activation (Ma et al., 2003; Willard et al., 2004). At the same time, binding of GDI proteins to G_{α} -GDP inhibits reassociation with $\beta\gamma$, promoting $\beta\gamma$ -selective signaling. All known GDI proteins contain a G protein regulatory (GPR, also known as GoLoco) domain that specifically binds and stabilizes G_{α} -GDP (Kimple et al., 2002; Natochin et al., 2002). Among several GDI proteins, AGS3 is known to have effects on striatal signaling by regulating the activation and deactivation of the $G_{\alpha i}$ protein and associated $\beta\gamma$ subunits (Takesono et al., 1999; De Vries et al., 2000a). AGS3 levels are upregulated during withdrawal from chronic cocaine (Bowers et al., 2003, 2004) and morphine (Fan et al., 2009) administration. In accordance with these observations, knockdown of AGS3 prevents cAMP superactivation during morphine withdrawal in the rat nucleus accumbens neurons (Peterson et al., 2000; Fan et al., 2009). Finally, introduction of AGS3 GPR motifs *in vivo* prevent $G_{\alpha i}$ signaling and promote drug seeking and locomotor sensitization (Bowers et al., 2004).

The second group of G protein regulators with emerging striatal functions is represented by proteins that catalyze GPCR-independent activation of G protein α subunits. These proteins act as a GEF for the G_{α} subunit by interacting with GDP-bound α subunits in the absence of $\beta\gamma$. They were initially discovered in mutant screens and suppression assays in *C. elegans* and termed RIC (resistant to inhibitors of cholinesterase; Miller et al., 1996; Hajdu-Cronin et al., 1999). Mammalian homologs of Ric-8, termed Ric-8A and Ric-8B, were found through a yeast two-hybrid screen with $G_{\alpha o}$ as bait (Tall et al., 2003) and shown to be a powerful GEF for the G_{α} subunits. Although the role of Ric-8A in the striatum has not been addressed specifically, initial evidence indicates that this protein serves as a modulator of both AGS3 (Thomas et al., 2008) and AC5 (Wang et al., 2007), suggesting its involvement in key steps of striatal signaling.

In conclusion, emerging picture indicates that numerous G protein pathways in the striatum are controlled by the host of the regulatory proteins, that affect the dynamics of signaling in the region and thus play critical roles in shaping behavioral responses mediated by the striatal neurons.

REFERENCES

- Abrams, P., Andersson, K. E., Buccafusco, J. J., Chapple, C., de Groat, W. C., Fryer, A. D., Kay, G., Laties, A., Nathanson, N. M., Pasricha, P. J., and Wein, A. J. (2006). Muscarinic receptors: their distribution and function in body systems, and the implications for treating overactive bladder. *Br. J. Pharmacol.* 148, 565–578.
- Akins, P. T., Surmeier, D. J., and Kitai, S. T. (1990). Muscarinic modulation of a transient K^{+} conductance in rat neostriatal neurons. *Nature* 344, 240–242.
- Ambrosio, A. F., Malva, J. O., Carvalho, A. P., and Carvalho, C. M. (1996). Modulation of Ca^{2+} channels by activation of adenosine A1 receptors in rat striatal glutamatergic nerve terminals. *Neurosci. Lett.* 220, 163–166.
- Anderson, G. R., Cao, Y., Davidson, S., Truong, H. V., Pravetoni, M., Thomas, M. J., Wickman, K., Giesler, G. J. Jr., and Martemyanov, K. A. (2010). R7BP complexes with RGS9-2 and RGS7 in the striatum differentially control motor learning and locomotor responses to cocaine. *Neuropsychopharmacology* 35, 1040–1050.
- Anderson, G. R., Lujan, R., and Martemyanov, K. A. (2009a). Changes in striatal signaling induce remodeling of RGS complexes containing Gbeta5 and R7BP subunits. *Mol. Cell. Biol.* 29, 3033–3044.
- Anderson, G. R., Posokhova, E., and Martemyanov, K. A. (2009b). The R7 RGS protein family: multi-subunit regulators of neuronal G protein signaling. *Cell Biochem. Biophys.* 54, 33–46.
- Anderson, G. R., Lujan, R., Semenov, A., Pravetoni, M., Posokhova, E. N., Song, J. H., Uversky, V., Chen, C. K., Wickman, K., and Martemyanov, K. A. (2007a). Expression and localization of RGS9-2/G5/R7BP complex *in vivo* is set by dynamic control of its constitutive degradation by cellular cysteine proteases. *J. Neurosci.* 27, 14117–14127.

- Anderson, G. R., Semenov, A., Song, J. H., and Martemyanov, K. A. (2007b). The membrane anchor R7BP controls the proteolytic stability of the striatal specific RGS protein, RGS9-2. *J. Biol. Chem.* 282, 4772–4781.
- Arbuthnott, G. W., and Wickens, J. (2007). Space, time and dopamine. *Trends Neurosci.* 30, 62–69.
- Augood, S. J., and Emson, P. C. (1994). Adenosine A2a receptor mRNA is expressed by enkephalin cells but not by somatostatin cells in rat striatum: a co-expression study. *Brain Res. Mol. Brain Res.* 22, 204–210.
- Azmitia, E. C., and Segal, M. (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.* 179, 641–667.
- Balcells-Olivero, M., and Vezina, P. (1997). Effects of naltrexone on amphetamine-induced locomotion and rearing: acute and repeated injections. *Psychopharmacology (Berl.)* 131, 230–238.
- Barnes, N. M., and Sharp, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacology* 38, 1083–1152.
- Basavarajappa, B. S. (2007). Neuropharmacology of the endocannabinoid signaling system-molecular mechanisms, biological actions and synaptic plasticity. *Curr. Neuropharmacol.* 5, 81–97.
- Bernard, V., Normand, E., and Bloch, B. (1992). Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. *J. Neurosci.* 12, 3591–3600.
- Biddlecome, G. H., Berstein, G., and Ross, E. M. (1996). Regulation of phospholipase C- β by Gq and m1 muscarinic cholinergic receptor. Steady-state balance of receptor-mediated activation and GTPase-activating protein-promoted deactivation. *J. Biol. Chem.* 271, 7999–8007.
- Bisogno, T., Ligresti, A., and Di Marzo, V. (2005). The endocannabinoid signalling system: biochemical aspects. *Pharmacol. Biochem. Behav.* 81, 224–238.
- Blundell, J., Hoang, C. V., Potts, B., Gold, S. J., and Powell, C. M. (2008). Motor coordination deficits in mice lacking RGS9. *Brain Res.* 1190, 78–85.
- Bolam, J. P., Hanley, J. J., Booth, P. A., and Bevan, M. D. (2000). Synaptic organisation of the basal ganglia. *J. Anat.* 196(Pt 4), 527–542.
- Bouhamdan, M., Yan, H. D., Yan, X. H., Bannan, M. J., and Andrade, R. (2006). Brain-specific regulator of G-protein signaling 9-2 selectively interacts with α -actinin-2 to regulate calcium-dependent inactivation of NMDA receptors. *J. Neurosci.* 26, 2522–2530.
- Bowers, M. S., Lake, R. W., McFarland, K., Peterson, Y. K., Lanier, S. M., Lapish, C. C., and Kalivas, P. W. (2003). AGS3: a G-Protein regulator of addiction-associated behaviors. *Ann. N. Y. Acad. Sci.* 1003, 356–357.
- Bowers, M. S., McFarland, K., Lake, R. W., Peterson, Y. K., Lapish, C. C., Gregory, M. L., Lanier, S. M., and Kalivas, P. W. (2004). Activator of G protein signaling 3: a gatekeeper of cocaine sensitization and drug seeking. *Neuron* 42, 269–281.
- Bradley, S. R., Standaert, D. G., Rhodes, K. J., Rees, H. D., Testa, C. M., Levey, A. I., and Conn, P. J. (1999). Immunohistochemical localization of subtype 4a metabotropic glutamate receptors in the rat and mouse basal ganglia. *J. Comp. Neurol.* 407, 33–46.
- Burchett, S. A., Bannan, M. J., and Granneman, J. G. (1999). RGS mRNA expression in rat striatum: modulation by dopamine receptors and effects of repeated amphetamine administration. *J. Neurochem.* 72, 1529–1533.
- Burchett, S. A., Volk, M. L., Bannan, M. J., and Granneman, J. G. (1998). Regulators of G protein signaling: rapid changes in mRNA abundance in response to amphetamine. *J. Neurochem.* 70, 2216–2219.
- Cabrera-Vera, T. M., Hernandez, S., Earls, L. R., Medkova, M., Sundgren-Andersson, A. K., Surmeier, D. J., and Hamm, H. E. (2004). RGS9-2 modulates D2 dopamine receptor-mediated Ca^{2+} channel inhibition in rat striatal cholinergic interneurons. *Proc. Natl. Acad. Sci. U.S.A.* 101, 16339–16344.
- Calabresi, P., Centonze, D., Gubellini, P., Pisani, A., and Bernardi, G. (1998a). Endogenous ACh enhances striatal NMDA-responses via M1-like muscarinic receptors and PKC activation. *Eur. J. Neurosci.* 10, 2887–2895.
- Calabresi, P., Centonze, D., Pisani, A., Sancesario, G., North, R. A., and Bernardi, G. (1998b). Muscarinic IPSPs in rat striatal cholinergic interneurons. *J. Physiol. (Lond.)* 510(Pt 2), 421–427.
- Calabresi, P., Centonze, D., Gubellini, P., Pisani, A., and Bernardi, G. (2000). Acetylcholine-mediated modulation of striatal function. *Trends Neurosci.* 23, 120–126.
- Cartmell, J., and Schoepp, D. D. (2000). Regulation of neurotransmitter release by metabotropic glutamate receptors. *J. Neurochem.* 75, 889–907.
- Caulfield, M. P., and Birdsall, N. J. (1998). International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* 50, 279–290.
- Celver, J., Sharma, M., and Koo, A. (2010). RGS9-2 mediates specific inhibition of agonist-induced internalization of D2-dopamine receptors. *J. Neurochem.* 114, 739–749.
- Cepeda, C., Wu, N., Andre, V. M., Cummings, D. M., and Levine, M. S. (2007). The corticostriatal pathway in Huntington's disease. *Prog. Neurobiol.* 81, 253–271.
- Charlton, J. J., Allen, P. B., Psifogorgou, K., Chakravarty, S., Gomes, I., Neve, R. L., Devi, L. A., Greengard, P., Nestler, E. J., and Zachariou, V. (2008). Multiple actions of spinophilin regulate mu opioid receptor function. *Neuron* 58, 238–247.
- Chase, T. N., and Oh, J. D. (2000). Striatal dopamine- and glutamate-mediated dysregulation in experimental parkinsonism. *Trends Neurosci.* 23, S86–S91.
- Chen, C., and Lin, S. C. (1998). The core domain of RGS16 retains G-protein binding and GAP activity in vitro, but is not functional in vivo. *FEBS Lett.* 422, 359–362.
- Chen, C. K., Burns, M. E., He, W., Wensel, T. G., Baylor, D. A., and Simon, M. I. (2000). Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9-1. *Nature* 403, 557–560.
- Chen, C. K., Eversole-Cire, P., Zhang, H. K., Mancino, V., Chen, Y. J., He, W., Wensel, T. G., and Simon, M. I. (2003). Instability of GGL domain-containing RGS proteins in mice lacking the G protein β -subunit G β 5. *Proc. Natl. Acad. Sci. U.S.A.* 100, 6604–6609.
- Cho, H., Park, C., Hwang, I. Y., Han, S. B., Schimmel, D., Despres, D., and Kehrl, J. H. (2008). Rgs5 targeting leads to chronic low blood pressure and a lean body habitus. *Mol. Cell Biol.* 28, 2590–2597.
- Cicchetti, F., Pensa, L., Wu, Y., and Parent, A. (2000). Chemical anatomy of striatal interneurons in normal individuals and in patients with Huntington's disease. *Brain Res. Brain Res. Rev.* 34, 80–101.
- Cifelli, C., Rose, R. A., Zhang, H., Voigtlaender-Bolz, J., Bolz, S. S., Backx, P. H., and Heximer, S. P. (2008). RGS4 regulates parasympathetic signaling and heart rate control in the sinoatrial node. *Circ. Res.* 103, 527–535.
- Conn, P. J., and Pin, J. P. (1997). Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.* 37, 205–237.
- Corti, C., Aldegheri, L., Somogyi, P., and Ferraguti, F. (2002). Distribution and synaptic localisation of the metabotropic glutamate receptor 4 (mGluR4) in the rodent CNS. *Neuroscience* 110, 403–420.
- Cunha, R. A. (2001). Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. *Neurochem. Int.* 38, 107–125.
- David, H. N., Ansseau, M., and Abraini, J. H. (2005). Dopamine-glutamate reciprocal modulation of release and motor responses in the rat caudate-putamen and nucleus accumbens of "intact" animals. *Brain Res. Brain Res. Rev.* 50, 336–360.
- De Klippel, N., Sarre, S., Ebinger, G., and Michotte, Y. (1993). Effect of M1- and M2-muscarinic drugs on striatal dopamine release and metabolism: an in vivo microdialysis study comparing normal and 6-hydroxydopamine-lesioned rats. *Brain Res.* 630, 57–64.
- De Vries, L., Fischer, T., Tronchere, H., Brothers, G. M., Strockbine, B., Siderovski, D. P., and Farquhar, M. G. (2000a). Activator of G protein signaling 3 is a guanine dissociation inhibitor for G α i subunits. *Proc. Natl. Acad. Sci. U.S.A.* 97, 14364–14369.
- De Vries, L., Zheng, B., Fischer, T., Elenko, E., and Farquhar, M. G. (2000b). The regulator of G protein signaling family. *Annu. Rev. Pharmacol. Toxicol.* 40, 235–271.
- De Vries, L., and Gist Farquhar, M. (1999). RGS proteins: more than just GAPs for heterotrimeric G proteins. *Trends Cell Biol.* 9, 138–144.
- Dhawan, B. N., Cesselin, F., Raghunath, R., Reisine, T., Bradley, P. B., Portoghesi, P. S., and Hamon, M. (1996). International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.* 48, 567–592.
- Di Matteo, V., Pierucci, M., Esposito, E., Crescimanno, G., Benigno, A., and Di Giovanni, G. (2008). Serotonin modulation of the basal ganglia circuitry: therapeutic implication for Parkinson's disease and other motor disorders. *Prog. Brain Res.* 172, 423–463.

- Diaz-Otanez, C. S., Capriles, N. R., and Cancela, L. M. (1997). D1 and D2 dopamine and opiate receptors are involved in the restraint stress-induced sensitization to the psychostimulant effects of amphetamine. *Pharmacol. Biochem. Behav.* 58, 9–14.
- DiBello, P. R., Garrison, T. R., Apanovitch, D. M., Hoffman, G., Shuey, D. J., Mason, K., Cockett, M. I., and Dohlmann, H. G. (1998). Selective uncoupling of RGS action by a single point mutation in the G protein α -subunit. *J. Biol. Chem.* 273, 5780–5784.
- Ding, J., Guzman, J. N., Tkatch, T., Chen, S., Goldberg, J. A., Ebert, P. J., Levitt, P., Wilson, C. J., Hamm, H. E., and Surmeier, D. J. (2006). RGS4-dependent attenuation of M4 autoreceptor function in striatal cholinergic interneurons following dopamine depletion. *Nat. Neurosci.* 9, 832–842.
- Dunwiddie, T. V., and Masino, S. A. (2001). The role and regulation of adenosine in the central nervous system. *Annu. Rev. Neurosci.* 24, 31–55.
- Dupre, D. J., Robitaille, M., Rebois, R. V., and Hebert, T. E. (2009). The role of Gbetagamma subunits in the organization, assembly, and function of GPCR signaling complexes. *Annu. Rev. Pharmacol. Toxicol.* 49, 31–56.
- El-Ghundi, M., O'Dowd, B. F., and George, S. R. (2007). Insights into the role of dopamine receptor systems in learning and memory. *Rev. Neurosci.* 18, 37–66.
- Everitt, B. J., and Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat. Neurosci.* 8, 1481–1489.
- Falk, T., Zhang, S., Erbe, E. L., and Sherman, S. J. (2006). Neurochemical and electrophysiological characteristics of rat striatal neurons in primary culture. *J. Comp. Neurol.* 494, 275–289.
- Fan, P., Jiang, Z., Diamond, I., and Yao, L. (2009). Up-regulation of AGS3 during morphine withdrawal promotes cAMP superactivation via adenylyl cyclase 5 and 7 in rat nucleus accumbens/striatal neurons. *Mol. Pharmacol.* 76, 526–533.
- Felder, C. C., and Glass, M. (1998). Cannabinoid receptors and their endogenous agonists. *Annu. Rev. Pharmacol. Toxicol.* 38, 179–200.
- Fernandez-Ruiz, J., Lastres-Becker, I., Cabranes, A., Gonzalez, S., and Ramos, J. A. (2002). Endocannabinoids and basal ganglia functionality. *Prostaglandins Leukot. Essent. Fatty Acids* 66, 257–267.
- Ferre, S., Karcz-Kubicha, M., Hope, B. T., Popoli, P., Burgueno, J., Gutierrez, M. A., Casado, V., Fuxe, K., Goldberg, S. R., Lluís, C., Franco, R., and Ciruela, F. (2002). Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11940–11945.
- Fink, J. S., Weaver, D. R., Rivkees, S. A., Peterfreund, R. A., Pollack, A. E., Adler, E. M., and Reppert, S. M. (1992). Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum. *Brain Res. Mol. Brain Res.* 14, 186–195.
- Fredholm, B. B., AP, I. J., Jacobson, K. A., Klotz, K. N., and Linden, J. (2001). International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* 53, 527–552.
- Fuxe, K., Marcellino, D., Genedani, S., and Agnati, L. (2007). Adenosine A(2A) receptors, dopamine D(2) receptors and their interactions in Parkinson's disease. *Mov. Disord.* 22, 1990–2017.
- Gabel, L. A., and Nisenbaum, E. S. (1999). Muscarinic receptors differentially modulate the persistent potassium current in striatal spiny projection neurons. *J. Neurophysiol.* 81, 1418–1423.
- Garzon, J., Rodriguez-Munoz, M., Lopez-Fando, A., and Sanchez-Blazquez, P. (2005a). Activation of mu-opioid receptors transfers control of G α subunits to the regulator of G-protein signaling RGS9-2: role in receptor desensitization. *J. Biol. Chem.* 280, 8951–8960.
- Garzon, J., Rodriguez-Munoz, M., Lopez-Fando, A., and Sanchez-Blazquez, P. (2005b). The RGS2 protein exists in a complex with mu-opioid receptors and regulates the desensitizing capacity of G α proteins. *Neuropsychopharmacology* 30, 1632–1648.
- Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J. Jr., and Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250, 1429–1432.
- Geurts, M., Hermans, E., and Maloteaux, J. M. (2002). Opposite modulation of regulators of G protein signalling-2 RGS2 and RGS4 expression by dopamine receptors in the rat striatum. *Neurosci. Lett.* 333, 146–150.
- Geurts, M., Maloteaux, J. M., and Hermans, E. (2003). Altered expression of regulators of G-protein signaling (RGS) mRNAs in the striatum of rats undergoing dopamine depletion. *Biochem. Pharmacol.* 66, 1163–1170.
- Gilman, A. G. (1987). G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* 56, 615–649.
- Glickstein, S. B., and Schmauss, C. (2001). Dopamine receptor functions: lessons from knockout mice [corrected]. *Pharmacol. Ther.* 91, 63–83.
- Gold, S. J., Hoang, C. V., Potts, B. W., Porras, G., Pioli, E., Kim, K. W., Nadjar, A., Qin, C., LaHoste, G. J., Li, Q., Bioulac, B. H., Waugh, J. L., Gurevich, E., Neve, R. L., and Bezard, E. (2007). RGS9-2 negatively modulates L-3,4-dihydroxyphenylalanine-induced dyskinesia in experimental Parkinson's disease. *J. Neurosci.* 27, 14338–14348.
- Gold, S. J., Ni, Y. G., Dohlmann, H. G., and Nestler, E. J. (1997). Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. *J. Neurosci.* 17, 8024–8037.
- Gonzalez-Nicolini, M. V., Berglind, W., Cole, K. S., Keogh, C. L., and McGinty, J. F. (2003). Local mu and delta opioid receptors regulate amphetamine-induced behavior and neuropeptide mRNA in the striatum. *Neuroscience* 121, 387–398.
- Gonzalez-Nicolini, V., and McGinty, J. F. (2002). Gene expression profile from the striatum of amphetamine-treated rats: a cDNA array and in situ hybridization histochemical study. *Brain Res. Gene Expr. Patterns* 1, 193–198.
- Graveland, G. A., and DiFiglia, M. (1985). The frequency and distribution of medium-sized neurons with indented nuclei in the primate and rodent neostriatum. *Brain Res.* 327, 307–311.
- Gray, A. M., Rawls, S. M., Shippenberg, T. S., and McGinty, J. F. (1999). The kappa-opioid agonist, U-69593, decreases acute amphetamine-evoked behaviors and calcium-dependent dialysate levels of dopamine and glutamate in the ventral striatum. *J. Neurochem.* 73, 1066–1074.
- Graybiel, A. M. (1990). Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci.* 13, 244–254.
- Graybiel, A. M. (2000). The basal ganglia. *Curr. Biol.* 10, R509–R511.
- Gubellini, P., Saulle, E., Centonze, D., Bonsi, P., Pisani, A., Bernardi, G., Conquet, F., and Calabresi, P. (2001). Selective involvement of mGlu1 receptors in corticostriatal LTD. *Neuropharmacology* 40, 839–846.
- Gubellini, P., Saulle, E., Centonze, D., Costa, C., Tropea, D., Bernardi, G., Conquet, F., and Calabresi, P. (2003). Corticostriatal LTP requires combined mGluR1 and mGluR5 activation. *Neuropharmacology* 44, 8–16.
- Hajdu-Cronin, Y. M., Chen, W. J., Patikoglou, G., Koelle, M. R., and Sternberg, P. W. (1999). Antagonism between G(o)alpha and G(q)alpha in *Caenorhabditis elegans*: the RGS protein EAT-16 is necessary for G(o)alpha signaling and regulates G(q)alpha activity. *Genes Dev.* 13, 1780–1793.
- Han, J., Mark, M. D., Li, X., Xie, M., Waka, S., Rettig, J., and Herlitze, S. (2006). RGS2 determines short-term synaptic plasticity in hippocampal neurons by regulating Gi/o-mediated inhibition of presynaptic Ca²⁺ channels. *Neuron* 51, 575–586.
- Han, M. H., Renthall, W., Ring, R. H., Rahman, Z., Psifogeorgou, K., Howland, D., Birnbaum, S., Young, K., Neve, R., Nestler, E. J., and Zachariou, V. (2010). Brain region specific actions of regulator of G protein signaling 4 oppose morphine reward and dependence but promote analgesia. *Biol. Psychiatry* 67, 761–769.
- Hart, M. J., Jiang, X., Kozasa, T., Roscoe, W., Singer, W. D., Gilman, A. G., Sternweis, P. C., and Bollag, G. (1998). Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by G α 13. *Science* 280, 2112–2114.
- Hasbi, A., Fan, T., Alijanian, M., Nguyen, T., Perreault, M. L., O'Dowd, B. F., and George, S. R. (2009). Calcium signaling cascade links dopamine D1-D2 receptor heteromer to striatal BDNF production and neuronal growth. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21377–21382.
- He, W., Lu, L., Zhang, X., El-Hodiri, H. M., Chen, C. K., Slep, K. C., Simon, M. I., Jamrich, M., and Wensel, T. G. (2000). Modules in the photoreceptor RGS9-1.GBETA 5L GTPase-accelerating protein complex control effector coupling, GTPase acceleration, protein folding, and stability. *J. Biol. Chem.* 275, 37093–37100.

- Heidbreder, C., Goldberg, S. R., and Shippenberg, T. S. (1993). Inhibition of cocaine-induced sensitization by the delta-opioid receptor antagonist naltrindole. *Eur. J. Pharmacol.* 243, 123–127.
- Heiman, M., Schaefer, A., Gong, S., Peterson, J. D., Day, M., Ramsey, K. E., Suarez-Farinas, M., Schwarz, C., Stephan, D. A., Surmeier, D. J., Greengard, P., and Heintz, N. (2008). A translational profiling approach for the molecular characterization of CNS cell types. *Cell* 135, 738–748.
- Hernandez-Lopez, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H., and Surmeier, D. J. (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca^{2+} currents and excitability via a novel PLC[β 1]-IP3-calcineurin-signaling cascade. *J. Neurosci.* 20, 8987–8995.
- Hersch, S. M., Gutekunst, C. A., Rees, H. D., Heilman, C. J., and Levey, A. I. (1994). Distribution of m1-m4 muscarinic receptor proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies. *J. Neurosci.* 14, 3351–3363.
- Heximer, S. P. (2004). RGS2-mediated regulation of Gqalpha. *Meth. Enzymol.* 390, 65–82.
- Heximer, S. P., Knutsen, R. H., Sun, X., Kaltenbronn, K. M., Rhee, M. H., Peng, N., Oliveira-dos-Santos, A., Penninger, J. M., Muslin, A. J., Steinberg, T. H., Wyss, J. M., Mecham, R. P., and Blumer, K. J. (2003). Hypertension and prolonged vasoconstrictor signaling in RGS2-deficient mice. *J. Clin. Invest.* 111, 445–452.
- Hollinger, S., and Hepler, J. R. (2002). Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. *Pharmacol. Rev.* 54, 527–559.
- Hooks, S. B., Waldo, G. L., Corbitt, J., Bodor, E. T., Krumins, A. M., and Harden, T. K. (2003). RGS6, RGS7, RGS9, and RGS11 stimulate GTPase activity of Gi family G-proteins with differential selectivity and maximal activity. *J. Biol. Chem.* 278, 10087–10093.
- Howe, A. R., and Surmeier, D. J. (1995). Muscarinic receptors modulate N-, P-, and L-type Ca^{2+} currents in rat striatal neurons through parallel pathways. *J. Neurosci.* 15, 458–469.
- Huang, C. C., Lo, S. W., and Hsu, K. S. (2001). Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. *J. Physiol. (Lond.)* 532, 731–748.
- Huang, X., Fu, Y., Charbeneau, R. A., Saunders, T. L., Taylor, D. K., Hankenson, K. D., Russell, M. W., D'Alecy, L. G., and Neubig, R. R. (2006). Pleiotropic phenotype of a genomic knock-in of an RGS-insensitive G184S Gna12 allele. *Mol. Cell. Biol.* 26, 6870–6879.
- Hyman, S. E., Malenka, R. C., and Nestler, E. J. (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu. Rev. Neurosci.* 29, 565–598.
- Ingi, T., Krumins, A. M., Chidiac, P., Brothers, G. M., Chung, S., Snow, B. E., Barnes, C. A., Lananhan, A. A., Siderovski, D. P., Ross, E. M., Gilman, A. G., and Worley, P. F. (1998). Dynamic regulation of RGS2 suggests a novel mechanism in G-protein signaling and neuronal plasticity. *J. Neurosci.* 18, 7178–7188.
- Ishii, M., and Kurachi, Y. (2006). Muscarinic acetylcholine receptors. *Curr. Pharm. Des.* 12, 3573–3581.
- Iwamoto, T., Okumura, S., Iwatsubo, K., Kawabe, J., Ohtsu, K., Sakai, I., Hashimoto, Y., Izumitani, A., Sango, K., Ajiki, K., Toya, Y., Umemura, S., Goshima, Y., Arai, N., Vatter, S. E., and Ishikawa, Y. (2003). Motor dysfunction in type 5 adenylyl cyclase-null mice. *J. Biol. Chem.* 278, 16936–16940.
- Jaber, M., Robinson, S. W., Missale, C., and Caron, M. G. (1996). Dopamine receptors and brain function. *Neuropharmacology* 35, 1503–1519.
- Jabourian, M., Venance, L., Bourgoin, S., Ozon, S., Perez, S., Godeheu, G., Glowinski, J., and Kemel, M. L. (2005). Functional mu opioid receptors are expressed in cholinergic interneurons of the rat dorsal striatum: territorial specificity and diurnal variation. *Eur. J. Neurosci.* 21, 3301–3309.
- Jenner, P. (2008). Molecular mechanisms of L-DOPA-induced dyskinesia. *Nat. Rev. Neurosci.* 9, 665–677.
- Jeong, S. W., and Ikeda, S. R. (2000). Endogenous regulator of G-protein signaling proteins modify N-type calcium channel modulation in rat sympathetic neurons. *J. Neurosci.* 20, 4489–4496.
- Jiang, Z. G., and North, R. A. (1992). Pre- and postsynaptic inhibition by opioids in rat striatum. *J. Neurosci.* 12, 356–361.
- Jong, Y. J., Kumar, V., and O'Malley, K. L. (2009). Intracellular metabotropic glutamate receptor 5 (mGluR5) activates signaling cascades distinct from cell surface counterparts. *J. Biol. Chem.* 284, 35827–35838.
- Joseph, J. D., Wang, Y. M., Miles, P. R., Budygin, E. A., Picetti, R., Gainetdinov, R. R., Caron, M. G., and Wightman, R. M. (2002). Dopamine autoreceptor regulation of release and uptake in mouse brain slices in the absence of D(3) receptors. *Neuroscience* 112, 39–49.
- Kelley, A. E., Bakshi, V. P., Haber, S. N., Steininger, T. L., Will, M. J., and Zhang, M. (2002). Opioid modulation of taste hedonics within the ventral striatum. *Physiol. Behav.* 76, 365–377.
- Kim, K. S., Lee, K. W., Im, J. Y., Yoo, J. Y., Kim, S. W., Lee, J. K., Nestler, E. J., and Han, P. L. (2006). Adenylyl cyclase type 5 (AC5) is an essential mediator of morphine action. *Proc. Natl. Acad. Sci. U.S.A.* 103, 3908–3913.
- Kimple, R. J., Kimple, M. E., Betts, L., Sondek, J., and Siderovski, D. P. (2002). Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits. *Nature* 416, 878–881.
- Koob, G. F., and Le Moal, M. (2008). Addiction and the brain antireward system. *Annu. Rev. Psychol.* 59, 29–53.
- Koos, T., and Tepper, J. M. (2002). Dual cholinergic control of fast-spiking interneurons in the neostriatum. *J. Neurosci.* 22, 529–535.
- Kosinski, C. M., Risso Bradley, S., Conn, P. J., Levey, A. I., Landwehrmeyer, G. B., Penney, J. B. Jr., Young, A. B., and Standaert, D. G. (1999). Localization of metabotropic glutamate receptor 7 mRNA and mGluR7a protein in the rat basal ganglia. *J. Comp. Neurol.* 415, 266–284.
- Kovoor, A., Seyffarth, P., Ebert, J., Barghshoon, S., Chen, C. K., Schwarz, S., Axelrod, J. D., Cheyette, B. N., Simon, M. I., Lester, H. A., and Schwarz, J. (2005). D2 dopamine receptors colocalize regulator of G-protein signaling 9-2 (RGS9-2) via the RGS9 DEP domain, and RGS9 knock-out mice develop dyskinesias associated with dopamine pathways. *J. Neurosci.* 25, 2157–2165.
- Kozasa, T., Jiang, X., Hart, M. J., Sternweis, P. M., Singer, W. D., Gilman, A. G., Bollag, G., and Sternweis, P. C. (1998). p115 RhoGEF, a GTPase activating protein for Galpha12 and Galpha13. *Science* 280, 2109–2111.
- Kreitzer, A. C., and Malenka, R. C. (2008). Striatal plasticity and basal ganglia circuit function. *Neuron* 60, 543–554.
- Kruzich, P. J., Suchland, K. L., and Grandy, D. K. (2004). Dopamine D4 receptor-deficient mice, congenic on the C57BL/6J background, are hypersensitive to amphetamine. *Synapse* 53, 131–139.
- Lan, K. L., Sarvazyan, N. A., Taussig, R., Mackenzie, R. G., DiBello, P. R., Dohman, H. G., and Neubig, R. R. (1998). A point mutation in Galphao and Galphai1 blocks interaction with regulator of G protein signaling proteins. *J. Biol. Chem.* 273, 12794–12797.
- Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczky, J., LeVine, R., McEwan, P., McKernan, K., Meldrum, J., Mesirov, J. P., Miranda, C., Morris, W., Naylor, J., Raymond, C., Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J. C., Mungall, A., Plumb, R., Ross, M., Shownkeen, R., Sims, S., Waterston, R. H., Wilson, R. K., Hillier, L. W., McPherson, J. D., Marra, M. A., Mardis, E. R., Fulton, L. A., Chinwalla, A. T., Pepin, K. H., Gish, W. R., Chissoe, S. L., Wendl, M. C., Delehaunty, K. D., Miner, T. L., Delehaunty, A., Kramer, J. B., Cook, L. L., Fulton, R. S., Johnson, D. L., Minx, P. J., Clifton, S. W., Hawkins, T., Branscomb, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J. F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., Gibbs, R. A., Muzny, D. M., Scherer, S. E., Bouck, J. B., Sodergren, E. J., Worley, K. C., Rives, C. M., Gorrell, J. H., Metzker, M. L., Naylor, S. L., Kucherlapati, R. S., Nelson, D. L., Weinstock, G. M., Sakaki, Y., Fujiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Weissbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Smith, D. R., Doucette-Stamm, L., Rubenfield, M., Weinstock, K., Lee, H. M., Dubois, J., Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Hood, L., Rowen, L., Madan, A., Qin, S., Davis, R. W., Federspiel, N. A., Abola, A. P., Proctor, M. J., Myers, R. M., Schmutz, J., Dickson,

- M., Grimwood, J., Cox, D. R., Olson, M. V., Kaul, R., Raymond, C., Shimizu, N., Kawasaki, K., Minoshima, S., Evans, G. A., Athanasiou, M., Schultz, R., Roe, B. A., Chen, F., Pan, H., Ramser, J., Lehrach, H., Reinhardt, R., McCombie, W. R., de la Bastide, M., Dedhia, N., Blöcker, H., Hornischer, K., Nord-siek, G., Agarwala, R., Aravind, L., Bailey, J. A., Bateman, A., Batzoglu, S., Birney, E., Bork, P., Brown, D. G., Burge, C. B., Cerutti, L., Chen, H. C., Church, D., Clamp, M., Copley, R. R., Doerks, T., Eddy, S. R., Eichler, E. E., Furey, T. S., Galagan, J., Gilbert, J. G., Harmon, C., Hayashizaki, Y., Haus-sler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L. S., Jones, T. A., Kasif, S., Kasprzyk, A., Kennedy, S., Kent, W. J., Kitts, P., Koonin, E. V., Korf, I., Kulp, D., Lancet, D., Lowe, T. M., McLysaght, A., Mikkelsen, T., Moran, J. V., Mulder, N., Pollara, V. J., Ponting, C. P., Schuler, G., Schultz, J., Slater, G., Smit, A. F., Stupka, E., Szustakowski, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y. I., Wolfe, K. H., Yang, S. P., Yeh, R. F., Collins, F., Guyer, M. S., Peterson, J., Felsenfeld, A., Wetterstrand, K. A., Patrinos, A., Morgan, M. J., de Jong, P., Catanese, J. J., Osoegawa, K., Shizuya, H., Choi, S., Chen, Y. J., and International Human Genome Sequencing Consortium. (2001). Initial sequencing and analysis of the human genome. *Nature* 409, 860–921.
- Law, P. Y., Wong, Y. H., and Loh, H. H. (2000). Molecular mechanisms and regulation of opioid receptor signaling. *Annu. Rev. Pharmacol. Toxicol.* 40, 389–430.
- Lee, J. K., McCoy, M. K., Harms, A. S., Ruhn, K. A., Gold, S. J., and Tansey, M. G. (2008). Regulator of G-protein signaling 10 promotes dopaminergic neuron survival via regulation of the microglial inflammatory response. *J. Neurosci.* 28, 8517–8528.
- Lee, K. W., Hong, J. H., Choi, I. Y., Che, Y., Lee, J. K., Yang, S. D., Song, C. W., Kang, H. S., Lee, J. H., Noh, J. S., Shin, H. S., and Han, P. L. (2002). Impaired D2 dopamine receptor function in mice lacking type 5 adenylyl cyclase. *J. Neurosci.* 22, 7931–7940.
- Lee, S. P., So, C. H., Rashid, A. J., Varghese, G., Cheng, R., Lanca, A. J., O'Dowd, B. F., and George, S. R. (2004). Dopamine D1 and D2 receptor Co-activation generates a novel phospholipase C-mediated calcium signal. *J. Biol. Chem.* 279, 35671–35678.
- Lefkowitz, R. J., Rajagopal, K., and Whalen, E. J. (2006). New roles for beta-arrestins in cell signaling: not just for seven-transmembrane receptors. *Mol. Cell* 24, 643–652.
- Lefkowitz, R. J., and Shenoy, S. K. (2005). Transduction of receptor signals by beta-arrestins. *Science* 308, 512–517.
- Levey, A. I., Kitt, C. A., Simonds, W. F., Price, D. L., and Brann, M. R. (1991). Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J. Neurosci.* 11, 3218–3226.
- Logue, S. F., Grauer, S. M., Paulsen, J., Graf, R., Taylor, N., Sung, M. A., Zhang, L., Hughes, Z., Pulito, V. L., Liu, F., Rosenzweig-Lipson, S., Brandon, N. J., Marquis, K. L., Bates, B., and Pausch, M. (2009). The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? *Mol. Cell. Neurosci.* 42, 438–447.
- Ma, H., Peterson, Y. K., Bernard, M. L., Lanier, S. M., and Graber, S. G. (2003). Influence of cytosolic AGS3 on receptor – G protein coupling. *Biochemistry* 42, 8085–8093.
- Mao, H., Zhao, Q., Daigle, M., Ghahremani, M. H., Chidiac, P., and Albert, P. R. (2004). RGS17/RGS22, a novel regulator of Gi/o, Gz, and Gq signaling. *J. Biol. Chem.* 279, 26314–26322.
- Maple, A. M., Perna, M. K., Parlaman, J. P., Stanwood, G. D., and Brown, R. W. (2007). Ontogenetic quip-irole treatment produces long-lasting decreases in the expression of Rgs9, but increases Rgs17 in the striatum, nucleus accumbens and frontal cortex. *Eur. J. Neurosci.* 26, 2532–2538.
- Marazziti, D., Mandillo, S., Di Pietro, C., Golini, E., Matteoni, R., and Tocchini-Valentini, G. P. (2007). GPR37 associates with the dopamine transporter to modulate dopamine uptake and behavioral responses to dopaminergic drugs. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9846–9851.
- Martemyanov, K. A., and Arshavsky, V. Y. (2002). Noncatalytic domains of RGS9-1.GBETA 5L play a decisive role in establishing its substrate specificity. *J. Biol. Chem.* 277, 32843–32848.
- Martemyanov, K. A., and Arshavsky, V. Y. (2009). Chapter 7 biology and functions of the RGS9 isoforms. *Prog. Mol. Biol. Transl. Sci.* 86, 205–227.
- Martemyanov, K. A., Hopp, J. A., and Arshavsky, V. Y. (2003a). Specificity of G protein-RGS protein recognition is regulated by affinity adapters. *Neuron* 38, 857–862.
- Martemyanov, K. A., Lishko, P. V., Calero, N., Keresztes, G., Sokolov, M., Strissel, K. J., Leskov, I. B., Hopp, J. A., Kolesnikov, A. V., Chen, C. K., Lem, J., Heller, S., Burns, M. E., and Arshavsky, V. Y. (2003b). The DEP domain determines subcellular targeting of the GTPase activating protein RGS9 in vivo. *J. Neurosci.* 23, 10175–10181.
- Martin-McCaffrey, L., Willard, F. S., Oliveira-dos-Santos, A. J., Natale, D. R., Snow, B. E., Kimple, R. J., Pajak, A., Watson, A. J., Dagnino, L., Penninger, J. M., Siderovski, D. P., and D'Souza, S. J. (2004). RGS14 is a mitotic spindle protein essential from the first division of the mammalian zygote. *Dev. Cell* 7, 763–769.
- Matamalas, M., Bertran-Gonzalez, J., Salomon, L., Degos, B., Deniau, J. M., Valjent, E., Herve, D., and Girault, J. A. (2009). Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. *PLoS ONE* 4, e4770. doi: 10.1371/journal.pone.0004770
- McGeorge, A. J., and Faull, R. L. (1989). The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience* 29, 503–537.
- Miller, K. G., Alfonso, A., Nguyen, M., Crowell, J. A., Johnson, C. D., and Rand, J. B. (1996). A genetic selection for *Caenorhabditis elegans* synaptic transmission mutants. *Proc. Natl. Acad. Sci. U.S.A.* 93, 12593–12598.
- Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., and Caron, M. G. (1998). Dopamine receptors: from structure to function. *Physiol. Rev.* 78, 189–225.
- Miura, M., Saino-Saito, S., Masuda, M., Kobayashi, K., and Aosaki, T. (2007). Compartment-specific modulation of GABAergic synaptic transmission by mu-opioid receptor in the mouse striatum with green fluorescent protein-expressing dopamine islands. *J. Neurosci.* 27, 9721–9728.
- Mizushima, K., Miyamoto, Y., Tsukahara, F., Hirai, M., Sakaki, Y., and Ito, T. (2000). A novel G-protein-coupled receptor gene expressed in striatum. *Genomics* 69, 314–321.
- Moukhes, H., Bosler, O., Bolam, J. P., Vallee, A., Umbriaco, D., Geffard, M., and Doucet, G. (1997). Quantitative and morphometric data indicate precise cellular interactions between serotonin terminals and postsynaptic targets in rat substantia nigra. *Neuroscience* 76, 1159–1171.
- Nakanishi, S., Nakajima, Y., Masu, M., Ueda, Y., Nakahara, K., Watanabe, D., Yamaguchi, S., Kawabata, S., and Okada, M. (1998). Glutamate receptors: brain function and signal transduction. *Brain Res. Brain Res. Rev.* 26, 230–235.
- Natochin, M., Gasimov, K. G., and Artemyev, N. O. (2002). A GPR-protein interaction surface of Gi(alpha): implications for the mechanism of GDP-release inhibition. *Biochemistry* 41, 258–265.
- Nestler, E. J., Alreja, M., and Aghajanian, G. K. (1999). Molecular control of locus coeruleus neurotransmission. *Biol. Psychiatry* 46, 1131–1139.
- Nestler, E. J., and Carlezon, W. A. Jr. (2006). The mesolimbic dopamine reward circuit in depression. *Biol. Psychiatry* 59, 1151–1159.
- Nichols, D. E., and Nichols, C. D. (2008). Serotonin receptors. *Chem. Rev.* 108, 1614–1641.
- Oldham, W. M., and Hamm, H. E. (2008). Heterotrimeric G protein activation by G-protein-coupled receptors. *Nat. Rev. Mol. Cell Biol.* 9, 60–71.
- Paquet, M., and Smith, Y. (2003). Group I metabotropic glutamate receptors in the monkey striatum: subsynaptic association with glutamatergic and dopaminergic afferents. *J. Neurosci.* 23, 7659–7669.
- Paulsen, R. H., Woodson, J., Liu, Z., and Ross, E. M. (1996). Carboxyl-terminal fragments of phospholipase C-beta1 with intrinsic Gq GTPase-activating protein (GAP) activity. *J. Biol. Chem.* 271, 26622–26629.
- Perez, S., Tierney, A., Deniau, J. M., and Kemel, M. L. (2007). Tachykinin regulation of cholinergic transmission in the limbic/prefrontal territory of the rat dorsal striatum: implication of new neurokinine 1-sensitive receptor binding site and interaction with enkephalin/mu opioid receptor transmission. *J. Neurochem.* 103, 2153–2163.
- Pertwee, R. G. (1997). Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol. Ther.* 74, 129–180.
- Peterson, Y. K., Bernard, M. L., Ma, H., Hazard, S. III, Graber, S. G., and Lanier, S. M. (2000). Stabilization of the GDP-bound conformation of Galpha by a peptide derived from the G-protein regulatory motif of AGS3. *J. Biol. Chem.* 275, 33193–33196.
- Picconi, B., Pisani, A., Barone, I., Bonsi, P., Centonze, D., Bernardi, G., and Calabresi, P. (2005). Pathological synaptic plasticity in the striatum: implications for Parkinson's disease. *Neurotoxicology* 26, 779–783.
- Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. (2002). Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* 3, 639–650.

- Piomelli, D. (2003). The molecular logic of endocannabinoid signalling. *Nat. Rev. Neurosci.* 4, 873–884.
- Pisani, A., Calabresi, P., Centonze, D., and Bernardi, G. (1997). Enhancement of NMDA responses by group I metabotropic glutamate receptor activation in striatal neurones. *Br. J. Pharmacol.* 120, 1007–1014.
- Pisani, A., Gubellini, P., Bonsi, P., Conquet, F., Picconi, B., Centonze, D., Bernardi, G., and Calabresi, P. (2001). Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. *Neuroscience* 106, 579–587.
- Posokhova, E., Wydeven, N., Allen, K. L., Wickman, K., and Martemyanov, K. A. (2010). RGS6/Gss5 complex accelerates IKACH gating kinetics in atrial myocytes and modulates parasympathetic regulation of heart rate. *Circ. Res.* 107, 1350–1354.
- Premont, R. T., and Gainetdinov, R. R. (2007). Physiological roles of G protein-coupled receptor kinases and arrestins. *Annu. Rev. Physiol.* 69, 511–534.
- Prezeau, L., Carrette, J., Helpap, B., Curry, K., Pin, J. P., and Bockaert, J. (1994). Pharmacological characterization of metabotropic glutamate receptors in several types of brain cells in primary cultures. *Mol. Pharmacol.* 45, 570–577.
- Psifogougou, K., Papakosta, P., Russo, S. J., Neve, R. L., Kardassis, D., Gold, S. J., and Zachariou, V. (2007). RGS9-2 is a negative modulator of mu-opioid receptor function. *J. Neurochem.* 103, 617–625.
- Rahman, Z., Schwarz, J., Gold, S. J., Zachariou, V., Wein, M. N., Choi, K. H., Kovoov, A., Chen, C. K., DiLeone, R. J., Schwarz, S. C., Selley, D. E., Sim-Selley, L. J., Barrot, M., Luedtke, R. R., Self, D., Neve, R. L., Lester, H. A., Simon, M. I., and Nestler, E. J. (2003). RGS9 modulates dopamine signaling in the basal ganglia. *Neuron* 38, 941–952.
- Rascol, O., and Fabre, N. (2001). Dyskinesia: L-dopa-induced and tardive dyskinesia. *Clin. Neuropharmacol.* 24, 313–323.
- Rawls, S. M., and McGinty, J. F. (1998). Muscarinic receptors regulate extracellular glutamate levels in the rat striatum: an in vivo microdialysis study. *J. Pharmacol. Exp. Ther.* 286, 91–98.
- Rawls, S. M., and McGinty, J. F. (2000). Delta opioid receptors regulate calcium-dependent, amphetamine-evoked glutamate levels in the rat striatum: an in vivo microdialysis study. *Brain Res.* 861, 296–304.
- Ribeiro, J. A., Sebastiao, A. M., and de Mendonca, A. (2002). Adenosine receptors in the nervous system: pathophysiological implications. *Prog. Neurobiol.* 68, 377–392.
- Robinet, E. A., Geurts, M., Maloteaux, J. M., and Pauwels, P. J. (2001). Chronic treatment with certain antipsychotic drugs preserves upregulation of regulator of G-protein signalling 2 mRNA in rat striatum as opposed to c-fos mRNA. *Neurosci. Lett.* 307, 45–48.
- Ross, E. M., and Wilkie, T. M. (2000). GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annu. Rev. Biochem.* 69, 795–827.
- Rubinstein, M., Phillips, T. J., Bunzow, J. R., Falzone, T. L., Dziewczapolski, G., Zhang, G., Fang, Y., Larson, J. L., McDougall, J. A., Chester, J. A., Saez, C., Pugsley, T. A., Gershnik, O., Low, M. J., and Grandy, D. K. (1997). Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. *Cell* 90, 991–1001.
- Salah-Uddin, H., Thomas, D. R., Davies, C. H., Hagan, J. J., Wood, M. D., Watson, J. M., and Challiss, R. A. (2008). Pharmacological assessment of m1 muscarinic acetylcholine receptor-gq/11 protein coupling in membranes prepared from postmortem human brain tissue. *J. Pharmacol. Exp. Ther.* 325, 869–874.
- Sanchez, G., Coletti, N., Vazquez, P., Cervenansky, C., Aguirre, A., Quillfeldt, J. A., Jerusalinsky, D., and Kornisiuk, E. (2009). Muscarinic inhibition of hippocampal and striatal adenylyl cyclase is mainly due to the M(4) receptor. *Neurochem. Res.* 34, 1363–1371.
- Sanchez-Blazquez, P., Rodriguez-Diaz, M., Lopez-Fando, A., Rodriguez-Munoz, M., and Garzon, J. (2003). The GBeta5 subunit that associates with the R7 subfamily of RGS proteins regulates mu-opioid effects. *Neuropharmacology* 45, 82–95.
- Sara, S. J. (2009). The locus coeruleus and noradrenergic modulation of cognition. *Nat. Rev. Neurosci.* 10, 211–223.
- Schad, C. A., Justice, J. B. Jr., and Holtzman, S. G. (1996). Differential effects of delta- and mu-opioid receptor antagonists on the amphetamine-induced increase in extracellular dopamine in striatum and nucleus accumbens. *J. Neurochem.* 67, 2292–2299.
- Schiff, M. L., Siderovski, D. P., Jordan, J. D., Brothers, G., Snow, B., De Vries, L., Ortiz, D. F., and Diverse-Pierluissi, M. (2000). Tyrosine-kinase-dependent recruitment of RGS12 to the N-type calcium channel. *Nature* 408, 723–727.
- Schiffmann, S. N., Fisone, G., Moresco, R., Cunha, R. A., and Ferre, S. (2007). Adenosine A2A receptors and basal ganglia physiology. *Prog. Neurobiol.* 83, 277–292.
- Schoepp, D. D. (2001). Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. *J. Pharmacol. Exp. Ther.* 299, 12–20.
- Schwendt, M., Gold, S. J., and McGinty, J. F. (2006). Acute amphetamine down-regulates RGS4 mRNA and protein expression in rat forebrain: distinct roles of D1 and D2 dopamine receptors. *J. Neurochem.* 96, 1606–1615.
- Schwendt, M., Hearing, M. C., See, R. E., and McGinty, J. F. (2007). Chronic cocaine reduces RGS4 mRNA in rat prefrontal cortex and dorsal striatum. *Neuroreport* 18, 1261–1265.
- Schwendt, M., and McGinty, J. F. (2007). Regulator of G-protein signaling 4 interacts with metabotropic glutamate receptor subtype 5 in rat striatum: relevance to amphetamine behavioral sensitization. *J. Pharmacol. Exp. Ther.* 323, 650–657.
- Seeman, P., Ko, F., Jack, E., Greenstein, R., and Dean, B. (2007). Consistent with dopamine supersensitivity, RGS9 expression is diminished in the amphetamine-treated animal model of schizophrenia and in postmortem schizophrenia brain. *Synapse* 61, 303–309.
- Shu, F. J., Ramineni, S., and Hepler, J. R. (2010). RGS14 is a multifunctional scaffold that integrates G protein and Ras/Raf MAPkinase signalling pathways. *Cell. Signal.* 22, 366–376.
- Shuen, J. A., Chen, M., Gloss, B., and Calakos, N. (2008). Drd1a-tTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. *J. Neurosci.* 28, 2681–2685.
- Siderovski, D. P., Strockbine, B., and Behe, C. I. (1999). Whither goest the RGS proteins? *Crit. Rev. Biochem. Mol. Biol.* 34, 215–251.
- Siderovski, D. P., and Willard, F. S. (2005). The GAPs, GEFs, and GDI of heterotrimeric G-protein alpha subunits. *Int. J. Biol. Sci.* 1, 51–66.
- Silverman, J. L., and Koenig, J. I. (2007). Evidence for the involvement of ERbeta and RGS9-2 in 17-beta estradiol enhancement of amphetamine-induced place preference behavior. *Horm. Behav.* 52, 146–155.
- Sinnarajah, S., Dessauer, C. W., Srikanth, D., Chen, J., Yuen, J., Yilma, S., Dennis, J. C., Morrison, E. E., Vodyanoy, V., and Kehrl, J. H. (2001). RGS2 regulates signal transduction in olfactory neurons by attenuating activation of adenylyl cyclase III. *Nature* 409, 1051–1055.
- Smith, Y., and Kiehl, J. Z. (2000). Anatomy of the dopamine system in the basal ganglia. *Trends Neurosci.* 23, S28–S33.
- Smolders, I., Bogaert, L., Ebinger, G., and Michotte, Y. (1997). Muscarinic modulation of striatal dopamine, glutamate, and GABA release, as measured with in vivo microdialysis. *J. Neurochem.* 68, 1942–1948.
- Song, W. J., Tkatch, T., and Surmeier, D. J. (2000). Adenosine receptor expression and modulation of Ca(2+) channels in rat striatal cholinergic interneurons. *J. Neurophysiol.* 83, 322–332.
- Spadoni, F., Martella, G., Martorana, A., Lavaroni, F., D'Angelo, V., Bernardi, G., and Stefani, A. (2004). Opioid-mediated modulation of calcium currents in striatal and pallidal neurons following reserpine treatment: focus on kappa response. *Synapse* 51, 194–205.
- Spanagel, R., Herz, A., and Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc. Natl. Acad. Sci. U.S.A.* 89, 2046–2050.
- Srinivasa, S. P., Bernstein, L. S., Blumer, K. J., and Linder, M. E. (1998). Plasma membrane localization is required for RGS4 function in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* 95, 5584–5589.
- Stanwood, G. D., Parlaman, J. P., and Levitt, P. (2006). Genetic or pharmacological inactivation of the dopamine D1 receptor differentially alters the expression of regulator of G-protein signalling (Rgs) transcripts. *Eur. J. Neurosci.* 24, 806–818.
- Sun, X., Kaltenbronn, K. M., Steinberg, T. H., and Blumer, K. J. (2005). RGS2 is a mediator of nitric oxide action on blood pressure and vasoconstrictor signaling. *Mol. Pharmacol.* 67, 631–639.
- Surmeier, D. J., Bargas, J., Hemmings, H. C. Jr., Nairn, A. C., and Greengard, P. (1995). Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* 14, 385–397.

- Surmeier, D. J., Ding, J., Day, M., Wang, Z., and Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci.* 30, 228–235.
- Suzuki, T., Miura, M., Nishimura, K., and Aosaki, T. (2001). Dopamine-dependent synaptic plasticity in the striatal cholinergic interneurons. *J. Neurosci.* 21, 6492–6501.
- Swanson, C. J., Baker, D. A., Carson, D., Worley, P. F., and Kalivas, P. W. (2001). Repeated cocaine administration attenuates group I metabotropic glutamate receptor-mediated glutamate release and behavioral activation: a potential role for Homer. *J. Neurosci.* 21, 9043–9052.
- Takesono, A., Cismowski, M. J., Ribas, C., Bernard, M., Chung, P., Hazard, S. III, Duzic, E., and Lanier, S. M. (1999). Receptor-independent activators of heterotrimeric G-protein signaling pathways. *J. Biol. Chem.* 274, 33202–33205.
- Talbot, J. N., Jutkiewicz, E. M., Graves, S. J., Clemans, C. F., Nicol, M. R., Mortensen, R. M., Huang, X., Neubig, R. R., and Traynor, J. R. (2010). RGS inhibition at G(α)12 selectively potentiates 5-HT1A-mediated antidepressant effects. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11086–11091.
- Tall, G. G., Krumins, A. M., and Gilman, A. G. (2003). Mammalian Ric-8A (synembryn) is a heterotrimeric Gα protein guanine nucleotide exchange factor. *J. Biol. Chem.* 278, 8356–8362.
- Tamaru, Y., Nomura, S., Mizuno, N., and Shigemoto, R. (2001). Distribution of metabotropic glutamate receptor mGluR3 in the mouse CNS: differential location relative to pre- and postsynaptic sites. *Neuroscience* 106, 481–503.
- Taymans, J. M., Leysen, J. E., and Langlois, X. (2003). Striatal gene expression of RGS2 and RGS4 is specifically mediated by dopamine D1 and D2 receptors: clues for RGS2 and RGS4 functions. *J. Neurochem.* 84, 1118–1127.
- Tekumalla, P. K., Calon, F., Rahman, Z., Birdi, S., Rajput, A. H., Hornykiewicz, O., Di Paolo, T., Bedard, P. J., and Nestler, E. J. (2001). Elevated levels of DeltaFosB and RGS9 in striatum in Parkinson's disease. *Biol. Psychiatry* 50, 813–816.
- Tepper, J. M., Koos, T., and Wilson, C. J. (2004). GABAergic microcircuits in the neostriatum. *Trends Neurosci.* 27, 662–669.
- Testa, C. M., Standaert, D. G., Landwehrmeyer, G. B., Penney, J. B. Jr., and Young, A. B. (1995). Differential expression of mGluR5 metabotropic glutamate receptor mRNA by rat striatal neurons. *J. Comp. Neurol.* 354, 241–252.
- Thanos, P. K., Bermeo, C., Rubinstein, M., Suchland, K. L., Wang, G. J., Grandy, D. K., and Volkow, N. D. (2010). Conditioned place preference and locomotor activity in response to methylphenidate, amphetamine and cocaine in mice lacking dopamine D4 receptors. *J. Psychopharmacol. (Oxford)* 24, 897–904.
- Thomas, C. J., Tall, G. G., Adhikari, A., and Sprang, S. R. (2008). Ric-8A catalyzes guanine nucleotide exchange on Gαphail bound to the GPR/GoLoco exchange inhibitor AGS3. *J. Biol. Chem.* 283, 23150–23160.
- Thomas, E. A., Danielson, P. E., and Sutcliffe, J. G. (1998). RGS9: a regulator of G-protein signalling with specific expression in rat and mouse striatum. *J. Neurosci. Res.* 52, 118–124.
- Van Ree, J. M., Niesink, R. J., Van Wolfswinkel, L., Ramsey, N. F., Kornet, M. M., Van Furth, W. R., Vanderschuren, L. J., Gerrits, M. A., and Van den Berg, C. L. (2000). Endogenous opioids and reward. *Eur. J. Pharmacol.* 405, 89–101.
- Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A., Gocayne, J. D., Amanatides, P., Ballew, R. M., Huson, D. H., Wortman, J. R., Zhang, Q., Kodira, C. D., Zheng, X. H., Chen, L., Skupski, M., Subramanian, G., Thomas, P. D., Zhang, J., Gabor Miklos, G. L., Nelson, C., Broder, S., Clark, A. G., Nadeau, J., McKusick, V. A., Zinder, N., Levine, A. J., Roberts, R. J., Simon, M., Slayman, C., Hunkapiller, M., Bolanos, R., Delcher, A., Dew, I., Fasulo, D., Flanigan, M., Florea, L., Halpern, A., Hanchenalli, S., Kravitz, S., Levy, S., Mobarry, C., Reinert, K., Remington, K., Abu-Threideh, J., Beasley, E., Bid-dick, K., Bonazzi, V., Brandon, R., Cargill, M., Chandramouliswaran, I., Charlab, R., Chaturvedi, K., Deng, Z., Di Francesco, V., Dunn, P., Eilbeck, K., Evangelista, C., Gabrielian, A. E., Gan, W., Ge, W., Gong, F., Gu, Z., Guan, P., Heiman, T. J., Higgins, M. E., Ji, R. R., Ke, Z., Ketchum, K. A., Lai, Z., Lei, Y., Li, Z., Li, J., Liang, Y., Lin, X., Lu, F., Merkulov, G. V., Milshina, N., Moore, H. M., Naik, A. K., Narayan, V. A., Neelam, B., Nusskern, D., Rusch, D. B., Salzberg, S., Shao, W., Shue, B., Sun, J., Wang, Z., Wang, A., Wang, X., Wang, J., Wei, M., Wides, R., Xiao, C., Yan, C., Yao, A., Ye, J., Zhan, M., Zhang, W., Zhang, H., Zhao, Q., Zheng, L., Zhong, F., Zhong, W., Zhu, S., Zhao, S., Gilbert, D., Baumhueter, S., Spier, G., Carter, C., Cravchik, A., Woodage, T., Ali, F., An, H., Awe, A., Baldwin, D., Baden, H., Barnstead, M., Barrow, I., Beeson, K., Busam, D., Carver, A., Center, A., Cheng, M. L., Curry, L., Danaher, S., Davenport, L., Desilets, R., Dietz, S., Dodson, K., Doup, L., Ferreira, S., Garg, N., Gluecksmann, A., Hart, B., Haynes, J., Haynes, C., Heiner, C., Hladun, S., Hostin, D., Houck, J., Howland, T., Ibegwam, C., Johnson, J., Kalush, F., Kline, L., Koduru, S., Love, A., Mann, F., May, D., McCawley, S., McIntosh, T., McMullen, I., Moy, M., Moy, L., Murphy, B., Nelson, K., Pfannkuch, C., Pratts, E., Puri, V., Qureshi, H., Reardon, M., Rodriguez, R., Rogers, Y. H., Romblad, D., Ruhfel, B., Scott, R., Sitter, C., Smallwood, M., Stewart, E., Strong, R., Suh, E., Thomas, R., Tint, N. N., Tse, S., Vech, C., Wang, G., Wetter, J., Williams, S., Williams, M., Windsor, S., Winn-Deen, E., Wolfe, K., Zaveri, J., Zaveri, K., Abril, J. F., Guigó, R., Campbell, M. J., Sjlander, K. V., Karlak, B., Kejariwal, A., Mi, H., Lazareva, B., Hattori, T., Narechania, A., Diemer, K., Muruganujan, A., Guo, N., Sato, S., Bafna, V., Istrail, S., Lippert, R., Schwartz, R., Walenz, B., Yooseph, S., Allen, D., Basu, A., Baxendale, J., Blick, L., Caminha, M., Carnes-Stine, J., Caulk, P., Chiang, Y. H., Coyne, M., Dahlke, C., Mays, A., Dombroski, M., Donnelly, M., Ely, D., Esparham, S., Fosler, C., Gire, H., Glanowski, S., Glasser, K., Glodek, A., Gorokhov, M., Graham, K., Gropman, B., Harris, M., Heil, J., Henderson, S., Hoover, J., Jennings, D., Jordan, C., Jordan, J., Kasha, J., Kagan, L., Kraft, C., Levitsky, A., Lewis, M., Liu, X., Lopez, J., Ma, D., Majoros, W., McDaniel, J., Murphy, S., Newman, M., Nguyen, T., Nguyen, N., Nodell, M., Pan, S., Peck, J., Peterson, M., Rowe, W., Sanders, R., Scott, J., Simpson, M., Smith, T., Sprague, A., Stockwell, T., Turner, R., Venter, E., Wang, M., Wen, M., Wu, D., Wu, M., Xia, A., Zandieh, A., and Zhu, X. (2001). The sequence of the human genome. *Science* 291, 1304–1351.
- Wang, S. C., Lai, H. L., Chiu, Y. T., Ou, R., Huang, C. L., and Chern, Y. (2007). Regulation of type V adenylylate cyclase by Ric8a, a guanine nucleotide exchange factor. *Biochem. J.* 406, 383–388.
- Weiner, D. M., Levey, A. I., and Brann, M. R. (1990). Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. *Proc. Natl. Acad. Sci. U.S.A.* 87, 7050–7054.
- Wess, J. (2004). Muscarinic acetylcholine receptor knockout mice: novel phenotypes and clinical implications. *Annu. Rev. Pharmacol. Toxicol.* 44, 423–450.
- Willard, F. S., Kimple, R. J., and Siderovski, D. P. (2004). Return of the GDI: the GoLoco motif in cell division. *Annu. Rev. Biochem.* 73, 925–951.
- Willard, M. D., Willard, F. S., Li, X., Cappell, S. D., Snider, W. D., and Siderovski, D. P. (2007). Selective role for RGS12 as a Ras/Raf/MEK scaffold in nerve growth factor-mediated differentiation. *EMBO J.* 26, 2029–2040.
- Xie, K., Allen, K. L., Kourrich, S., Colon-Saez, J., Thomas, M. J., Wickman, K., and Martemyanov, K. A. (2010). Gbet5 recruits R7 RGS proteins to GIRK channels to regulate the timing of neuronal inhibitory signaling. *Nat. Neurosci.* 13, 661–663.
- Xie, Z., Geiger, T. R., Johnson, E. N., Nyborg, J. K., and Druey, K. M. (2008). RGS13 acts as a nuclear repressor of CREB. *Mol. Cell* 31, 660–670.
- Xu, M., Mizobe, F., Yamamoto, T., and Kato, T. (1989). Differential effects of M1- and M2-muscarinic drugs on striatal dopamine release and metabolism in freely moving rats. *Brain Res.* 495, 232–242.
- Yan, Z., and Surmeier, D. J. (1996). Muscarinic (m2/m4) receptors reduce N- and P-type Ca2+ currents in rat neostriatal cholinergic interneurons through a fast, membrane-delimited, G-protein pathway. *J. Neurosci.* 16, 2592–2604.
- Zachariou, V., Georgescu, D., Sanchez, N., Rahman, Z., DiLeone, R., Berton, O., Neve, R. L., Sim-Selley, L. J., Selley, D. E., Gold, S. J., and Nestler, E. J. (2003). Essential role for RGS9 in opiate action. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13656–13661.
- Zerangue, N., and Jan, L. Y. (1998). G-protein signaling: fine-tuning signaling kinetics. *Curr. Biol.* 8, R313–R316.
- Zhang, D., Zhang, L., Tang, Y., Zhang, Q., Lou, D., Sharp, F. R., Zhang, J., and Xu, M. (2005a). Repeated cocaine administration induces gene expression changes through the dopamine D1 receptors. *Neuropsychopharmacology* 30, 1443–1454.
- Zhang, Y., James, M., Middleton, F. A., and Davis, R. L. (2005b).

- Transcriptional analysis of multiple brain regions in Parkinson's disease supports the involvement of specific protein processing, energy metabolism, and signaling pathways, and suggests novel disease mechanisms. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 137B, 5–16.
- Zhang, K., Howes, K. A., He, W., Bronson, J. D., Pettenati, M. J., Chen, C., Palczewski, K., Wensel, T. G., and Baehr, W. (1999). Structure, alternative splicing, and expression of the human RGS9 gene. *Gene* 240, 23–34.
- Zhang, W., Yamada, M., Gomeza, J., Basile, A. S., and Wess, J. (2002). Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1-M5 muscarinic receptor knock-out mice. *J. Neurosci.* 22, 6347–6352.
- Zhou, F. M., Wilson, C. J., and Dani, J. A. (2002). Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J. Neurobiol.* 53, 590–605.
- Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
- Received: 14 May 2011; accepted: 23 July 2011; published online: 08 August 2011.
- Citation: Xie K and Martemyanov KA (2011) Control of striatal signaling by G protein regulators. *Front. Neuroanat.* 5:49. doi: 10.3389/fnana.2011.00049
- Copyright © 2011 Xie and Martemyanov. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Role of beta-arrestin 2 downstream of dopamine receptors in the basal ganglia

Thomas Del'Guidice, Morgane Lemasson and Jean-Martin Beaulieu*

Department of Psychiatry and Neuroscience, Faculty of Medicine, Centre de Recherche Université Laval Robert-Giffard, Québec, QC, Canada

Edited by:

Emmanuel Valjent, Université Montpellier 1 & 2, France

Reviewed by:

Takehsi Kaneko, Kyoto University, Japan

Gordon Arbuthnott, Okinawa Institute of Science and Technology, Japan

Laura M. Bohn, The Scripps Research Institute, US Minor Outlying Islands
Stefano Marullo, Institut Cochin, France

*Correspondence:

Jean-Martin Beaulieu, Centre de Recherche Université Laval Robert Giffard, 2601 Chemin de la Canardière, Suite F-6500, Beauport, Québec, QC, Canada G1J 2G3.
e-mail: martin.beaulieu@crulrg.ulaval.ca

Multifunctional scaffolding protein beta-arrestins (β Arr) and the G protein-receptor kinases are involved in the desensitization of several G protein-coupled receptors (GPCR). However, arrestins can also contribute to GPCR signaling independently from G proteins. In this review, we focus on the role of β Arr in the regulation of dopamine receptor functions in the striatum. First, we present *in vivo* evidence supporting a role for these proteins in the regulation of dopamine receptor desensitization. Second, we provide an overview of the roles of β Arr2 in the regulation of extracellular-signal-regulated kinases/MAP kinases and Akt/GSK3 signaling pathways downstream of the D1 and D2 dopamine receptors. Thereafter, we examine the possible involvement of β Arr-mediated signaling in the action of dopaminergic drugs used for the treatment of mental disorders. Finally, we focus on different potential cellular proteins regulated by β Arr-mediated signaling which could contribute to the regulation of behavioral responses to dopamine. Overall, the identification of a cell signaling function for β Arr downstream of dopamine receptors underscores the intricate complexity of the intertwined mechanisms regulating and mediating cell signaling in the basal ganglia. Understanding these mechanisms may lead to a better comprehension of the several roles played by these structures in the regulation of mood and to the development of new psychoactive drugs having better therapeutic efficacy.

Keywords: dopamine, arrestin, glycogen synthase kinase, Akt/protein kinase B, MAP kinase, striatum, biased ligand pharmacology, G protein-coupled receptors

INTRODUCTION

Dopamine is a catecholamine neurotransmitter that has several functions in the brain including the regulation of locomotion, cognition, emotions, and endocrine secretion (for review see Carlsson, 2001). Consequently, abnormal dopamine signaling could be implicated in many neuropsychiatric disorders such as schizophrenia, bipolar disorder, depression, Parkinson's disease, and drug abuse. This broad functional implication of the dopaminergic system is, at least in part, explained by its prominent cerebral innervation.

Anatomically, dopamine neurons extend widely in the central nervous system and could be dissociated in five sub-systems: nigrostriatal, mesolimbic, mesocortical, tuberoinfundibular, and tuberohypophysial (Bannon et al., 1983; Bjorklund and Stenevi, 1984). Among these, one of the major dopamine containing regions is the nigrostriatal pathway, composed of dopamine neurons originating from substantia nigra and ventral tegmental area that project respectively to the caudate putamen and nucleus accumbens, among other areas (Gerfen and Surmeier, 2011).

Dopamine signaling is mediated by two different groups of G protein-coupled receptors (GPCR). The first category, the D1 family, comprises the D1- and D5-receptors (D1R and D5R). The second family, named D2-class receptors, is formed by the D3R, D4R along with the short and long splices variants of the D2R (for a review see Missale et al., 1998; Beaulieu and Gainetdinov, 2011). In the basal ganglia, D1R is mostly expressed by GABAergic medium

spiny neurons (MSN) of the caudate putamen (striatum) while D2R are found in both MSN and dopamine neurons. Recent studies using bacterial artificial chromosome (BAC) transgenic mice expressing specific reporters (e.g., eGFP and/or dtTomato) under the control of the D1R or D2R gene promoters, revealed a high level of segregation of D1R- and D2R-containing MSN (Figure 1) of the striatum and nucleus accumbens (Shuen et al., 2008; Valjent et al., 2009). These data confirmed that MSN could be separated into two subgroups according to their projection areas and the type of dopaminergic receptors that they express. One of these subgroups includes MSN of the direct striatonigral pathway, which mostly express D1R, and project to the medial globus pallidus and substantia nigra pars reticulata. The second group is composed of MSN of the "indirect" striatopallidal track that project to the lateral globus pallidus and express preferentially D2R (Gerfen and Surmeier, 2011). In the striatum, activation of D1R appears to facilitate transmission along the direct pathway while activation of post-synaptic D2R would inhibit transmission along the indirect pathway (Parent et al., 2000; Kravitz et al., 2010). However, it is noteworthy that a small subpopulation of MSN synthesizes both D1R and D2R and may have different dopamine receptor signaling properties (Rashid et al., 2007; Valjent et al., 2009).

Functionally, dopamine receptors signal through G protein-dependent cellular processes. D1R is mostly coupled to *Gas*/olf proteins and stimulate the activity of adenylate cyclase and the production of the second messenger cAMP (Spano et al., 1978;

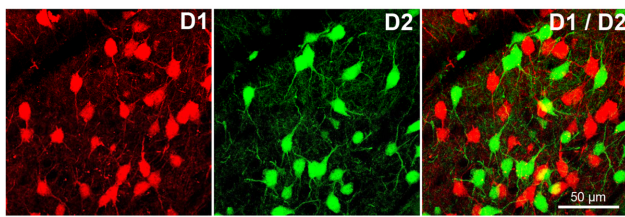


FIGURE 1 | D1 and D2 receptors are segregated in different cells in the striatum and in the nucleus accumbens. The first image (left) shows striatal cells of the direct striatonigral pathway expressing D1 receptors (specific reporter dtTomato under the control of D1R promoter). Second image (middle) shows striatal cells of the indirect striatopallidal track expressing D2 receptors (specific reporter eGFP under the control of D2R promoter). The third picture (right) shows the lack of colocalization of D1 and D2 receptors in striatal cells (yellow parts of cells are cell overlap artifacts).

Kebabian and Calne, 1979). In contrast, D2R is associated to G α i/o proteins and inhibit the production of cAMP. The coupling of dopamine receptors to different G proteins allows this neurotransmitter to act on multiple cell signaling molecules such as kinases and phosphatases (Beaulieu and Gainetdinov, 2011). As for other GPCR, activation of dopamine receptors is quickly followed by their rapid phosphorylation by GPCR kinases (G protein-receptor kinases, GRK; Premont and Gainetdinov, 2007). This results in the recruitment of multifunctional scaffolding proteins termed beta-arrestin 1 and beta-arrestin 2 (β Arr1 and β Arr2) in order to desensitize and internalize GPCR (Gainetdinov et al., 2004; Shenoy and Lefkowitz, 2005). The recruitment of at least β Arr2 to D1R and D2R has also been reported to activate cellular signaling in a G protein-independent manner by inducing the formation of functional protein complexes in which β Arr acts as a scaffold for different kinases and phosphatases (Beaulieu et al., 2005; Urs et al., 2011).

In this review, we provide an overview of our current understanding of the different contributions of β Arr to dopamine receptor functions putting an emphasis on studies conducted directly in the mouse striatum. First, we present an outline of *in vivo* studies of the involvement of β Arr and GRK in dopamine receptor desensitization and internalization. We then summarize different lines of evidence supporting the implication of β Arr-dependent signaling events downstream of both D1R and D2R. Thereafter, we examine the possible involvement of β Arr and its molecular interactors in the action of drugs used for treatment of psychiatric disorders. Finally, we discuss briefly evidences pointing toward possible molecular mechanisms through which β Arr-mediated signaling can contribute to the regulation of neuronal functions by dopamine.

A ROLE FOR BETA-ARRESTIN IN THE REGULATION OF DOPAMINE RECEPTOR FUNCTIONS

Following receptor stimulation, homologous GPCR desensitization constitutes a crucial mechanism to protect cells against overstimulation of the receptors. This phenomenon begins with the phosphorylation at specific sites of the activated GPCR by GRK. This first step is followed by the recruitment and the binding of the multifunctional adaptor proteins arrestins (Lohse et al.,

1990a; Pitcher et al., 1998; Pierce and Lefkowitz, 2001; Gainetdinov et al., 2004; Premont, 2005). Association of β Arr to GPCR prevents further G protein activation therefore ensuing receptor desensitization (Lohse et al., 1990b). β Arr also promote receptor internalization from the cellular membrane to the cytoplasm through the subsequent binding of arrestins to the clathrin adaptor protein adaptin (AP2; Laporte et al., 2002) and to clathrin itself (Krupnick et al., 1997). This process triggers clathrin-mediated endocytosis of the receptor and either its subsequent recycling to the cell surface or degradation (Figure 2; Ferguson et al., 1996; Ferguson, 2001; Claing et al., 2002; Claing and Laporte, 2005). Dopamine receptor activity is modulated by desensitization via β Arr1 and β Arr2. Evidences that support the involvement of β Arr1 in the regulation of D1R and D2R, come from *in vitro* studies (Kim et al., 2001; Oakley et al., 2001). Furthermore, research conducted in heterologous cellular systems also indicates a role for β Arr2 in desensitization of D1R, D2R, and D3R (Kim et al., 2001; Oakley et al., 2001; Gainetdinov et al., 2004; Lan et al., 2009). Thus, β Arr proteins could serve as key regulators in dopamine receptor activity.

In vivo studies using different enhancers of dopamine neurotransmission (cocaine and amphetamine) and dopamine agonists (apomorphine), have resulted in a more complex picture of the role of arrestins in dopamine receptor functions (Gainetdinov et al., 2004; Beaulieu et al., 2009). The psychostimulants cocaine and amphetamine both affect the functions of the dopamine transporter (DAT) and induce an increase of extracellular dopamine levels, resulting in over-stimulation of dopamine receptors. In normal mice, enhancement of striatal dopaminergic activity following acute administration of these drugs leads to a hyperactive locomotion phenotype. Impairment of desensitization by deleting one of its mediators, GRK6, in GRK6-knock-out mice (GRK6-KO), exacerbates psychostimulant-induced hyperactivity as compared to wild-type (WT) animals (Gainetdinov et al., 2003). Furthermore, characterization of dopamine receptor functions in these mice has shown that a lack of GRK6 results in reduced D2R desensitization. This indicates that impaired desensitization of dopamine receptors in mice lacking GRK6 actively contributes to enhanced locomotor response to psychostimulants acting on dopaminergic neurotransmission.

Surprisingly, inactivation of other components of the desensitization machinery such as β Arr1 and β Arr2 does not reproduce the behavioral effects observed in GRK6-KO mice after dopaminergic drugs exposure. Indeed, β Arr1-KO and β Arr2-KO mice both display a reduced behavioral responsiveness to the non-selective dopamine agonist apomorphine (Gainetdinov et al., 2004; Beaulieu et al., 2005). In addition, reduced locomotor responsiveness to amphetamine has also been reported in β Arr2-KO mice (Beaulieu et al., 2005) while behavioral responsiveness to cocaine does not appear to be affected by an absence of either β Arr1 or β Arr2 (Bohn et al., 2003; Gainetdinov et al., 2004). β Arr2 also appears to contribute to the dopamine-dependent effect of the opiate drug morphine. Administration of morphine in mice produces locomotor hyperactivity by increasing dopamine release from the presynaptic terminals (Kuschinsky and Hornykiewicz, 1974). Interestingly, this behavioral effect of enhanced dopamine neurotransmission resulting from morphine administration has

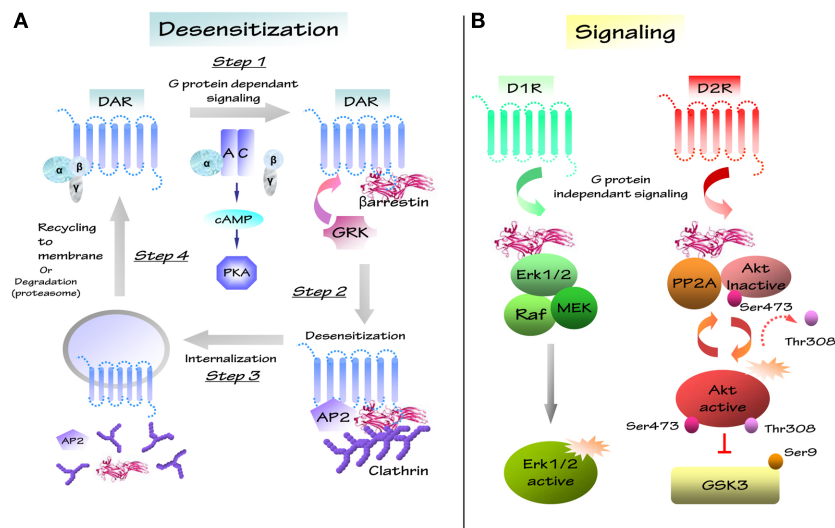


FIGURE 2 | Schematic representation of dopaminergic desensitization and signaling. (A) After stimulation, GPCR/DAR regulates different second messengers like adenylate cyclase (AC), cyclic adenosine monophosphate (cAMP), protein kinase A (PKA) in a G protein-dependent way (step1). Signaling induces the recruitment of G protein-receptor kinases family (GRK) and of multifunctional scaffolding protein β arrestin, which induce the formation of the receptor/protein complex (GPCR/AP2/ β arrestin/clathrin; step2). GPCR-associated protein complex is

internalized in clathrin coated-pits and complex is disassembled (step3). Internalized GPCR is recycled to membrane or degraded by proteasome (step4). **(B)** D1 and D2 receptors can regulate several molecular pathways through different G protein-independent signalling. D1 receptor (D1R) may regulates protein complex composed of β arrestin 2/Erk/Raf/MEK and induces Erk activation. D2 receptor (D2R) modulates the Akt/ β arrestin 2/PP2A protein complex and the Akt downstream substrate GSK3 that is inhibited by Akt.

also been reported to be reduced in mice deficient for β Arr2 (Bohn et al., 2003). Finally, the locomotor consequences of a genetic enhancement of dopamine tones also appear to be partially counteracted by a loss of β Arr2. In mice, genetic deletion of the DAT abolishes dopamine reuptake (Jones et al., 1998). This induces an increase of extra synaptic dopamine levels and subsequent hyperactive phenotype (Giros et al., 1996; Gainetdinov et al., 1999). However, double mutant mice lacking both β Arr2 and the DAT display a reduction in this novelty-induced locomotor hyperactivity phenotype (Beaulieu et al., 2005). Overall, results from these different studies indicate that a lack of β Arr1 or β Arr2 leads to a reduction in the locomotor response to dopamine receptor stimulation. The only major exception is locomotor response to acute cocaine, which is not overtly affected by a lack of either β Arr1 or β Arr2. The reasons these discrepancies remain obscure. One possibility is that acute cocaine administration may trigger locomotor responses by acting also on other neurotransmitter systems. In line with this, it is of interest that acute but not chronic locomotor response to cocaine has been shown to be severely reduced in mice expressing low levels of the glutamate/NMDA receptor subunit NR1 while locomotor responses to amphetamine were left essentially unaffected in these mice (Ramsey et al., 2008).

In conclusion, while β AR and GRK appear to play a role in dopamine receptor desensitization and internalization in cultured cells systems, this role is only clearly supported at the behavioral level by data obtained in GRK6-KO mice. In contrast, lack of β Arr has either a minimal effect or reduced the behavioral responsiveness to certain drugs acting on dopamine functions therefore suggesting that β Arr may be implicated in

different cellular processes regulating dopaminergic signaling and locomotor behaviors (Beaulieu et al., 2005, 2007a; Urs et al., 2011).

REGULATION OF ERK/MAP KINASE SIGNALING BY β Arr2 AND D1R

Dopamine receptor-regulated behaviors are frequently associated to the activation of extracellular-signal-regulated kinases (ERK)/MAP kinase signaling (Beaulieu et al., 2006; Girault et al., 2007; Beaulieu and Gainetdinov, 2011). The serine threonine kinase ERK is positively regulated following its phosphorylation by the MAP kinase-kinase, MAPK/ERK kinase (MEK) that is itself activated by the MAP kinase-kinase-kinase RAF following the activation of different types of receptors including several receptor tyrosine kinases (RTK) and GPCR (Girault et al., 2007). Different lines of evidence have demonstrated the importance of ERK-mediated signaling in the maintenance of dopamine receptor functions. For instance, activation of D1R has been shown to activate ERK in the mouse striatum while conversely inhibition of ERK leads to reductions of hyper-locomotor responses in DAT-KO mice or following administration of amphetamine or cocaine (Berhow et al., 1996; Valjent et al., 2000, 2006a; Beaulieu et al., 2006; Girault et al., 2007). Furthermore, activation of ERK by dopamine receptors also appears to play an important role in long-term changes in synaptic plasticity in response to drug of abuse (Miller and Marshall, 2005) or in response to L-DOPA therapy for Parkinson's disease (Santini et al., 2007).

In transfected cells, β Arr2 can support the formation of a mitogen activated protein kinase (MAP kinase) complex composed of β Arr2 and the MAP kinase pathway kinases RAF, MEK, and ERK in response to GPCR activation (Ahn et al., 2004; Shenoy

and Lefkowitz, 2005) resulting in an activation of ERK (Luttrell et al., 2001; Wei et al., 2004; Luttrell and Gesty-Palmer, 2010). Therefore, β Arr2 may serve to promote ERK-mediated signaling underlying dopamine-regulated behaviors. Recent evidence suggests that β Arr2 may participate in locomotor response and activation of ERK in response to D1R stimulation. Urs et al. (2011) have reported that acute morphine administration increases the interaction between β Arr2 and ERK in the striatum of WT but not of D1R-KO mice as measured by co-immunoprecipitation assays. In addition, morphine-dependent locomotion and activation of striatal ERK2 by morphine were curtailed in mice lacking either the D1R or β Arr2. Finally, co-administration of the indirect ERK inhibitor SL327 along with morphine to WT mice also reduced the locomotor response to morphine. This suggests that the D1R may regulate ERK signaling and may contribute to the dopamine-mediated locomotor responses to morphine by acting via a β Arr2-dependent mechanism. However, there is also strong evidence for a role of canonical G protein/cAMP-mediated mechanisms in the regulation of ERK activity by D1R (Girault et al., 2007). Therefore, results obtained in this study following administration of SL327 or in D1R-KO mice may also reflect an effect of these experimental conditions on other D1R receptor signaling mechanisms and further studies are needed to establish the contribution of G protein and β Arr2 in the regulation of ERK activity and behavior by D1R.

THE REGULATION OF Akt AND GSK3 BY DOPAMINE AND BETA-ARRESTIN 2

Over the years, several lines of evidence have pointed toward the contribution of β Arr-mediated mechanisms in the regulation of Akt and GSK3, two serine/threonine kinases, by dopamine.

Akt, also termed protein kinase B, is known to be involved in various cellular processes such as transcription, glucose metabolism, proliferation, migration, and with insulin and neurotrophin action through the stimulation of RTK (Cross et al., 1995; Alessi et al., 1996; Scheid and Woodgett, 2001). Activation of RTK and some GPCR regulates the phosphoinositide-3 kinase (PI3K), which converts phosphatidyl-inositol-2-phosphate (PIP2) to phosphatidyl-inositol-3-phosphate (PIP3; For review, Martelli et al., 2010). Then, the newly formed PIP3 interacts with the pleckstrin homology domain of Akt, inducing its recruitment to the plasma membrane. This cellular process results in the phosphorylation of Akt at threonine 308 and serine 473 residues by two phosphatidyl-dependent kinases, PDK1 and PDK2/ricor-mTOR, respectively (Scheid and Woodgett, 2001; Jacinto et al., 2006). Once activated, Akt phosphorylates in turn several substrates including GSK3 (Rossig et al., 2002).

Originally known for its role in the control of glycogenesis in response to insulin, GSK3 is implicated in numerous physiological processes such as embryonic development, cell differentiation, cell survival, Wnt signaling, apoptosis as well as serotonin, and dopamine receptor signaling (Woodgett, 2003; Beaulieu et al., 2004, 2008b; Li et al., 2004). Mammalian cells express two isoforms of GSK3, GSK3 α , and GSK3 β , which are constitutively active and can phosphorylate several cellular substrates (Woodgett, 1990). GSK3 α and GSK3 β are negatively regulated by several kinases involved in PI3K and MAP kinases signaling pathways (Frame

and Cohen, 2001). Additionally, Akt phosphorylation inhibits the two isoforms of GSK3 in response to growth factors and hormones, including BDNF, IGF, and insulin (Yamada et al., 2002; Altar et al., 2008). Specifically, Akt phosphorylates specific residues serine 21 for GSK3 α and serine 9 for GSK3 β that are located in the N-terminal domains of both GSK3 isoforms (Stambolic and Woodgett, 1994; Frame and Cohen, 2001). Therefore, Akt is an important regulator of various signaling cascades involving GSK3.

Experiments using DAT-KO mice, dopamine depletion or dopamine receptors agonist/antagonists have provided converging evidence for a negative regulation of Akt resulting in an activation of GSK3 isoforms by D2-class receptors in mammals and other vertebrates (Beaulieu et al., 2004; Bychkov et al., 2007; Chen et al., 2007; Souza et al., 2011). Consequently, pharmacological D2-class receptors antagonists induce Akt activation and subsequent GSK3 inhibition (Beaulieu et al., 2004; Emamian et al., 2004). Furthermore, experiments conducted using mice lacking different dopamine receptors showed that a loss of D2R but not D1R prevented the inactivation of striatal Akt by drugs acting on dopamine neurotransmission (Beaulieu et al., 2007b). In contrast, mice deficient for the D3R exhibit a reduced responsiveness of Akt phosphorylation to dopaminergic drugs. Therefore, D2R would be crucial for the inhibition of Akt by dopamine, while the D3R appears to potentiate the D2R dopamine response (Beaulieu et al., 2007b).

It has been demonstrated that β Arr2 is actively involved in dopamine-regulated Akt inhibition (Beaulieu et al., 2004, 2005). The role of β Arr2 in mediating the regulation of Akt and GSK3 by D2R is supported both by behavioral and direct *in vivo* biochemical observations in pharmacological and genetic models of enhanced dopamine neurotransmission. At the behavioral level, β Arr2-KO mice present lower locomotor activity in response to the dopamine-dependent action of amphetamine and apomorphine (Gainetdinov et al., 2004; Beaulieu et al., 2005). In line with this, increased locomotor activity observed in mice lacking the DAT is also antagonized by the absence of β Arr2 in double knockout animals (Beaulieu et al., 2005). At the biochemical level, both amphetamine and apomorphine have been shown to inhibit the phosphorylation and activation of Akt in the striatum of WT mice while these two drugs failed to inhibit Akt in β Arr2-KO mice. Furthermore, genetically increased dopaminergic tones were also shown not to affect Akt activity in mice deficient for both DAT and β Arr2, suggesting an important role of this scaffolding protein in Akt regulation by dopamine. Further characterization of the molecular mechanisms underlying the regulation of Akt by D2R following receptor stimulation has shown that β Arr2 is involved in the formation of a protein complex composed of Akt, β Arr2, and protein phosphatase 2A (PP2A; Beaulieu et al., 2005). Once in the complex, PP2A dephosphorylates and deactivates Akt, resulting in the activation of GSK3 (Beaulieu et al., 2004, 2005).

One interesting observation emerging from the regulation of this pathway is a difference of kinetics that points toward the existence of two modalities of cell signaling responses to slow synaptic neurotransmission. Indeed, inhibition of Akt is a slower and more persistent phenomenon than events mediated by the cAMP-PKA pathway (Beaulieu et al., 2004, 2005, 2007a). On the one hand, administration of amphetamine in mice induces

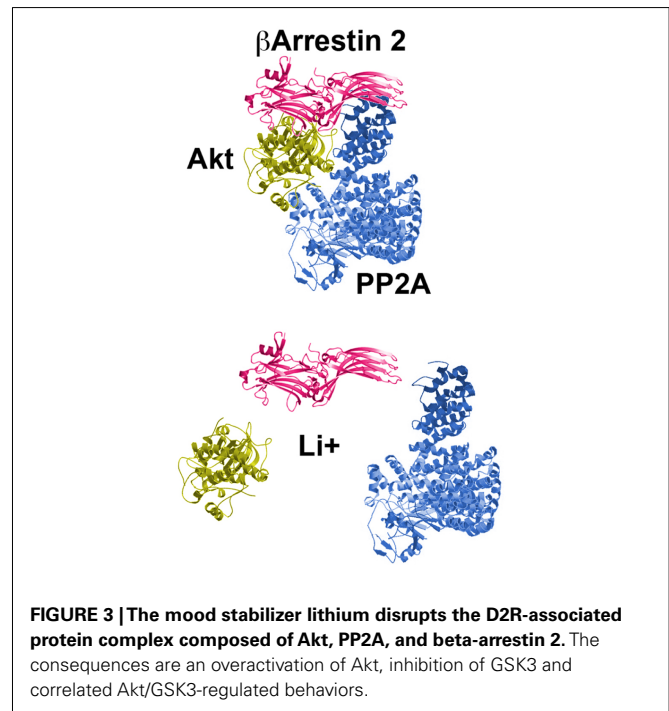
a cAMP-dependent phosphorylation of ERK and DARPP-32 during the first 30 min after drug administration (Valjent et al., 2005). On the other hand, Akt inhibition following acute treatment with amphetamine becomes significant after 30 min and persists over the duration of the drug behavioral effects (Beaulieu et al., 2007a, 2005). These different kinetics suggest that dopamine-regulated behaviors and action of dopaminergic drugs depend, at least in part, from different and temporally successive waves of GPCR signaling mediated respectively by G proteins and the β Arr2.

ARRESTIN/Akt/GSK3, DOPAMINE SIGNALING AS A TARGET FOR MOOD STABILIZERS

The most prescribed and studied mood stabilizer, lithium, is known for its therapeutic effects in the treatment of bipolar disorders (Cade, 1949; Schou et al., 1954; Phiel and Klein, 2001; Blanco et al., 2002). However more than 60 years after its introduction, the molecular mechanisms underlying the therapeutic actions of lithium are still unknown. Among other proposed mechanisms, lithium can directly and indirectly inhibit the activity of GSK3 (Stambolic et al., 1996; O'Brien and Klein, 2009). In mice, indirect inhibition of GSK3 occurs following the activation of its direct regulator Akt (Chalecka-Franaszek and Chuang, 1999; Beaulieu et al., 2004, 2008a). It has recently been demonstrated that this effect of lithium on the activity of Akt could be mediated by β Arr2, at least in the mouse striatum (Beaulieu et al., 2008a). Biochemically, lithium disrupts the β Arr2/Akt/PP2A signaling complex that is regulated by D2R (Figure 3). This leads to an overactivation of Akt and subsequent indirect GSK3 inhibition. In β Arr2-KO mice, lithium fails to inhibit GSK3 and to activate Akt as it does in the striatum of WT mice (Beaulieu et al., 2008a). Furthermore, in these mutant mice, chronic treatment with lithium does not exert antimanic and antidepressant-like effects seen in control animals. This finding provides strong correlative evidence that lithium could exert some of its biochemical and behavioral effects by interfering with the dopamine associated- β Arr2 signaling complex involved in the regulation of Akt and GSK3. This possible mechanism of action is further supported by recent observations indicating that expression of the Akt isoform Akt1 is also essential for the regulation of some behaviors by lithium in mice (Pan et al., 2011). Interestingly, other mood stabilizers such as valproic acid and lamotrigine might also modulate Akt/GSK3 signaling (Chen et al., 1999; Li et al., 2002; Beaulieu and Caron, 2008). Indeed, both mood stabilizers decrease the phosphorylation of GSK3 substrates (Gould and Manji, 2005). However, these different drugs have to be further studied to investigate a possible involvement of Akt/GSK3 signaling in their therapeutic effects.

A ROLE FOR Akt AND GSK3-MEDIATED DOPAMINE SIGNALING IN THE ACTION OF ANTIPSYCHOTICS

Antipsychotics are a heterogeneous family of drugs used for the treatment of several psychiatric disorders, mostly schizophrenia. Among these drugs, so called first generation or “typical” antipsychotics like haloperidol and chlorpromazine are believed to exert most of their therapeutic action in schizophrenia by blocking D2-class receptors (Snyder, 1976). In addition, more recent second generation or “atypical” antipsychotics (AA) like clozapine have been shown to retain D2-class receptor antagonist functions while



also acting on other “non-dopamine” receptor types (Meltzer, 1991; Kapur and Remington, 2001). Considering the role of D2R in regulating the β Arr2/Akt/GSK3 pathway, it is not surprising that antipsychotics with D2R antagonist properties have been reported to activate Akt and inhibit GSK3 *in vivo* (Emamian et al., 2004; Alimohamad et al., 2005; Beaulieu et al., 2007a; Li et al., 2007). Among these, haloperidol prevents the inhibition of Akt and concomitant activation of GSK3 by dopamine (Beaulieu et al., 2004; Emamian et al., 2004). Similarly AA such as clozapine, olanzapine, risperidone, quetiapine, and ziprasidone, have been shown to activate Akt or mimic its activity by increasing GSK3 α and GSK3 β phosphorylation in the striatum, cortex, and hippocampus (Alimohamad et al., 2005; Li et al., 2007; Beaulieu et al., 2009). Unfortunately, the functional involvement of β Arr2 has not been explored in these *in vivo* studies. However, *in vitro* experiments using bioluminescent resonance energy transfer (BRET) have suggested that both typical and AA may induce some of their effects by antagonizing the recruitment of β Arr2 to the D2R long isoform (D2_LR; Masri et al., 2008). More specifically, this study has shown three different effects of antipsychotics on the inhibition of cAMP-synthesis and arrestin recruitment in response to D2_LR stimulation. Some drugs, like haloperidol, antagonized both the regulation of cAMP and the recruitment of β Arr2 to the D2_LR with a similar efficacy. In contrast, other drugs like clozapine appeared to be stronger antagonists of β Arr2 recruitment than of cAMP modulation. Finally, other antipsychotics like aripiprazole acted as partial antagonists of cAMP regulation while fully inhibiting β Arr2 recruitment. Taken together, these results suggest that antipsychotics share common antagonistic properties toward D2R/ β Arr2-mediated signaling while having different effects on the control of cAMP production by this same receptor, at least in transfected cells. It would thus be interesting to explore the physiological action of each of these drugs on Akt and GSK3 *in vivo*

to establish whether the effects of these psychoactive drugs are closely related to D2R/ β Arr2/Akt/GSK3 signaling.

It should be noted however, the implication of D2R in the regulation of Akt and GSK3 by AA can also be explained by other mechanisms. One alternative could involve serotonin neurotransmission. Indeed, most AAs are 5-HT_{2A} receptors antagonists (Kapur and Remington, 2001). Interestingly, the 5-HT_{2A} agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) activates GSK3 *in vivo* (Li et al., 2004) while AA have the opposite effect on the activity of this kinase (Li et al., 2007). Furthermore, enhancers of serotonin neurotransmissions like serotonin reuptake inhibitors and fenfluramine or genetic manipulations decreasing serotonin synthesis have also been shown to affect GSK3 activity *in vivo* (Li et al., 2007; Beaulieu et al., 2008b; **Figure 4**). Additional characterization is therefore needed to determine the relative contribution of dopamine and serotonin receptors in the modulation of Akt/GSK3 by AA drugs and to define the roles that they may have in psychotic disorders.

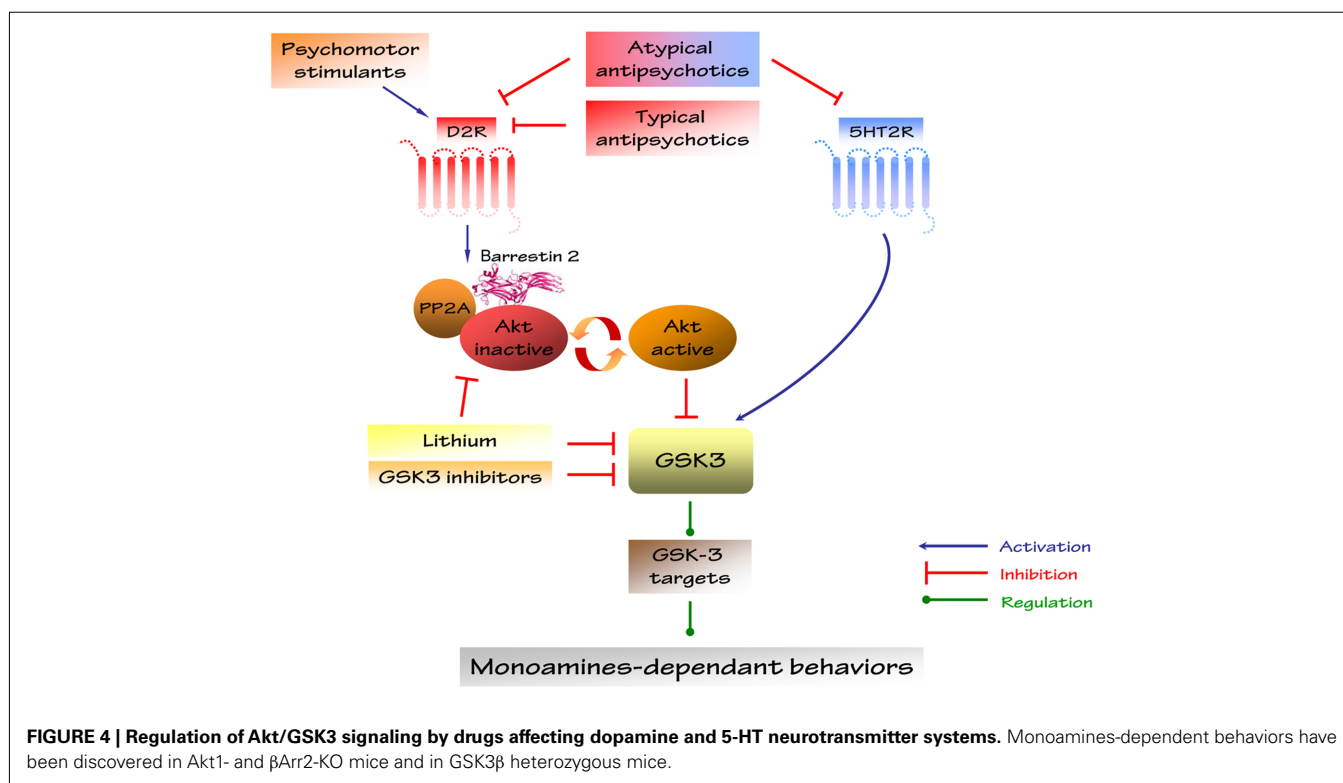
MOLECULAR TARGETS OF DOPAMINE REGULATED BY β Arr2, Akt, AND GSK3 SIGNALING

While several lines of evidence indicate a role for β Arr2, Akt, and GSK3 in dopamine receptor signaling, there is little information on the nature of the molecular targets of these kinases that are affected by dopamine receptors in the basal ganglia or other brain regions. Akt and GSK3 have various substrates involved in numerous cellular processes linked to mental illnesses-associated physiological functions like cytoskeleton organization, trafficking, cell survival, apoptosis, and DNA transcription (Frame and Cohen, 2001; Woodgett, 2001). Here, we present three different molecular

systems that are regulated by GSK3 and for which there are some evidences for direct involvement of dopamine receptor signaling: the direct GSK3 substrate β -catenin, ionotropic glutamate receptors, and the regulation of circadian rhythms.

β -CATENIN

β -catenin has multiple roles in the cell. This protein functions as a transcription factor and a scaffolding protein, which anchors the actin cytoskeleton as a mediator of adherent junctions. β -catenin is a common component of the Wnt and Akt/GSK3 signaling pathway (For review: Freyberg et al., 2010). In the absence of Wnt stimulation, β -catenin forms a complex with GSK3 and several other proteins. This induces the phosphorylation of β -catenin by GSK3 and then its ubiquitination and proteasomal degradation (Doble and Woodgett, 2003). Conversely, activation of the Wnt receptor Frizzled leads to the disruption of this complex by the protein Dishevelled (Dvl). Free β -catenin can then translocate to the nucleus and affect gene expression (Fukushima et al., 2001). Interestingly, the regulation of the Akt/GSK3 signaling cascade by D2R and β Arr2 may affect β -catenin activity. Interestingly, chronic treatment with the mood stabilizer lithium in mice increases β -catenin levels in different brain regions such as the amygdala, striatum, hypothalamus, and hippocampus (O'Brien et al., 2004; Beaulieu et al., 2008a). It is has been shown that increased levels of striatal β -catenin in response to lithium is dependent upon the expression of β Arr2 therefore suggesting that changes in β -catenin in response to lithium may arise from a disruption of β Arr2-mediated D2R signaling (Beaulieu et al., 2008a). In addition, overexpression of β -catenin in mice reproduces the behavioral effects of the GSK3 inhibition



by lithium on dopamine-dependent locomotor hyperactivity and in tests evaluating antidepressant and anxiolytic effects of drugs in rodents (Gould et al., 2008). The functional consequences of these drugs could be mediated by the striatum, since the β -catenin forebrain-specific conditional knock-out mouse shows little behavioral changes (Gould et al., 2008). However, this still has to be established.

GLUTAMATE RECEPTORS

The modulation of GSK3 signaling by dopamine receptors also appears to regulate some functions of NMDA ionotropic glutamate receptors and associated synaptic plasticity (Chen et al., 2007). In particular, GSK3 activity modulates the development of long-term depression (LTD) in rat hippocampal slices, two processes regulated by ionotropic glutamate receptors (Zhu et al., 2007; Peineau et al., 2008). Furthermore, Akt/GSK3-mediated signaling appears to be responsible for the regulation of NMDA receptors by D2R in response to high levels of dopamine in the rat frontal cortex (Li et al., 2009). Briefly, application of high concentrations of dopamine induces a reduction of NMDA current associated with the internalization of the NMDA receptor subunit NR2B both in cortical slice preparations and *in vivo*. The effect of elevated dopamine on NMDA receptor functions is dependent of D2R, GSK3, and PP2A and is not affected by inhibition of G proteins, therefore suggesting an involvement of β Arr2-mediated signaling (Li et al., 2009). However, the contribution of β Arr2 to the regulation of NMDA receptor functions as a result of elevated dopamine levels has not been confirmed. Also, it remains unclear whether this type of regulation occurs in the striatum or if it is restricted to cortical neurons.

REGULATION OF CLOCK GENE SIGNALING

The deregulation of circadian rhythm is thought to be an integral part of human mental diseases like bipolar disorders and in particular seasonal affective disorders (Benedetti et al., 2004; Mansour et al., 2005). One of the first studies associating GSK3 to the regulation of circadian rhythm has been made in *drosophila*. Yuan et al. (2005) have shown that the fly GSK3 ortholog, Shaggy, is regulated by serotonin and affects the circadian cycle. Then, numerous studies have demonstrated the regulation of clock genes by GSK3 in mammals (Iitaka et al., 2005; Lamont et al., 2007). For example, the GSK3 inhibitor lithium affects the transcription of the clock gene *Bmal1* (Lamont et al., 2007). In line with this, GSK3 β can also regulate mammalian circadian protein functions *in vitro* (Iitaka et al., 2005). Interestingly, circadian rhythm-regulated gene expression and associated behaviors can also be modulated by the D2R, which is known to regulate GSK3 (Doi et al., 2006; Yujnovsky et al., 2006). D2R is highly expressed in the retina and plays an important role in neural adaptation to light (Doi et al., 2006). Accordingly, activation of D2R stimulates CLOCK:BMAL1 functions through MAP Kinases pathways and could possibly regulate circadian gene expression through β Arr2/Akt/GSK3 signaling (Sahar et al., 2010). This suggests a putative relevant link between circadian rhythm and dopamine receptor functions.

CONCLUSION AND PERSPECTIVES

The relatively recent identification of G protein-independent β Arr-mediated signaling of GPCR has radically changed our

perception of the roles played by these molecules in dopamine receptor functions. From simple desensitization molecules, β Arr are now seen as polyvalent GPCR-associated scaffolding proteins having complex and multiple functions in regulating the final outcome of receptor stimulation (Wei et al., 2003; Gesty-Palmer et al., 2006). Extensive and elegant research efforts have shown how downstream targets of dopamine receptors such as DARPP-32 can act as integrators and coincidence detectors modulating the outcome of dopamine receptor signaling in function of the activation of other neurotransmitter systems (Svenningsson et al., 2004). When put in this context, the dual functions of β Arr add an extra layer of complexity to dopamine receptor signaling. Indeed the co-occurrence of G protein-dependent and β Arr-mediated responses reveals that dopamine receptors also exhibit signaling diversity. Through such diversity, a given receptor may regulate several modalities of signaling under different conditions or during different periods of its activation cycle. This can have several important physiological consequences and raises many pending questions that will have to be addressed further in future research.

First, signal diversity downstream of dopamine receptors may have a considerable impact on the development of pharmacological approaches to manipulate dopamine receptor functions. The assumption that a receptor is engaged in exclusively one type of signaling event regardless of its cellular composition has been the basis of the definition of the intrinsic drug efficacy (Galandrin et al., 2007). However, a context where dopamine receptor can elicit different cell signaling responses involving G protein, β Arr, or other signaling molecules has led to the suggestion that it may be possible to generate new biased ligands for dopamine receptors that would modulate one or another cell signaling mechanism preferentially (Beaulieu et al., 2007a). It is believed that such biased agonism can have important implications for drug development because intracellular signaling events mediated by these parallel pathways may have distinct physiological consequences. As discussed above, D2R has been shown to be one of the best examples of this duality in receptor signaling, because it is involved in the regulation of the G protein-mediated functions and the β Arr2-mediated Akt/GSK3 signaling cascade (Beaulieu et al., 2009). Although there are no known D2R ligands affecting specifically one or the other of these signaling modalities, it may be possible to develop drugs to selectively target one of these pathways. In fact, the ability of the mood stabilizer drug lithium to disrupt β Arr2-mediated Akt/GSK3 signaling and to suppress the behavioral effects that are related to enhanced dopaminergic transmission provides the first evidence for the activity of clinically effective compounds on β Arr2 scaffolded signaling complexes downstream of the D2R (Beaulieu et al., 2008a, 2009; Rajagopal et al., 2010).

Prior to develop drugs acting preferentially on one or another cell signaling response, it would be important to decipher what is the relative role of these different modalities of signaling in regulating the final physiological outcome of dopamine receptor activation. This can be particularly important in the context of MAP kinase regulation. Indeed, ERK can be regulated by both G protein/DARPP-32 and β Arr2-mediated signaling events

downstream of D1R (Valjent et al., 2006b; Urs et al., 2011). Considering the important role of this kinase in regulating both acute and long-term consequences of dopamine receptor signaling (Beaulieu et al., 2006; Valjent et al., 2006b), it would be important to establish what is the level of segregation (if any) between the functions exerted by ERK when it is activated by either mechanisms. This can be important since association with a β Arr signaling complex may affect the subcellular localization of activated ERK and therefore change the nature of its substrates (Pierce and Lefkowitz, 2001). The same observations can also be made for the regulation of Akt and GSK3 by D2R since alternative mechanisms such as the transactivation of the BDNF receptor TrkB by dopamine receptors (Swift et al., 2011) may also affect Akt and GSK3 activity in specific neuronal populations.

Another pending issue is the nature of the contribution of β Arr1 in mediating dopamine receptor signaling. As of today, most of the attention has been focused on the role played by β Arr2 in both D1R- and D2R-mediated signaling. However, behavioral evidence indicates that β Arr1-KO mice also exhibit behavioral responses to dopamine drugs that are compatible with a major role of β Arr1 in mediating some signaling responses in addition to its probable function in canonical homologous receptor desensitization (Gainetdinov et al., 2004).

REFERENCES

- Ahn, S., Shenoy, S. K., Wei, H., and Lefkowitz, R. J. (2004). Differential kinetic and spatial patterns of beta-arrestin and G protein-mediated ERK activation by the angiotensin II receptor. *J. Biol. Chem.* 279, 35518–35525.
- Alessi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P., and Hemmings, B. A. (1996). Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J.* 15, 6541–6551.
- Alimohamad, H., Rajakumar, N., Seah, Y. H., and Rushlow, W. (2005). Antipsychotics alter the protein expression levels of beta-catenin and GSK-3 in the rat medial prefrontal cortex and striatum. *Biol. Psychiatry* 57, 533–542.
- Altar, C. A., Hunt, R. A., Jurata, L. W., Webster, M. J., Derby, E., Gallagher, P., Lemire, A., Brockman, J., and Laeng, P. (2008). Insulin, IGF-1, and muscarinic agonists modulate schizophrenia-associated genes in human neuroblastoma cells. *Biol. Psychiatry* 64, 1077–1087.
- Bannon, M. J., Wolf, M. E., and Roth, R. H. (1983). Pharmacology of dopamine neurons innervating the prefrontal, cingulate and piriform cortices. *Eur. J. Pharmacol.* 92, 119–125.
- Beaulieu, J. M., and Caron, M. G. (2008). Looking at lithium: molecular moods and complex behaviour. *Mol. Interv.* 8, 230–241.
- Beaulieu, J. M., and Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol. Rev.* 63, 182–217.
- Beaulieu, J. M., Gainetdinov, R. R., and Caron, M. G. (2007a). The Akt-GSK-3 signaling cascade in the actions of dopamine. *Trends Pharmacol. Sci.* 28, 166–172.
- Beaulieu, J. M., Tirotta, E., Sotnikova, T. D., Masri, B., Salahpour, A., Gainetdinov, R. R., Borrelli, E., and Caron, M. G. (2007b). Regulation of Akt signaling by D2 and D3 dopamine receptors in vivo. *J. Neurosci.* 27, 881–885.
- Beaulieu, J. M., Gainetdinov, R. R., and Caron, M. G. (2009). Akt/GSK3 signaling in the action of psychotropic drugs. *Annu. Rev. Pharmacol. Toxicol.* 49, 327–347.
- Beaulieu, J. M., Marion, S., Rodriguiz, R. M., Medvedev, I. O., Sotnikova, T. D., Ghisi, V., Wetsel, W. C., Lefkowitz, R. J., Gainetdinov, R. R., and Caron, M. G. (2008a). A beta-arrestin 2 signaling complex mediates lithium action on behavior. *Cell* 132, 125–136.
- Beaulieu, J. M., Zhang, X., Rodriguiz, R. M., Sotnikova, T. D., Cools, M. J., Wetsel, W. C., Gainetdinov, R. R., and Caron, M. G. (2008b). Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. *Proc. Natl. Acad. Sci. U.S.A.* 105, 1333–1338.
- Beaulieu, J. M., Sotnikova, T. D., Gainetdinov, R. R., and Caron, M. G. (2006). Paradoxical striatal cellular signaling responses to psychostimulants in hyperactive mice. *J. Biol. Chem.* 281, 32072–32080.
- Beaulieu, J. M., Sotnikova, T. D., Marion, S., Lefkowitz, R. J., Gainetdinov, R. R., and Caron, M. G. (2005). An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell* 122, 261–273.
- Beaulieu, J. M., Sotnikova, T. D., Yao, W. D., Kockeritz, L., Woodgett, J. R., Gainetdinov, R. R., and Caron, M. G. (2004). Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc. Natl. Acad. Sci. U.S.A.* 101, 5099–5104.
- Benedetti, F., Bernasconi, A., Lorenzi, C., Pontiggia, A., Serretti, A., Colombo, C., and Smeraldi, E. (2004). A single nucleotide polymorphism in glycogen synthase kinase 3-beta promoter gene influences onset of illness in patients affected by bipolar disorder. *Neurosci. Lett.* 355, 37–40.
- Berhow, M. T., Hiroi, N., and Nestler, E. J. (1996). Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. *J. Neurosci.* 16, 4707–4715.
- Bjorklund, A., and Stenevi, U. (1984). Intracerebral neural implants: neuronal replacement and reconstruction of damaged circuitries. *Annu. Rev. Neurosci.* 7, 279–308.
- Blanco, C., Laje, G., Olsson, M., Marcus, S. C., and Pincus, H. A. (2002). Trends in the treatment of bipolar disorder by outpatient psychiatrists. *Am. J. Psychiatry* 159, 1005–1010.
- Bohn, L. M., Gainetdinov, R. R., Sotnikova, T. D., Medvedev, I. O., Lefkowitz, R. J., Dykstra, L. A., and Caron, M. G. (2003). Enhanced rewarding properties of morphine, but not cocaine, in beta(arrestin)-2 knock-out mice. *J. Neurosci.* 23, 10265–10273.
- Bychkov, E., Ahmed, M. R., Dalby, K. N., and Gurevich, E. V. (2007). Dopamine depletion and subsequent treatment with L-DOPA, but not the long-lived dopamine agonist pergolide, enhances activity of the Akt pathway in the rat striatum. *J. Neurochem.* 102, 699–711.
- Cade, J. F. (1949). Lithium salts in the treatment of psychotic excitement. *Med. J. Aust.* 2, 349–352.
- Carlsson, A. (2001). A paradigm shift in brain research. *Science* 294, 1021–1024.
- Chalecka-Franaszek, E., and Chuang, D. M. (1999). Lithium activates the serine/threonine kinase Akt-1 and suppresses glutamate-induced inhibition of Akt-1 activity in neurons. *Proc. Natl. Acad. Sci. U.S.A.* 96, 8745–8750.
- Chen, G., Huang, L. D., Jiang, Y. M., and Manji, H. K. (1999). The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. *J. Neurochem.* 72, 1327–1330.

In conclusion, the identification of cell signaling functions associated to β Arr downstream of dopamine receptors underscores the intricate complexity of the intertwined mechanisms regulating and mediating neuronal communication in the basal ganglia. This is an essential step to understand the several roles played by these structures in the regulation of mood, cognition, affect, and movement. Furthermore, understanding these mechanisms may lead to the development of new drugs having the desired therapeutic effect and avoiding aversive and undesired side effects.

ACKNOWLEDGMENTS

This review was supported by an Operating Grant (NSA 93798) from Canadian Institutes of Health Research (CIHR) to Jean-Martin Beaulieu. Jean-Martin Beaulieu is also supported by a Career development award from the Human Frontier Science Program, a National Alliance for Research on Schizophrenia and Depression (NARSAD), Young Investigator Award and hold a Canada research Chair in Molecular Psychiatry. Thomas Del'Guidice is a recipient of fellowships from the Fondation Robert Giffard and The Centre de Recherche sur le Cerveau, le Comportement et la Neuropsychiatrie (CRCN). Morgane Lemasson is a recipient of the Fonds de la Recherche et de la Santé du Québec (FRSQ).

- Chen, P., Gu, Z., Liu, W., and Yan, Z. (2007). Glycogen synthase kinase 3 regulates *N*-methyl-D-aspartate receptor channel trafficking and function in cortical neurons. *Mol. Pharmacol.* 72, 40–51.
- Claing, A., and Laporte, S. A. (2005). Novel roles for arrestins in G protein-coupled receptor biology and drug discovery. *Curr. Opin. Drug Discov. Devel.* 8, 585–589.
- Claing, A., Laporte, S. A., Caron, M. G., and Lefkowitz, R. J. (2002). Endocytosis of G protein-coupled receptors: roles of G protein-coupled receptor kinases and beta-arrestin proteins. *Prog. Neurobiol.* 66, 61–79.
- Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M., and Hemmings, B. A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 378, 785–789.
- Doble, B. W., and Woodgett, J. R. (2003). GSK-3: tricks of the trade for a multi-tasking kinase. *J. Cell. Sci.* 116(Pt 7), 1175–1186.
- Doi, M., Yujnovsky, I., Hirayama, J., Malerba, M., Tirotta, E., Sassone-Corsi, P., and Borrelli, E. (2006). Impaired light masking in dopamine D2 receptor-null mice. *Nat. Neurosci.* 9, 732–734.
- Emamian, E. S., Hall, D., Birnbaum, M. J., Karayiorgou, M., and Gogos, J. A. (2004). Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat. Genet.* 36, 131–137.
- Ferguson, S. S. (2001). Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol. Rev.* 53, 1–24.
- Ferguson, S. S., Downey, W. E. III, Colapietro, A. M., Barak, L. S., Ménard, L., and Caron, M. G. (1996). Role of beta-arrestin in mediating agonist-promoted G protein-coupled receptor internalization. *Science* 271, 363–366.
- Frame, S., and Cohen, P. (2001). GSK3 takes centre stage more than 20 years after its discovery. *Biochem. J.* 359(Pt 1), 1–16.
- Freyberg, Z., Ferrando, S. J., and Javitch, J. A. (2010). Roles of the Akt/GSK-3 and Wnt signaling pathways in schizophrenia and antipsychotic drug action. *Am. J. Psychiatry* 167, 388–396.
- Fukumoto, S., Hsieh, C. M., Maemura, K., Layne, M. D., Yet, S. F., Lee, K. H., Matsui, T., Rosenzweig, A., Taylor, W. G., Rubin, J. S., Perrella, M. A., and Lee, M. E. (2001). Akt participation in the Wnt signaling pathway through Dishevelled. *J. Biol. Chem.* 276, 17479–17483.
- Gainetdinov, R. R., Bohn, L. M., Sotnikova, T. D., Cyr, M., Laakso, A., Macrae, A. D., Torres, G. E., Kim, K. M., Lefkowitz, R. J., Caron, M. G., and Premont, R. T. (2003). Dopaminergic supersensitivity in G protein-coupled receptor kinase 6-deficient mice. *Neuron* 38, 291–303.
- Gainetdinov, R. R., Premont, R. T., Bohn, L. M., Lefkowitz, R. J., and Caron, M. G. (2004). Desensitization of G protein-coupled receptors and neuronal functions. *Annu. Rev. Neurosci.* 27, 107–144.
- Gainetdinov, R. R., Wetsel, W. C., Jones, S. R., Levin, E. D., Jaber, M., and Caron, M. G. (1999). Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science* 283, 397–401.
- Galandrin, S., Oligny-Longpré, G., and Bouvier, M. (2007). The evasive nature of drug efficacy: implications for drug discovery. *Trends Pharmacol. Sci.* 28, 423–430.
- Gerfen, C. R., and Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. *Annu. Rev. Neurosci.* 34, 441–466.
- Gesty-Palmer, D., Chen, M., Reiter, E., Ahn, S., Nelson, C. D., Wang, S., Eckhardt, A. E., Cowan, C. L., Spurney, R. F., Luttrel, L. M., and Lefkowitz, R. J. (2006). Distinct beta-arrestin and G protein-dependent pathways for parathyroid hormone receptor-stimulated ERK1/2 activation. *J. Biol. Chem.* 281, 10856–10864.
- Girault, J. A., Valjent, E., Caboche, J., and Hervé, D. (2007). ERK2: a logical AND gate critical for drug-induced plasticity? *Curr. Opin. Pharmacol.* 7, 77–85.
- Giros, B., Jaber, M., Jones, S. R., Wightman, R. M., and Caron, M. G. (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* 379, 606–612.
- Gould, T. D., K. C. O'Donnell, Picchini, A. M., Dow, E. R., Chen, G., and Manji, H. K. (2008). Generation and behavioral characterization of beta-catenin forebrain-specific conditional knock-out mice. *Behav. Brain Res.* 189, 117–125.
- Gould, T. D., and Manji, H. K. (2005). Glycogen synthase kinase-3: a putative molecular target for lithium mimetic drugs. *Neuropsychopharmacology* 30, 1223–1237.
- Iitaka, C., Miyazaki, K., Akaike, T., and Ishida, N. (2005). A role for glycogen synthase kinase-3beta in the mammalian circadian clock. *J. Biol. Chem.* 280, 29397–29402.
- Jacinto, E., Facchinetti, V., Liu, D., Soto, N., Wei, S., Jung, S. Y., Huang, Q., Qin, J., and Su, B. (2006). SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell* 127, 125–137.
- Jones, S. R., Gainetdinov, R. R., Wightman, R. M., and Caron, M. G. (1998). Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. *J. Neurosci.* 18, 1979–1986.
- Kapur, S., and Remington, G. (2001). Atypical antipsychotics: new directions and new challenges in the treatment of schizophrenia. *Annu. Rev. Med.* 52, 503–517.
- Kebabian, J. W., and Calne, D. B. (1979). Multiple receptors for dopamine. *Nature* 277, 93–96.
- Kim, K. M., Valenzano, K. J., Robinson, S. R., Yao, W. D., Barak, L. S., and Caron, M. G. (2001). Differential regulation of the dopamine D2 and D3 receptors by G protein-coupled receptor kinases and beta-arrestins. *J. Biol. Chem.* 276, 37409–37414.
- Kravitz, A. V., Freeze, B. S., Parker, P. R., Kay, K., Thwin, M. T., Deisseroth, K., and Kreitzer, A. C. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature* 466, 622–626.
- Krupnick, J. G., Goodman, O. B. Jr., Keen, J. H., and Benovic, J. L. (1997). Arrestin/clathrin interaction. Localization of the clathrin binding domain of nonvisual arrestins to the carboxy terminus. *J. Biol. Chem.* 272, 15011–15016.
- Kuschinsky, K., and Hornykiewicz, O. (1974). Effects of morphine on striatal dopamine metabolism: possible mechanism of its opposite effect on locomotor activity in rats and mice. *Eur. J. Pharmacol.* 26, 41–50.
- Lamont, E. W., Legault-Coutu, D., Cermakian, N., and Boivin, D. B. (2007). The role of circadian clock genes in mental disorders. *Dialogues Clin. Neurosci.* 9, 333–342.
- Lan, H., Teeter, M. M., Gurevich, V. V., and Neve, K. A. (2009). An intracellular loop 2 amino acid residue determines differential binding of arrestin to the dopamine D2 and D3 receptors. *Mol. Pharmacol.* 75, 19–26.
- Laporte, S. A., Miller, W. E., Kim, K. M., and Caron, M. G. (2002). beta-Arrestin/AP-2 interaction in G protein-coupled receptor internalization: identification of a beta-arrestin binding site in beta 2-adaptin. *J. Biol. Chem.* 277, 9247–9254.
- Li, X., Bijur, G. N., and Jope, R. S. (2002). Glycogen synthase kinase-3beta, mood stabilizers, and neuroprotection. *Bipolar Disord.* 4, 137–144.
- Li, X., Rosborough, K. M., Friedman, A. B., Zhu, W., and Roth, K. A. (2007). Regulation of mouse brain glycogen synthase kinase-3 by atypical antipsychotics. *Int. J. Neuropsychopharmacol.* 10, 7–19.
- Li, X., Zhu, W., Roh, M. S., Friedman, A. B., Rosborough, K., and Jope, R. S. (2004). In vivo regulation of glycogen synthase kinase-3beta (GSK3beta) by serotonergic activity in mouse brain. *Neuropsychopharmacology* 29, 1426–1431.
- Li, Y. C., Xi, D., Roman, J., Huang, Y. Q., and Gao, W. J. (2009). Activation of glycogen synthase kinase-3 beta is required for hyperdopamine and D2 receptor-mediated inhibition of synaptic NMDA receptor function in the rat prefrontal cortex. *J. Neurosci.* 29, 15551–15563.
- Lohse, M. J., Benovic, J. L., Caron, M. G., and Lefkowitz, R. J. (1990a). Multiple pathways of rapid beta 2-adrenergic receptor desensitization. Delineation with specific inhibitors. *J. Biol. Chem.* 265, 3202–3211.
- Lohse, M. J., Benovic, J. L., Codina, J., Caron, M. G., and Lefkowitz, R. J. (1990b). beta-Arrestin: a protein that regulates beta-adrenergic receptor function. *Science* 248, 1547–1550.
- Luttrel, L. M., and Gesty-Palmer, D. (2010). Beyond desensitization: physiological relevance of arrestin-dependent signaling. *Pharmacol. Rev.* 62, 305–330.
- Luttrel, L. M., Roudabush, F. L., Choy, E. W., Miller, W. E., Field, M. E., Pierce, K. L., and Lefkowitz, R. J. (2001). Activation and targeting of extracellular signal-regulated kinases by beta-arrestin scaffolds. *Proc. Natl. Acad. Sci. U.S.A.* 98, 2449–2454.
- Mansour, H. A., Wood, J., Chowdari, K. V., Dayal, M., Thase, M. E., Kupfer, D. J., Monk, T. H., Devlin, B., and Nimgaonkar, V. L. (2005). Circadian phase variation in bipolar I disorder. *Chronobiol. Int.* 22, 571–584.
- Martelli, A. M., Evangelisti, C., Chiarini, F., and McCubrey, J. A. (2010). The phosphatidylinositol 3-kinase/Akt/mTOR signaling network as a therapeutic target in acute myelogenous leukemia patients. *Oncotarget* 1, 89–103.
- Masri, B., Salahpour, A., Didrikson, M., Ghisi, V., Beaulieu, J. M., Gainetdinov, R. R., and Caron, M. G. (2008). Antagonism of dopamine D2 receptor/beta-arrestin 2 interaction is a common property of clinically effective antipsychotics. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13656–13661.

- Meltzer, H. Y. (1991). The mechanism of action of novel antipsychotic drugs. *Schizophr. Bull.* 17, 263–287.
- Miller, C. A., and Marshall, J. F. (2005). Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. *Neuron* 47, 873–884.
- Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., and Caron, M. G. (1998). Dopamine receptors: from structure to function. *Physiol. Rev.* 78, 189–225.
- Oakley, R. H., Laporte, S. A., Holt, J. A., Barak, L. S., and Caron, M. G. (2001). Molecular determinants underlying the formation of stable intracellular G protein-coupled receptor-beta-arrestin complexes after receptor endocytosis*. *J. Biol. Chem.* 276, 19452–19460.
- O'Brien, W. T., Harper, A. D., Jové, F., Woodgett, J. R., Maretto, S., Piccolo, S., and Klein, P. S. (2004). Glycogen synthase kinase-3beta haploinsufficiency mimics the behavioral and molecular effects of lithium. *J. Neurosci.* 24, 6791–6798.
- O'Brien, W. T., and Klein, P. S. (2009). Validating GSK3 as an in vivo target of lithium action. *Biochem. Soc. Trans.* 37(Pt 5), 1133–1138.
- Pan, J. Q., Lewis, M. C., Ketterman, J. K., Clore, E. L., Riley, M., Richards, K. R., Berry-Scott, E., Liu, X., Wagner, F. F., Holson, E. B., Neve, R. L., Biechele, T. L., Moon, R. T., Scolnick, E. M., Petryshen, T. L., and Haggarty, S. J. (2011). AKT kinase activity is required for lithium to modulate mood-related behaviors in mice. *Neuropsychopharmacology* 36, 1397–1411.
- Parent, M. B., Habib, M. K., and Baker, G. B. (2000). Time-dependent changes in brain monoamine oxidase activity and in brain levels of monoamines and amino acids following acute administration of the antidepressant/antipanic drug phenelzine. *Biochem. Pharmacol.* 59, 1253–1263.
- Peineau, S., Bradley, C., Taghibiglou, C., Doherty, A., Bortolotto, Z. A., Wang, Y. T., and Collingridge, G. L. (2008). The role of GSK-3 in synaptic plasticity. *Br. J. Pharmacol.* 153(Suppl. 1), S428–37.
- Phiel, C. J., and Klein, P. S. (2001). Molecular targets of lithium action. *Annu. Rev. Pharmacol. Toxicol.* 41, 789–813.
- Pierce, K. L., and Lefkowitz, R. J. (2001). Classical and new roles of beta-arrestins in the regulation of G-protein-coupled receptors. *Nat. Rev. Neurosci.* 2, 727–733.
- Pitcher, J. A., Hall, R. A., Daaka, Y., Zhang, J., Ferguson, S. S., Hester, S., Miller, S., Caron, M. G., Lefkowitz, R. J., and Barak, L. S. (1998). The G protein-coupled receptor kinase 2 is a microtubule-associated protein kinase that phosphorylates tubulin. *J. Biol. Chem.* 273, 12316–12324.
- Premont, R. T. (2005). Once and future signaling: G protein-coupled receptor kinase control of neuronal sensitivity. *Neuromolecular Med.* 7, 129–147.
- Premont, R. T., and Gainetdinov, R. R. (2007). Physiological roles of G protein-coupled receptor kinases and arrestins. *Annu. Rev. Physiol.* 69, 511–534.
- Rajagopal, S., Rajagopal, K., and Lefkowitz, R. J. (2010). Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat. Rev. Drug Discov.* 9, 373–386.
- Ramsey, A. J., Laakso, A., Cyr, M., Sotnikova, T. D., Salahpour, A., Medvedev, I. O., Dykstra, L. A., Gainetdinov, R. R., and Caron, M. G. (2008). Genetic NMDA receptor deficiency disrupts acute and chronic effects of cocaine but not amphetamine. *Neuropsychopharmacology* 33, 2701–2714.
- Rashid, A. J., So, C. H., Kong, M. M., Furtak, T., El-Ghundi, M., Cheng, R., O'Dowd, B. F., and George, S. R. (2007). D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 104, 654–659.
- Rossig, L., Badorff, C., Holzmänn, Y., Zeiher, A. M., and Dimmeler, S. (2002). Glycogen synthase kinase-3 couples AKT-dependent signaling to the regulation of p21Cip1 degradation. *J. Biol. Chem.* 277, 9684–9689.
- Sahar, S., Zocchi, L., Kinoshita, C., Borrelli, E., and Sassone-Corsi, P. (2010). Regulation of BMAL1 protein stability and circadian function by GSK3beta-mediated phosphorylation. *PLoS ONE* 5, e8561. doi: 10.1371/journal.pone.0008561
- Santini, E., Valjent, E., Usiello, A., Carta, M., Borgkvist, A., Girault, J. A., Hervé, D., Greengard, P., and Fisone, G. (2007). Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *J. Neurosci.* 27, 6995–7005.
- Scheid, M. P., and Woodgett, J. R. (2001). PKB/AKT: functional insights from genetic models. *Nat. Rev. Mol. Cell Biol.* 2, 760–768.
- Schou, M., Juel-Nielsen, N., Stromgren, E., and Voldby, H. (1954). The treatment of manic psychoses by the administration of lithium salts. *J. Neurol. Neurosurg. Psychiatr.* 17, 250–260.
- Shenoy, S. K., and Lefkowitz, R. J. (2005). Angiotensin II-stimulated signaling through G proteins and beta-arrestin. *Sci. STKE* 2005, cm14.
- Shuen, J. A., Chen, M., Gloss, B., and Calakos, N. (2008). Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. *J. Neurosci.* 28, 2681–2685.
- Snyder, S. H. (1976). The dopamine hypothesis of schizophrenia: focus on the dopamine receptor. *Am. J. Psychiatry* 133, 197–202.
- Souza, B. R., Romano-Silva, M. A., and Tropepe, V. (2011). Dopamine D2 receptor activity modulates Akt signaling and alters GABAergic neuron development and motor behavior in zebrafish larvae. *J. Neurosci.* 31, 5512–5525.
- Spano, P. F., Govoni, S., and Trabucchi, M. (1978). Studies on the pharmacological properties of dopamine receptors in various areas of the central nervous system. *Adv. Biochem. Psychopharmacol.* 19, 155–165.
- Stambolic, V., Ruel, L., and Woodgett, J. R. (1996). Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. *Curr. Biol.* 6, 1664–1668.
- Stambolic, V., and Woodgett, J. R. (1994). Mitogen inactivation of glycogen synthase kinase-3 beta in intact cells via serine 9 phosphorylation. *Biochem. J.* 303(Pt 3), 701–704.
- Svenningsson, P., Nishi, A., Fisone, G., Girault, J. A., Nairn, A. C., and Greengard, P. (2004). DARPP-32: an integrator of neurotransmission. *Annu. Rev. Pharmacol. Toxicol.* 44, 269–296.
- Swift, J. L., Godin, A. G., Doré, K., Frelaud, L., Bouchard, N., Nimmo, C., Sergeev, M., De Koninck, Y., Wiseman, P. W., and Beaulieu, J. M. (2011). Quantification of receptor tyrosine kinase transactivation through direct dimerization and surface density measurements in single cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7016–7021.
- Urs, N. M., Daigle, T. L., and Caron, M. G. (2011). A dopamine D1 receptor-dependent beta-arrestin signaling complex potentially regulates morphine-induced psychomotor activation but not reward in mice. *Neuropsychopharmacology* 36, 551–558.
- Valjent, E., Bertran-Gonzalez, J., Hervé, D., Fisone, G., and Girault, J. A. (2009). Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci.* 32, 538–547.
- Valjent, E., Corvol, J. C., Pages, C., Besson, M. J., Maldonado, R., and Caboche, J. (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine rewarding properties. *J. Neurosci.* 20, 8701–8709.
- Valjent, E., Corvol, J. C., Trzaskos, J. M., Girault, J. A., and Hervé, D. (2006a). Role of the ERK pathway in psychostimulant-induced locomotor sensitization. *BMC Neurosci.* 7, 20. doi: 10.1186/1471-2202-7-20
- Valjent, E., Corbille, A. G., Bertran-Gonzalez, J., Hervé, D., and Girault, J. A. (2006b). Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2932–2937.
- Valjent, E., Herve, D., and Girault, J. A. (2005). Drugs of abuse, protein phosphatases, and ERK pathway. *Med. Sci. (Paris)* 21, 453–454.
- Wei, H., Ahn, S., Barnes, W. G., and Lefkowitz, R. J. (2004). Stable interaction between beta-arrestin 2 and angiotensin type 1A receptor is required for beta-arrestin 2-mediated activation of extracellular signal-regulated kinases 1 and 2. *J. Biol. Chem.* 279, 48255–48261.
- Wei, H., Ahn, S., Shenoy, S. K., Karnik, S. S., Hunyady, L., Luttrell, L. M., and Lefkowitz, R. J. (2003). Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc. Natl. Acad. Sci. U.S.A.* 100, 10782–10787.
- Woodgett, J. R. (1990). Molecular cloning and expression of glycogen synthase kinase-3/factor A. *EMBO J.* 9, 2431–2438.
- Woodgett, J. R. (2001). Judging a protein by more than its name: GSK-3. *Sci. STKE* 2001, re12.
- Woodgett, J. R. (2003). Physiological roles of glycogen synthase kinase-3: potential as a therapeutic target for diabetes and other disorders. *Curr. Drug Targets Immune Endocr. Metabol. Disord.* 3, 281–290.
- Yamada, T., Katagiri, H., Asano, T., Tsuru, M., Inukai, K., Ono, H., Kodama, T., Kikuchi, M., and Oka, Y. (2002). Role of PDK1 in insulin-signaling pathway for glucose metabolism in 3T3-L1 adipocytes. *Am. J. Physiol. Endocrinol. Metab.* 282, E1385–E1394.

- Yuan, Q., Lin, F., Zheng, X., and Sehgal, A. (2005). Serotonin modulates circadian entrainment in *Drosophila*. *Neuron* 47, 115–127.
- Yujnovsky, I., Hirayama, J., Doi, M., Borrelli, E., and Sassone-Corsi, P. (2006). Signaling mediated by the dopamine D2 receptor potentiates circadian regulation by CLOCK:BMAL1. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6386–6391.
- Zhu, L. Q., Wang, S. H., Liu, D., Yin, Y. Y., Tian, Q., Wang, X. C., Wang, Q., Chen, J. G., and Wang, J. Z. (2007). Activation of glycogen synthase kinase-3 inhibits long-term potentiation with synapse-associated impairments. *J. Neurosci.* 27, 12211–12220.
- Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
- Received: 13 May 2011; paper pending published: 06 June 2011; accepted: 17 August 2011; published online: 06 September 2011.
- Citation: Del'Guidice T, Lemasson M and Beaulieu J-M (2011) Role of beta-arrestin 2 downstream of dopamine receptors in the basal ganglia. *Front. Neuroanat.* 5:58. doi: 10.3389/fnana.2011.00058
- Copyright © 2011 Del'Guidice, Lemasson and Beaulieu. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Signaling from the cytoplasm to the nucleus in striatal medium-sized spiny neurons

Miriam Matamales^{1,2,3†} and Jean-Antoine Girault^{1,2,3*}

¹ UMR-S 839, Inserm, Paris, France

² Université Pierre et Marie Curie, Paris, France

³ Institut du Fer à Moulin, Paris, France

Edited by:

Emmanuel Valjent, Université
Montpellier 1 & 2, France

Reviewed by:

Eric J. Nestler, Mount Sinai School of
Medicine, USA

Michael R. Kreutz, Leibniz-Institute for
Neurobiology, Germany

*Correspondence:

Jean-Antoine Girault, Institut du Fer à
Moulin, Inserm UPMC UMR-S 839,
17 rue du Fer à Moulin, 75005 Paris,
France.

e-mail: jean-antoine.girault@inserm.fr

†Present address:

Miriam Matamales, Alzheimer's &
Parkinson's Disease Laboratory,
Brain & Mind Research Institute,
University of Sydney, 100 Mallett St,
Camperdown, NSW 2050, Australia

Striatal medium-sized spiny neurons (MSNs) receive massive glutamate inputs from the cerebral cortex and thalamus and are a major target of dopamine projections. Interaction between glutamate and dopamine signaling is crucial for the control of movement and reward-driven learning, and its alterations are implicated in several neuropsychiatric disorders including Parkinson's disease and drug addiction. Long-lasting forms of synaptic plasticity are thought to depend on transcription of gene products that alter the structure and/or function of neurons. Although multiple signal transduction pathways regulate transcription, little is known about signal transmission between the cytoplasm and the nucleus of striatal neurons and its regulation. Here we review the current knowledge of the signaling cascades that target the nucleus of MSNs, most of which are activated by cAMP and/or Ca²⁺. We outline the mechanisms by which signals originating at the plasma membrane and amplified in the cytoplasm are relayed to the nucleus, through the regulation of several protein kinases and phosphatases and transport through the nuclear pore. We also summarize the identified mechanisms of transcription regulation and chromatin remodeling in MSNs that appear to be important for behavioral adaptations, and discuss their relationships with epigenetic regulation.

Keywords: medium-sized spiny neurons, dopamine, glutamate, protein phosphorylation, cytonuclear shuttling, transcription, chromatin remodeling, epigenetic regulation

WHY IS CYTONUCLEAR SIGNALING IMPORTANT IN NEURONS?

In interphasic eukaryotic cells the genetic material, the DNA, is segregated from the rest of the cell by the double membrane of the nuclear envelope which separates the nucleus from the cytoplasm. Communication between the two compartments is tightly regulated and essentially restricted to highly organized nuclear pores (Hoelz et al., 2011). The cytoplasm is the site of housekeeping functions common to all cell types, as well as highly specialized activities, such as those characteristic of synapses. Thus, the regulation of gene expression is under two opposite constraints. On the one hand the pattern of gene expression characteristic of the differentiated state of the cell must be very stable. On the other hand, fine tuning of gene expression is constantly required for the cell to adapt to its environment and respond to new demands.

In neurons gene transcription and protein synthesis have been known for many years to be necessary for the long-lasting changes that underlie learning and memory (Goelet et al., 1986; Black et al., 1987). The pattern of gene transcription depends on the dynamic interplay of numerous transcription factors, binding to promoter, enhancer, and silencer regions. It also depends on the organization of the chromatin, including covalent modification of nucleotides, such as cytidine methylation, and post-translational modifications of histones, which form the core of nucleosomes around which DNA is wrapped. These modifications control the accessibility

of DNA and the recruitment of transcription factors and other proteins through their specialized targeting domains. The resulting chromatin remodeling may provide the basis for "epigenetic" control of long-lasting neuronal adaptations (see Levenson and Sweatt, 2005; Borrelli et al., 2008; Day and Sweatt, 2010; Riccio, 2010, for reviews). The use of the word epigenetic has been the source of debates however. As originally defined, and recently reasserted, "An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence" (Berger et al., 2009). According to this stringent definition neurons, like other post-mitotic cells, could not undergo new epigenetic alterations (except perhaps by erasing existing epigenetic marks. . .). The word is often used in a less strict sense to designate long-lasting chromatin covalent modifications, a trend that has raised some criticisms as it may have misleading implications in terms of underlying mechanisms (Ptashne, 2007). At any rate a wealth of recent findings summarized in this review underlines the importance of the regulation of covalent modifications of histones and DNA by cytonuclear signaling pathways in striatal neurons.

A striking feature of most chromatin modifications is that they are highly dynamic, implying efficient regulatory mechanisms and active control mechanisms if they are to be maintained for long periods of time (Henikoff, 2008). In addition, the chromatin is topographically organized due to a highly complex network of

long range interactions, which are only starting to be deciphered in non-neuronal cells (Fraser and Bickmore, 2007). This higher level of chromatin organization is still largely a *terra incognita* in neurons. Interestingly, the patterns of heterochromatin staining, which result from the organization of the nuclear material, differ widely between neurons and other cell types and between different neuronal populations (Ramon y Cajal, 1899) but their significance and regulation have received little attention so far. In the striatum, this pattern allows to differentiate medium-sized spiny neurons (MSNs) from other cells (Matamales et al., 2009) and recent work suggests that the geometry and organization of the nucleus can undergo rapid and dramatic alterations in response to synaptic activity (Wittmann et al., 2009; Maze et al., 2011). Therefore deciphering the control of nuclear functions and organization in neurons represents a major challenge for understanding the neurobiology of learning and memory and brain plasticity.

An important issue to be addressed is how synaptic activity modifies gene transcription and chromatin remodeling. It has been proposed that information transmitted from synapses can alter nuclear function through at least two different pathways (see Deisseroth and Tsien, 2002; Adams and Dudek, 2005; Cohen and Greenberg, 2008; Jordan and Kreutz, 2009, for reviews and discussion): (i) a “synapse-to-nucleus” pathway in which proteins can move from activated synapses to the nucleus and control nuclear events such as transcription; (ii) a “soma-to-nucleus” pathway without direct transport of macromolecules from the synapses, but in which action potentials and second messengers such as Ca^{2+} or cAMP generated in the perikaryon control nuclear signaling. In the context of learning, it has been contended that the “soma-to-nucleus” model might be the most relevant mechanism because it is faster and may involve a cell-type specific threshold of activation, preventing the activation of other irrelevant genes (Adams and Dudek, 2005). However, it is likely that the two types of pathways act in combination over different time scales to achieve a precise tuning of nuclear function.

In this review we focus on MSNs, which are the most prevalent cell type of the striatum, comprising about 95% of striatal neurons in rodents (Tepper and Bolam, 2004). Since these neurons play an essential role in the circuits of the basal ganglia and are thought to undergo enduring adaptations important for procedural memory (Kreitzer, 2009), it is important to decipher the cytonuclear signaling pathways that trigger long-lasting changes in MSNs. Such changes are also likely to underlie some of the dysfunctions of the basal ganglia, in conditions such as drug addiction, L-DOPA-induced dyskinesia in Parkinson's disease, and, presumably, several other neuropsychiatric disorders.

MAJOR SIGNALING PATHWAYS IN MSNs

The striatum is the main entry station of the basal ganglia and MSNs are its sole output neurons. MSNs utilize GABA as their major neurotransmitter, making the striatum a large inhibitory structure (Yoshida and Pecht, 1971). MSNs receive excitatory glutamatergic inputs from the cortex and the thalamus, which contact dendritic spines and are modulated by dopamine (DA) released from the terminals of midbrain DA neurons (Bennett and Wilson, 2000). They also receive various other types of afferences including inhibitory synapses originating from other MSNs and

GABAergic interneurons, cholinergic inputs from large cholinergic interneurons, and several others such as serotonin, and various neuropeptides. Most of the present knowledge of gene regulation in the striatum results however from the study of dopamine and glutamate signaling, for which we provide a brief overview.

DOPAMINE RECEPTORS SIGNALING

The actions of dopamine in the striatum are mediated mainly by the D1 and D2 types of DA receptors (D1R and D2R) that are coupled to different G proteins having opposite effects on the production of cAMP (Kebabian and Calne, 1979). D2Rs have a high affinity for DA and are tonically activated by low basal concentrations of DA in the extracellular space, whereas D1Rs are thought to be stimulated following burst firing of DA neurons which is more efficient to release DA (Creese et al., 1983; Grace, 1991). D1Rs are coupled to the striatal-enriched $\text{G}\alpha_{\text{olf}}$ that stimulates adenylyl cyclase activity, thus increasing intracellular cAMP levels (Herve et al., 1993; Corvol et al., 2001; **Figure 1**). The principal target of cAMP is the cAMP-dependent protein kinase (PKA; Miyamoto et al., 1968) and D1R-induced PKA activation modulates multiple voltage- and ligand-gated ion channels, thereby modifying the effectiveness of synaptic inputs (Greengard et al., 1999; Nicola et al., 2000). In contrast to the phasic stimulation of D1Rs, D2Rs exert a tonic inhibitory effect on membrane potential and adenylyl cyclase, thereby opposing other receptors expressed in the same neurons such as A2a adenosine receptors. There is also a biphasic coupling of D2R with the Akt/GSK3 pathway, which includes an early activation (Brami-Cherrier et al., 2002) and a delayed inhibition linked to protein phosphatase 2A (PP2A) recruitment (Beaulieu et al., 2005). Although the Akt/GSK3 pathway is known to exert important effects on nuclear functions in other cell types (Hur and Zhou, 2010), little is known about such effects in the striatum. As initially proposed more than 20 years ago (Gerfen et al., 1990), D1Rs and D2Rs are remarkably segregated in striatonigral and striatopallidal neurons, respectively, with little overlap (see Valjent et al., 2009; Bertran-Gonzalez et al., 2010, for recent reviews). Interestingly, it has been shown that D1R–D2R heteromers can generate a specific Gq/Ca^{2+} -mediated signaling resulting in brain-derived neurotrophic factor (BDNF) induction (Hasbi et al., 2009). The physiological importance of this effect, which is expected to take place in the small proportion of MSNs coexpressing both receptors, remains to be fully evaluated. D3Rs are predominantly expressed in the ventral striatum (Diaz et al., 1995).

THE ERK PATHWAY AND SYNERGISM BETWEEN D1R AND GLUTAMATE RECEPTORS

Glutamate released by corticostriatal or corticothalamic neurons can act on several types of ionotropic and metabotropic receptors. One of their major effects is to induce an increase in cytosolic Ca^{2+} which triggers the rapid activation of the extracellular signal-regulated kinase (ERK; Sgambato et al., 1998; Vanhoutte et al., 1999; Pascoli et al., 2011). ERK is part of a signal transduction module highly conserved among eukaryotes that is involved in the control of many cellular processes including cell growth, differentiation and neuronal plasticity (Thomas and Huganir, 2004). This module comprises three enzymes acting in a cascade

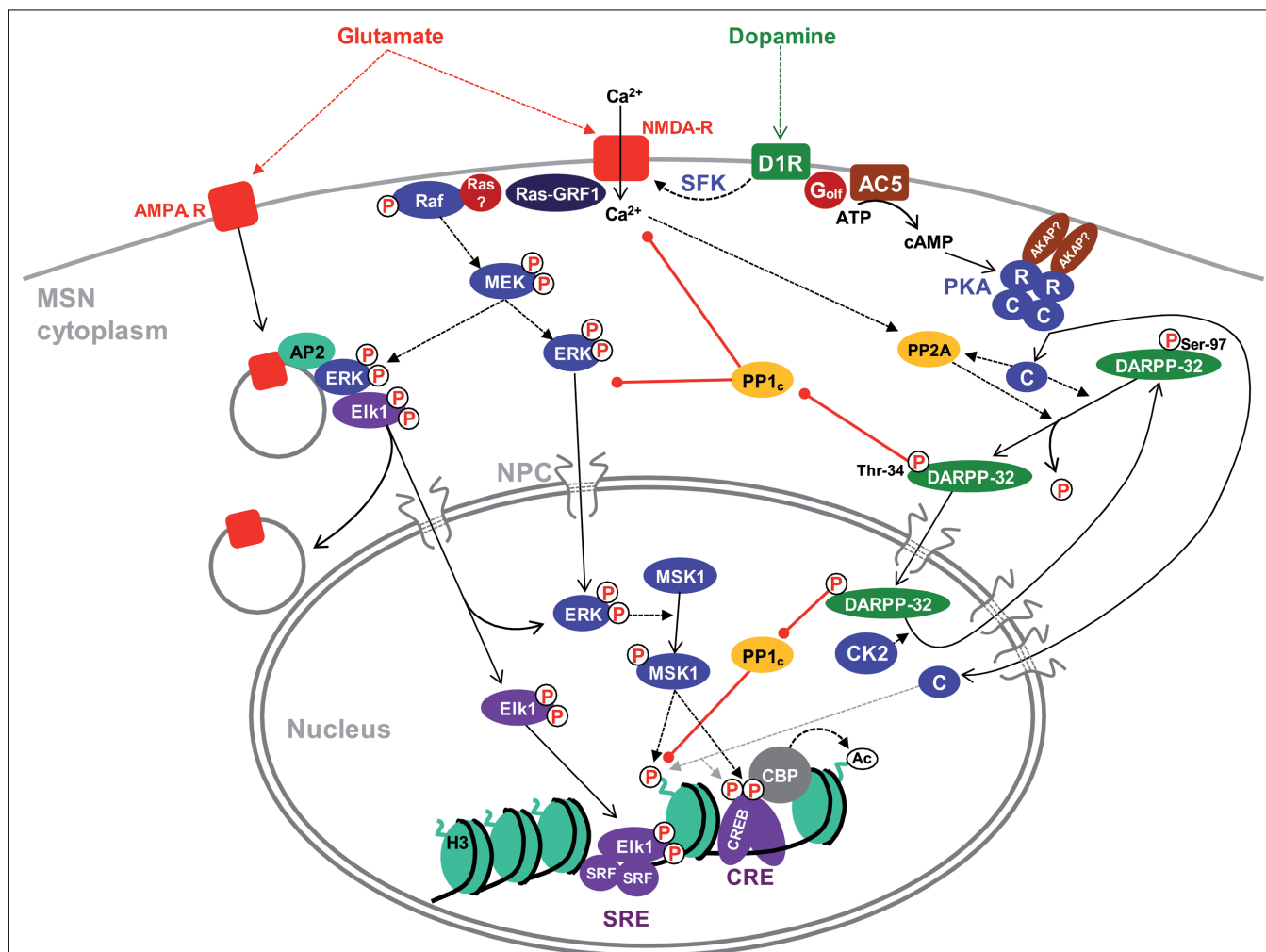


FIGURE 1 | Cytonuclear signaling pathways in MSNs. The indicated pathways have mostly been identified in D1R-expressing MSNs, although similar cascades exist in D2-expressing cells with some differences (Bertran-Gonzalez et al., 2009). The precise location of involved receptors (i.e., perikaryon vs dendrites and spines) is not indicated because it has not been determined experimentally in most cases. Activation of ERK by glutamate involves increases in cytosolic Ca^{2+} which stimulates Ras-GRF1 and possibly other guanine nucleotide exchange factors. A major source of Ca^{2+} is the activation of NMDAR by glutamate. This effect is strongly potentiated by stimulation of D1R by dopamine through (i) a cAMP-independent pathway involving Src-family tyrosine kinases (SFK); (ii) an amplification loop with inhibition of PP1 by phospho-Thr-34-DARPP-32. PP1 acts both upstream of ERK (e.g., by dephosphorylating NR1 subunits of NMDAR) and by activating the tyrosine phosphatase STEP which dephosphorylates the regulatory tyrosine of ERK (not shown). In striatal neurons in culture, active ERK associates with endocytosed AMPAR through adaptor protein 2 (AP2) together with the transcription factor Elk1 and this complex appears to be important for nuclear import of activated ERK and Elk1. D1R activates adenylyl

cyclase (mostly AC5 isoform) through the stimulatory heterotrimeric G_{olf} protein containing the α_{olf} and γ subunits. The inactive PKA heterotetramer is usually anchored through AKAPs, yet to be characterized in MSNs. cAMP binds to the regulatory subunits (R) and releases the catalytic subunits (C). C subunits phosphorylate numerous substrates in the cytoplasm and diffuse to the nucleus where they can phosphorylate resident proteins such as CREB or histone H3. In MSNs however, a nuclear resident protein kinase, ERK-activated MSK1, plays a major role in CREB and H3 phosphorylation. CREB recruits CBP which has histone acetyltransferase activity. PKA phosphorylates DARPP-32 on Thr-34, turning it into a potent inhibitor of PP1 catalytic subunit (PP1c). DARPP-32 continuously cycles between the cytoplasm and the nucleus and its nuclear export is facilitated by phosphorylation of Ser-97 by CK2, which is present in the cytoplasm and nucleus of MSNs. PKA increases nuclear DARPP-32 by inducing the dephosphorylation of Ser-97 by PP2A. Dotted arrows indicate influences or catalysis, solid arrows displacement. Red arrows with a round end indicate inhibition. NPC, nuclear pore complex. See text for references.

of sequential activatory phosphorylation: the upstream kinases (MAPK/ERK-kinase-kinases, MEKKs) phosphorylate and activate the MAPK/ERK-kinases (MEKs). MEKs are dual specificity kinases that trigger the activation of MAPKs by phosphorylating a threonine (Thr) and a tyrosine (Tyr) residue in their activation loop. In the striatum, it is well documented that drugs of

abuse that share the capacity to increase extracellular DA levels (Di Chiara et al., 1998), induce a robust increase in ERK phosphorylation in MSNs (Valjent et al., 2000, 2004). Activation of the ERK pathway requires D1R (Valjent et al., 2000, 2004, 2005) and is antagonized by D3R in regions where this receptor is expressed (Zhang et al., 2004). Interestingly, ERK activation

appears to be also dependent on glutamate *N*-methyl-D-aspartate receptors (NMDARs) since antagonists for these receptors block ERK phosphorylation in MSNs (Valjent et al., 2000, 2001, 2005). This provides the basis for coincidence detection between the DA and glutamate pathways, which is predicted to specifically identify neurons activated in relation to rewarding stimuli (Girault et al., 2007). Several molecular mechanisms of interaction between D1R and NMDAR converge for this effect (**Figure 1**): (i) a PKA-independent pathway involving Src-family non-receptor tyrosine kinases (Pascoli et al., 2011); (ii) a cAMP-PKA mediated effect through inhibition of the serine/threonine phosphatase PP1 (see below) and of the striatal-enriched tyrosine phosphatase STEP (Valjent et al., 2005). Activation of the ERK pathway in response to Ca^{2+} influx in MSNs results to a large extent from the activation of a Ca^{2+} /calmodulin-regulated guanine nucleotide exchange factor (GEF) termed Ras-guanine nucleotide releasing factor 1 (Ras-GRF1; Fasano et al., 2009). In addition, two other GEFs, stimulated by calcium and diacylglycerol, CalDAG-GEFI and CalDAG-GEFII (also known as RasGRP1/2) are expressed in MSNs and likely to be involved in the control of the ERK pathway (Toki et al., 2001).

Besides the PKA and ERK pathways, other signaling cascades are likely to be involved in the regulation of nuclear functions but have received comparatively less attention so far in striatal neurons. They include the Ca^{2+} /calmodulin-activated protein kinases (CaMKs) which play a key role in the regulation of gene expression in other neuronal types (Deisseroth and Tsien, 2002; Cohen and Greenberg, 2008). CaMKII is implicated in the activation of ERK (see Wang et al., 2007 for a review) and CaMKIV, which is predominantly nuclear, negatively regulates responses to cocaine in the MSNs (Bilbao et al., 2008).

THE ROLE OF PROTEIN PHOSPHATASES IN SIGNALING IN MSNs

The phosphorylation state of PKA and ERK substrates also depends on the activity of serine/threonine protein phosphatases. Well-characterized protein phosphatases in the striatum include protein phosphatase 1 (PP1 or PPP1), 2A (PP2A or PPP2) and 2B (PP2B or calcineurin or PPP3). PP1 catalytic subunit (PP1c) is a ~36kDa globular protein highly conserved in eukaryotes. Among the four PP1c isoforms (α , β/δ , γ 1, and γ 2) expressed in the mammalian brain, PP1 α and PP1 γ 1 are highly expressed in the striatum, whereas PP1 β/δ is less abundant (Da Cruz e Silva et al., 1995). PP1c subcellular localization and substrate specificity are controlled by multiple targeting subunits, which regulate its activity in combination with endogenous inhibitors (Bollen et al., 2010). DA- and cAMP-regulated phosphoprotein Mr ~32,000 (DARPP-32 or PPP1 regulatory subunit 1B – PPP1R1B) is an endogenous inhibitor of PP1 particularly enriched in striatal MSNs (Walaas et al., 1983; Hemmings et al., 1984; Ouimet et al., 1984). DARPP-32 activity is modulated by multiple neurotransmitters and is involved in the response to many neurotransmitters and psychoactive drugs (Svenningsson et al., 2004). Phosphorylation of specific Thr and serine (Ser) residues determines DARPP-32 overall function. For instance, phosphorylation of Thr-34 by PKA or cGMP-dependent protein kinase turns DARPP-32 into a very potent inhibitor of PP1c (Hemmings et al., 1984). Inhibition of PP1c by DARPP-32 amplifies PKA signaling by enhancing the phosphorylation of those of its targets that are substrates of

PP1, constituting a positive feed-forward mechanism by which the D1R DA signal is amplified (Svenningsson et al., 2004). DARPP-32 is also involved in cross-talks between signaling pathways and appears to be essential for ERK activation in response to drugs of abuse, as this activation is prevented in DARPP-32 knockout and in knockin Thr-34-Ala point mutant mice (Valjent et al., 2005). Altogether, the dopamine-dependent activation of the phosphatase inhibitor DARPP-32, directly or indirectly amplifies both PKA and ERK signaling cascades in MSNs.

BASIC MECHANISMS OF PROTEIN TRAFFIC BETWEEN THE CYTOPLASM AND THE NUCLEUS

Stimuli-triggered signaling cascades can promote the relocation of effector molecules from the cytoplasm to the nucleus where they exert direct or indirect effects on the expression of specific target genes. Conversely, termination of nuclear signaling can be facilitated by an efficient export of nuclear factors out of the nucleus. Thus, nuclear traffic provides a means of temporal and spatial control of signaling cascades and is tightly regulated by numerous mechanisms and at multiple levels (see Kaffman and O'Shea, 1999; Poon and Jans, 2005; Terry et al., 2007, for reviews). Because it is central for understanding cytonuclear signaling, we briefly overview the basic principles of nuclear import and export mechanisms, although this knowledge has been acquired in other cell types and little is known about the possible specificities of striatal neurons in this matter.

THE NUCLEAR PORE COMPLEX

Cytonuclear traffic takes place through the nuclear pore complex (NPC) that mediates bidirectional transport between the cytoplasm and the nucleus (Hoelz et al., 2011). The nuclear pore complex is a huge macromolecular assembly (diameter ~100 nm) that penetrates the double lipid bilayer of the nuclear envelope and is constructed by multiple copies of ~30 different proteins called nucleoporins, functionally conserved from yeast to mammals. Interestingly, a point mutation of nucleoporin-62 (Nup62) is responsible for a rare autosomal recessive infantile bilateral striatal necrosis (Basel-Vanagaite et al., 2006), although the mechanisms of the lesions and the basis for their striatal specificity are not known. Diffusion channels through the NPC have a calculated diameter of ~9–10 nm and allow the free passage of macromolecules of up to ~40 kDa (Hoelz et al., 2011). A diffusion barrier is formed by extended natively unfolded nucleoporin segments that contain numerous Phe-Gly repeats (Patel et al., 2007). This barrier prevents free diffusion of molecules larger than 40 kDa through the NPC. Translocation of such molecules is an energy-dependent process mediated by soluble transport receptors belonging to the family of the karyopherins, which interact with the Phe-Gly repeats through poorly understood mechanisms, and with specific sequences on their cargoes (see Mosammaparast and Pemberton, 2004; Hoelz et al., 2011, for reviews). Members of the karyopherin- β (Kap β) family, which share some sequence and structure homologies, are also termed importins and exportins depending on whether they mediate import into or export out of the nucleus, respectively. Kap β proteins bind to their cargoes via specific recognition of amino acid targeting sequences termed nuclear localization signal (NLS) for nuclear import and nuclear export signal (NES)

for export (Xu et al., 2010; **Figure 2**). Both importins and exportins interact with the small G-protein Ran, which, in its intranuclear GTP-bound form, induces dissociation of importin complexes and association of cargoes with exportin (Pemberton and Paschal, 2005).

NUCLEAR IMPORT AND EXPORT

The nuclear import cycle is mostly driven by a heterodimeric carrier composed by importin- β (Imp β) and its adaptor importin- α (Imp α , a member of karyopherin- α family). Imp α interact with their cargo through classical NLSs which contain one (monopartite) or two (bipartite) clusters of basic residues separated by 10–12 variable residues (Xu et al., 2010). Imp β can also bind directly some cargoes (Pemberton and Paschal, 2005). In the cytoplasm, Imp α binds the cargo and forms a heterodimeric complex with Imp β . The carrier-cargo complex then docks on the near cytoplasmic side of the NPC and exchanges sequential, low-affinity interactions with nucleoporins that allow the complex to advance through the NPC to the nucleoplasm by a mechanism still poorly understood. In the nucleus, Imp α / β -cargo complex dissociates when RanGTP binds Imp β , releasing the cargo from the transport complex (Lee

et al., 2005). Multiple mechanisms for regulation of nuclear import have been described (see Poon and Jans, 2005; Nardozzi et al., 2010, for reviews). Interestingly, importins have been localized to distal axons and dendrites in several types of neurons, including *Aplysia* sensory motor neurons, and rodent hippocampal and sensory neurons (Hanz et al., 2003; Thompson et al., 2004). Moreover, recent work has indicated that importin-mediated transport plays a specialized role in neurons in which it not only translocates proteins from the cell soma to the nucleus, but also carries signals from distal compartments to the nucleus during development, as well as in the adult nervous system (Jordan and Kreutz, 2009). Several studies have shown how signals such as Imp β :cargo complexes, kinases and transcription factors move to the nucleus upon synaptic activation (Martin et al., 1997; Meffert et al., 2003). In the striatum the levels of Imp α -1 are increased following lesion of DA neurons (Wang et al., 2004), but the significance of this observation is not known.

The best-characterized nuclear export pathway is mediated by the *chromosome region maintenance 1* protein (CRM1; also known as exportin-1), although CRM1-independent pathways have been identified for various cargoes (Lischka et al., 2001; Chen et al., 2004;

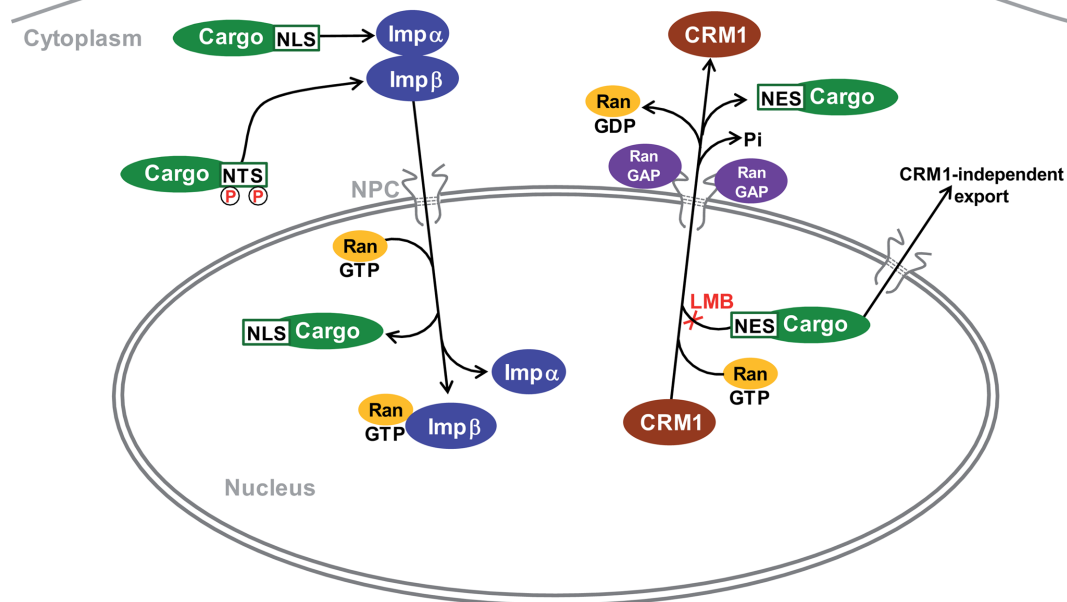


FIGURE 2 | Mechanisms for import and export of proteins to and from the nucleus. The major identified cytonuclear traffic pathways have been characterized in non-neuronal cells and their specifics in MSNs are not known. Transport is mediated by karyopherins which are also known as importins (Imp) and exportins, depending on their function. Proteins are imported by binding to Imp α through a basic nuclear localization sequence (NLS). Some others bind directly to Imp β . For example, proteins with nuclear translocation sequence (NTS Ser/Thr-Pro-Ser/Thr), which is activated by phosphorylation, bind to Imp7 (Chuderland et al., 2008). The complex moves through the nuclear pore complex (NPC) to the nucleoplasm where its dissociation is induced by RanGTP. The best-characterized nuclear export pathway is mediated by the exportin CRM1. Proteins containing a

hydrophobic nuclear export sequence (NES) bind to CRM1 in the presence of RanGTP. Following translocation the complex is dissociated through hydrolysis of GTP by Ran GTPase-activating proteins (Ran GAPs) enriched on the cytoplasmic side of the NPC. Leptomycin B (LMB) prevents binding of NES to CRM1. CRM1-independent export pathways are poorly characterized. RanGDP is imported from the cytoplasm to the nucleus where GDP is replaced by GTP by specific guanine nucleotide exchange factors (GEFs, not shown). Following carrier-cargo dissociation, the empty importins or exportins return to their compartment of origin through specific pathways that are not indicated on the scheme. References can be found in the text and in (Pemberton and Paschal, 2005; Poon and Jans, 2005; Nardozzi et al., 2010).

Eulalio et al., 2006; **Figure 2**). The majority of CRM1 export substrates contain a NES that consists in a short amino acid sequence of regularly spaced hydrophobic residues, of which leucine is the most abundant (Xu et al., 2010). CRM1 binds cooperatively to RanGTP and its export cargo, leading to the formation of a trimeric transport complex in the nucleus that is translocated through the NPC to the cytoplasm (Askjaer et al., 1998). RanGTP within the export complex is hydrolyzed by specific nucleoporins with GTPase-activating properties (Ran-GAPs), allowing dissociation of the export complex on the cytoplasmic side of the NPC (Pemberton and Paschal, 2005). The antifungal antibiotic leptomycin B (LMB) is a powerful tool to distinguish *bona fide* CRM1-exported substrates among other leucine-rich proteins since it specifically inhibits CRM1 nuclear export by occupation of the NES binding site (Kudo et al., 1999). The use of LMB has thus revealed that many proteins undergo a permanent cytonuclear trafficking and that their apparent cytoplasmic localization results from their nuclear export being more efficient than their import. This dynamic equilibrium can be the target of many regulations. In the next sections we will examine how such regulations apply to signaling proteins important in the striatum, using mostly information obtained in other cell types since very few studies have addressed directly these issues in MSNs.

NUCLEAR TRANSLOCATION OF PROTEIN KINASES IMPORTANT FOR SIGNALING IN MSNs

PKA NUCLEAR TRANSLOCATION

Inactive PKA is a tetrameric holoenzyme consisting of two regulatory subunits (R) that bind and inhibit two catalytic subunits (C; Taylor et al., 1990). The interaction of R with C subunits stabilizes both molecules against proteolysis. A family of functionally related proteins known as A-kinase-anchoring proteins (AKAPs) plays an essential role in the distribution of PKA in specific intracellular domains (Wong and Scott, 2004). AKAPs are multivalent adaptor proteins that bind the R subunit of PKA and anchor the enzyme at distinct subcellular locations in close proximity to specific substrates and other signaling enzymes. Sequential and cooperative binding of cAMP to the two sites of each R subunit releases monomeric C subunits (Taylor et al., 2008; **Figure 1**). Dissociated C subunits can then phosphorylate Ser and Thr residues in numerous proteins containing the consensus amino acid sequence (R/K₂-x-S/T-Φ, where Φ is a hydrophobic residue). PKA can thus exert its function via a wide range of substrates spanning multiple neuronal compartments.

Although PKA is preferentially located in the cytoplasm in resting conditions, a significant fraction of the dissociated C subunit of PKA localizes to the nucleus upon increase of intracellular cAMP (Meinkoth et al., 1990). The cytoplasmic localization of PKA holoenzyme is achieved by tethering the R subunits to cytoplasmic AKAPs. In addition the quaternary complex is too big to diffuse into the nucleus and no active import mechanism has been described for the holoenzyme. In contrast, free C subunits can diffuse into the nucleus since they are small enough to pass through the NPC (Meinkoth et al., 1990; Harootunian et al., 1993). A strong and delayed activation of nuclear PKA-mediated phosphorylation is observed in neurons following

stimulation of membrane receptors that activate adenylyl cyclase (Gervasi et al., 2007). The functional importance of nuclear PKA was demonstrated using a specific inhibitor of this enzyme in the nucleus, which prevented the induction of long-lasting long-term potentiation in hippocampal slices (Matsushita et al., 2001).

Considering the fact that specific anchoring proteins tightly compartmentalize PKA subcellular localization, it is rather unlikely that PKA activated at the synapse can travel from distant processes to the nucleus. Instead, the rapid diffusion toward the cell body of the highly hydrophilic cAMP produced at the plasma membrane may allow the activation and translocation of PKA located in close proximity to the nucleus (Hempel et al., 1996). In some cell types, a pool of PKA tethered to the nuclear envelope by specific AKAPs is able to rapidly translocate to the nucleus upon cAMP increase (Wong and Scott, 2004). The anchoring of RIIβ to AKAP75, two proteins abundant in specific neuronal populations, appears to be an important factor for nuclear activation of PKA (Paolillo et al., 1999). In addition, nuclear anchoring proteins can retain the free C subunit of PKA and facilitate its nuclear function (Sastri et al., 2005), whereas binding to the protein kinase inhibitor (PKI) which has a strong NES enhances its active export by a CRM1-dependent mechanism (Fantozzi et al., 1994). However, little is known about the regulation of nuclear dynamics of PKA in neurons, in spite of its functional importance.

ERK NUCLEAR TRANSLOCATION

In neurons as in other cell types, in the absence of stimulation, ERK is inactive and mostly located in the cytoplasm, in dendrites and perikarya. Following activation of the signaling cascades that lead to MEK and ERK activation, phosphorylated ERK accumulates transiently in the nucleus, where it has important targets. This response has been well characterized in MSNs *in vivo*, in response to corticostriatal stimulation and drugs of abuse (Sgambato et al., 1998; Valjent et al., 2000). However, the molecular mechanisms controlling ERK localization have been mostly studied in non-neuronal cells. The association with MEK, which bears a NES is an important factor for maintaining ERK in the cytoplasm (Adachi et al., 2000). Passive diffusion of the two ERK isoforms (ERK1 and ERK2) that have a molecular mass of 44 and 42 kDa, respectively, is sufficient for their nuclear accumulation (Adachi et al., 1999; Burack and Shaw, 2005). In addition, it has been proposed that homodimerization was an important step for ERK nuclear import (Khokhlatchev et al., 1998; Adachi et al., 1999). However, recent studies using FRET and dimerization-deficient mutants have ruled out this hypothesis (Burack and Shaw, 2005; Lidke et al., 2010). ERK sequence does not contain any classical NLS, but includes a novel nuclear translocation signal (NTS) motif, Ser/Thr-Pro-Ser/Thr, which is active when phosphorylated by a variety of protein kinases, and mediates interaction with importin-7 (Chuderland et al., 2008). Interestingly, in hippocampal neurons, ERK nuclear translocation appears to require a combination of Ca²⁺ and cAMP (Impey et al., 1998) or PKA and TrkB signaling (Patterson et al., 2001). Although the mechanism of this synergism is not known, it could reflect the existence of regulatory phosphorylation, yet to be identified.

TARGETS OF NUCLEAR SIGNALING IN MSNs

The major nuclear target of signaling pathways in post-mitotic cells is the transcription machinery. Since the first studies on growth factor-regulated transcription in non-neuronal cells (Cochran et al., 1983), it is usual to differentiate immediate-early genes (IEGs) that are rapidly transcribed in response to stimuli and whose transcription does not require protein synthesis. The promoters of these genes contain specific response elements that are targeted by transcription factors regulated by various signaling pathways. Many IEGs are themselves transcription factors and their up-regulation accounts for the induction of late genes. Late genes are transcribed after a longer delay after the onset of the stimulus and their transcription is prevented by protein synthesis inhibitors that prevent IEGs translation. Thus, regulation of transcription includes changes in the levels, localization, and activity of transcription factors, as well as functionally associated alterations in chromatin structure and organization, which can either facilitate or inhibit the expression of specific genes. Some chromatin alterations are long-lasting and may be involved in stable neuronal adaptations. It should be emphasized that regulation of other types of nuclear functions may also be important in neurons such as RNA processing, including mRNA splicing. However, virtually nothing is known about the regulation of these nuclear processes in striatal neurons.

REGULATION OF TRANSCRIPTION FACTORS IN MSNs

The accumulation of active PKA and/or P-ERK in the nucleus leads to phosphorylation of several nuclear proteins involved in gene transcription. In neurons the most extensively studied transcription factor substrate of PKA is the cAMP response-element (CRE) binding protein (CREB). CREB is a member of the leucine-zipper transcription factors family that bind to a specific DNA sequence, CRE, found in one or several copies in the promoters of many genes (Shaywitz and Greenberg, 1999). CREB dimer binds constitutively to CRE sites, but is inactive. Its activity increases when it is phosphorylated on Ser-133, allowing its interaction with the co-activator CREB binding protein (CBP) or the related p300 (Goodman and Smolik, 2000). CBP mutations are responsible for Rubinstein-Taybi syndrome, a rare human genetic disorder characterized by mental retardation and physical abnormalities (Petrij et al., 1995). CBP possess a histone acetyltransferase (HAT) activity and interacts with the basic transcriptional machinery. CREB also interacts with the cAMP-regulated transcriptional co-activators (CRTC1-3, also called transducer of regulated CREB – ToRC – in *Drosophila melanogaster*; Altarejos and Montminy, 2011). CRTCs are important partners of CREB, activated in response to cAMP and Ca^{2+} , which both promote its nuclear accumulation. Interestingly, CRTC is upregulated in rats chronically treated with cocaine, through the up-regulation of microRNA-212 (miR-212; Hollander et al., 2010). In addition to PKA, Ser-133 is the target of many other protein kinases, including CaMK II/IV, p90 ribosomal S6 kinases 1/2 (RSK1/2), mitogen- and stress-activated kinases 1/2 (MSK1/2), and PKC (Johannessen and Moens, 2007). MSK1 is a nuclear substrate of ERK (Deak et al., 1998) enriched in the striatum (Heffron and Mandell, 2005). The role of MSK1 in CREB regulation in MSNs seems to be prominent since in mutant mice

lacking MSK1 many biochemical nuclear responses in response to cocaine, including CREB phosphorylation, were blocked and locomotor sensitization was decreased (Brami-Cherrier et al., 2005). In contrast, Zif-268 induction and cocaine conditioned place preference were not altered. Importantly, Δ FosB, a cocaine-induced transcription factor that is expressed at low levels following a single drug administration, but accumulates after repeated treatment due to its stability, plays a major role in morphological and behavioral responses to chronic cocaine (Nestler, 2008).

The transcription factor Elk1, a member of the Ets family, associates with two molecules of serum response factor (SRF) to form a ternary complex which binds to serum response elements (SRE) found in the promoter of many genes (Besnard et al., 2010; **Figure 1**). In MSNs Elk-1 is located in part in the cytoplasm and its nuclear accumulation depends on its phosphorylation by ERK (Vanhoutte et al., 1999). Moreover, ERK and Elk1 appear to be recruited from neurites to cell bodies and nucleus in response to glutamate stimulation, and to undergo retrograde trafficking with α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, following their clathrin-dependent endocytosis (Trifilieff et al., 2009; **Figure 1**). This interesting mechanism could provide an example of synapse-to nucleus signaling, although this remains to be directly demonstrated.

In addition to the ERK-mediated pathways, it should be mentioned that stimulation of D1R induces gene transcription through activation of L-type calcium channels and recruitment of the Ca^{2+} /calcineurin-regulated nuclear factor of activated T-cells (NFAT; Groth et al., 2008). A different type of cytoplasmic transcription factor which accumulates in the nucleus upon binding of its ligand, the glucocorticoid receptor (GR), also plays an important role in responses mediated by striatal neurons. Selective deletion of GR in neurons expressing the D1R promoter resulted in altered gene expression and behavioral responses induced by cocaine but not morphine (Barik et al., 2010). Many other transcription factors are likely to be important for controlling gene expression in MSNs but have so far received less attention.

REGULATION OF CHROMATIN REMODELING IN MSNs

Signaling in the nucleus not only controls the activity of transcription factors that mediate gene expression, but also changes chromatin structure thereby modifying the transcriptional rate of specific genes. Histones can undergo multiple post-translational modifications on their N-terminal tail, including phosphorylation, acetylation, methylation, ubiquitinylation, and sumoylation, which alter their interaction with DNA, and thus the degree of chromatin condensation (see Shilatiffard, 2006 for a review). Transition from condensed chromatin into a more relaxed state is needed to accommodate the molecular elements required for transcription. Chromatin remodeling is involved in many processes in the nervous system, during development and differentiation, but also learning and memory (Levenson and Sweatt, 2005). Recent research indicates that alterations of histone post-translational modifications may also be implicated in psychiatric disorders, including depression, schizophrenia, and drug addiction (Tsankova et al., 2007; Borrelli et al., 2008; Brami-Cherrier et al., 2009; Franklin and Mansuy, 2010).

Phosphorylation of histone H3 on Ser-10 is the most extensively studied chromatin post-translational modification in striatal neurons. The functional significance of this modification is not entirely clear however. It has been suggested that during the interphase, phosphorylation of H3 Ser-10 promotes detachment of specific regions from the heterochromatic scaffold to allow decondensation and gene expression (Johansen and Johansen, 2006). Ser-10 phosphorylation in combination with H3 Lys-14 methylation is associated with increased *c-Fos* transcription in hippocampal neurons in response to stimulation of various receptors including D1R (Crosio et al., 2003). Phosphorylation of H3 is increased in striatal neurons after acute treatment with cocaine, a response that requires MSK1 (Brami-Cherrier et al., 2005) and occurs selectively in striatonigral neurons (Bertran-Gonzalez et al., 2008). Importantly, chromatin immunoprecipitation (ChIP) demonstrated that this phosphorylation occurred specifically at the promoter region of IEGs (Kumar et al., 2005) through recruitment of CBP (Levine et al., 2005). Similar responses were observed in striatonigral neurons following L-DOPA treatment in dopamine-denervated striatum (Santini et al., 2007, 2009). Conversely, in striatopallidal neurons it is the blockade of D2 receptors which triggers H3 Ser-10 phosphorylation by unmasking the stimulation of the PKA pathway by A2a adenosine receptors, normally inhibited by D2R (Li et al., 2004; Bertran-Gonzalez et al., 2008, 2009). In this case, phosphorylation of H3 Ser-10 is not mediated by MSK1, and may depend directly on PKA and PP1 inhibition by DARPP-32. Additional modifications of H3 and H4 tails have been reported in similar conditions (Li et al., 2004; Brami-Cherrier et al., 2005; Kumar et al., 2005; Nicholas et al., 2008).

Recent work further supports the role of covalent modifications of histones in the long-lasting effect of cocaine and possibly other addictive drugs (LaPlant and Nestler, 2011). Genome-wide studies using ChIP with antibodies for polyacetylated H3 or H4, or dimethylated H3 revealed changes in numerous genes including a group of deacetylases, the sirtuins (Renthal et al., 2009). This study emphasized the breadth of drug-induced gene regulation and the interest of genome-wide studies in this context. Repeated cocaine administration reduced global levels of H3 Lys-9 dimethylation in the nucleus accumbens, through the repression of the G9a lysine dimethyltransferase in this brain region by Δ FosB (Maze et al., 2010). Using conditional mutagenesis and viral-mediated gene transfer, the authors found that G9a down-regulation increased the dendritic spine plasticity of nucleus accumbens MSNs and enhanced the preference for cocaine. Interestingly, di- and trimethylation of H3 Lys-9 create binding sites for chromodomain-containing proteins, including those of the heterochromatin protein 1 (HP1) family which promote transcriptional repression and genomic silencing (Bannister et al., 2001; Lachner et al., 2001). Accordingly, altered H3 Lys-9 trimethylation induced in the nucleus accumbens by repeated cocaine administration was associated with changes in heterochromatin (Maze et al., 2011). Overexpression in the ventral striatum of Setdb1 (Set domain, bifurcated 1), a methyltransferase specific for H3 Lys-9, down-regulated the expression of NR2B (Jiang et al., 2010). All these results identify regulation of H3 Lys-9 methylation as functionally important in the striatum.

DNA methylation on cytidine residues, preferentially located in CpG islands, is classically considered to be a very stable mark. However, in brain neurons DNA methylation appears to be reversible and to participate in memory formation and storage (Day and Sweatt, 2010). A recent work provides a possible mechanism for demethylation in this context, through a process that requires the base excision repair pathway (Guo et al., 2011). This involves a specific hydroxylase, TET1, which converts 5-methylcytosine to 5-hydroxymethylcytosine, which exists at significant levels in various brain regions (Kriaucionis and Heintz, 2009). The importance of DNA methylation in the striatum is indicated by the role of the methylase Dnmt3 in cocaine-induced spine alterations (LaPlant et al., 2010). Moreover the X-linked transcriptional repressor methyl CpG binding protein 2 (MeCP2), known for its role in a neurodevelopmental disorder, the Rett syndrome, regulates cocaine intake in rat (Im et al., 2010). This effect seems to depend on regulation of striatal BDNF levels in interaction with miR-212 (Im et al., 2010), which is itself upregulated by chronic cocaine, as mentioned above (Hollander et al., 2010). Moreover, amphetamine-induced spine plasticity and conditioned place preference were altered in MeCP2-deficient mice (Deng et al., 2010). Altogether these observations strongly support the importance of chromatin post-translational modifications and DNA methylation in the chronic effects of drugs of abuse in striatal neurons and suggest they may also be important for the long-lasting adaptations of these neurons in physiological or other pathological circumstances.

ROLE OF PROTEIN PHOSPHATASES IN THE REGULATION OF CHROMATIN RESPONSES IN MSNs

We have discussed above several protein kinases pathways which converge to the nucleus of MSNs where they control transcription factors. These pathways can also directly alter the state of phosphorylation of histones and other nuclear proteins including enzymes regulating the modifications of histones or DNA. The resulting changes in transcription can in turn have consequences on chromatin structure, as indicated by the effects of chronic drug treatments on the levels of several mRNAs coding for these enzymes (LaPlant et al., 2010; Maze et al., 2010). However much remains to be done to better understand these regulations. Recent work has uncovered the likely importance of the regulation of nuclear protein phosphatases in striatal neurons. The role of nuclear protein phosphatases has been extensively studied in non-neuronal tissues where these enzymes have been implicated in the regulation of multiple processes including gene transcription, RNA processing, cell cycle progression or signal termination (Bollen and Beullens, 2002; Moorhead et al., 2007). For example, PP1 is a major P-CREB phosphatase (Hagiwara et al., 1992) and regulates the dephosphorylation of P-Ser-10 H3 in mitotic cells (Hsu et al., 2000). Regulation of PP1 activity in the nucleus of hippocampal neurons alters histone post-translational modifications directly (phosphorylation) and indirectly through the control enzymes that modify acetylation and methylation, as well as CREB expression and phosphorylation associated with learning and memory (Koshibu et al., 2009). PP1 α appears to be the predominant PP1 isoform in the nucleus of MSNs (Ouimet et al., 1995), where it has the potential to antagonize the action of

dopamine and glutamate by dephosphorylating PKA and ERK targets. Recent work shows that PP1 activity can be modulated in the nucleus of MSNs through the regulated accumulation of phospho-Thr-34-DARPP-32 (Stipanovich et al., 2008). This study demonstrated that psychostimulant drugs (d-amphetamine and cocaine) and morphine, which act by different mechanisms but share the ability to increase extracellular dopamine in the striatum, as well as incentive learning, increased the nuclear concentration of DARPP-32. *In vitro* analysis of the mechanisms of DARPP-32 nuclear translocation showed that this protein is constantly shuttling between the cytoplasm and the nucleus, although it is predominantly localized in the cytoplasm as a result of an efficient CRM-1-dependent nuclear export (Stipanovich et al., 2008). This export requires phosphorylation of Ser-97 by casein kinase 2 (CK2) present in the nucleus and cytoplasm of MSNs (Figure 1). Upon D1R stimulation, Ser-97 is dephosphorylated, presumably through activation of a PKA-activated form of PP2A (Ahn et al., 2007; Stipanovich et al., 2008), causing a nuclear accumulation of DARPP-32. Ongoing work in our laboratory has revealed that additional mechanisms are involved in the control of DARPP-32 cytonuclear localization, providing the basis for its precise but complex regulation by multiple extracellular signals (Matamales and Girault, unpublished observations). It should be underlined that the control of DARPP-32 localization by regulation of Ser-97 phosphorylation is parallel to but independent from its phosphorylation on Thr-34, which is responsible for PP1 inhibition activity. When P-Thr34 DARPP-32 accumulates in the nucleus, it can facilitate the phosphorylation of histone H3 on Ser-10 (Stipanovich et al., 2008). In contrast, nuclear trapping of dephospho-Thr-34-DARPP-32 by mutation of Ser-97 to Ala decreases signaling responses including H3 phosphorylation (ibid and our unpublished observations). Thus, it is possible that in some circumstances nuclear accumulation of inactive DARPP-32 plays a role in down-regulating cytoplasmic responses, a hypothesis that needs to be explored.

The nuclear accumulation of the active form of a potent PP1 inhibitor may help to tilt the kinase and phosphatase equilibrium in favor of phosphorylation. Given the large number of potential substrates of PP1 in the nucleus, it is likely that the regulation of DARPP-32 trafficking has multiple consequences that have yet to be identified. For example, it has been shown that DARPP-32 binds directly to the splicing factor transformer 2-beta 1 (tra2-beta1) and changes the usage of tra2-beta1 dependent alternative exons in heterologous systems (Benderska et al., 2010). Therefore the link

between the nuclear localization of DARPP-32 and short term and long-term adaptations, including but not restricted to changes in gene transcription, clearly needs to be further investigated.

CONCLUSION AND PERSPECTIVES

We have provided here an overview of some mechanisms of cytonuclear signaling and regulation of nuclear functions and summarized the current knowledge of glutamate and dopamine signaling to the nucleus in MSNs (Figure 1). Much of our understanding of the basic mechanisms of cytonuclear signaling is inferred from work in other cellular systems since few studies have dissected the mechanisms and regulation of these pathways in striatal neurons so far. As a general principle the prevention of “background noise” kinase activity in the nucleus of unstimulated neurons is likely to be important to avoid meaningless nuclear responses. This is probably achieved through the regulated cytoplasmic sequestration of PKA, ERK, and other signaling molecules including some transcription factors, as well as by potent phosphatase activities in the nucleus. Upon receptor stimulation, and resulting cAMP and Ca²⁺ production, protein kinases are activated and accumulate in the nucleus, where they exert their effects directly or indirectly through resident nuclear kinases such as MSK1. Identified targets include transcription factors and chromatin components such as histones that modulate the rate of transcription. The silencing of phosphatase activity through activation and regulated nuclear translocation of the PP1 inhibiting form of DARPP-32 plays a complementary role in MSNs. Thus, the precise nuclear adaptations taking place in MSNs are likely to depend on a fine balance between multiple cytonuclear signaling pathways. Since the nuclear responses are important for long-term changes of behavioral responses we suggest that deciphering the rules of cytonuclear signaling in striatal neurons will be crucial to understand the learning mechanisms that involve the basal ganglia and their pathological alterations. This new field is only beginning to be explored in this context.

ACKNOWLEDGMENTS

Work in J. A. Girault's laboratory is supported by Inserm and UPMC and by grants from the *Fondation pour la recherche médicale* (FRM), the *Agence Nationale de la Recherche* (ANR), the *Fondation pour la Recherche sur le cerveau* (FRC), and the European Research Council. The group is affiliated to the *Ecole des Neurosciences de Paris Ile de France* (ENP). M. Matamales is currently supported by an EMBO Long-Term Fellowship (ALTF 1228-2010).

REFERENCES

- Adachi, M., Fukuda, M., and Nishida, E. (1999). Two co-existing mechanisms for nuclear import of MAP kinase: passive diffusion of a monomer and active transport of a dimer. *EMBO J.* 18, 5347–5358.
- Adachi, M., Fukuda, M., and Nishida, E. (2000). Nuclear export of MAP kinase (ERK) involves a MAP kinase (MEK)-dependent active transport mechanism. *J. Cell Biol.* 148, 849–856.
- Adams, J. P., and Dudek, S. M. (2005). Late-phase long-term potentiation: getting to the nucleus. *Nat. Rev. Neurosci.* 6, 737–743.
- Ahn, J. H., McAvoy, T., Rakhilin, S. V., Nishi, A., Greengard, P., and Nairn, A. C. (2007). Protein kinase A activates protein phosphatase 2A by phosphorylation of the B56delta subunit. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2979–2984.
- Altarejos, J. Y., and Montminy, M. (2011). CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nat. Rev. Mol. Cell Biol.* 12, 141–151.
- Askjaer, P., Jensen, T. H., Nilsson, J., Englmeier, L., and Kjems, J. (1998). The specificity of the CRM1-Rev nuclear export signal interaction is mediated by RanGTP. *J. Biol. Chem.* 273, 33414–33422.
- Bannister, A. J., Zegerman, P., Partridge, J. F., Miska, E. A., Thomas, J. O., Allshire, R. C., and Kouzarides, T. (2001). Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* 410, 120–124.
- Barik, J., Parnaudeau, S., Saint Amax, A. L., Guiard, B. P., Golib Dzib, J. F., Bocquet, O., Bailly, A., Benecke, A., and Tronche, F. (2010). Glucocorticoid receptors in dopaminergic neurons, key for cocaine, are dispensable for molecular and behavioral morphine responses. *Biol. Psychiatry* 68, 231–239.
- Basel-Vanagaite, L., Muncher, L., Straussberg, R., Pasmanik-Chor, M., Yahav, M., Rainshtein, L., Walsh, C. A., Magal, N., Taub, E., Drasinover, V., Shalev, H., Attia, R., Rechavi, G., Simon, A. J., and Shohat, M. (2006). Mutated nup62 causes autosomal recessive infantile bilateral

- striatal necrosis. *Ann. Neurol.* 60, 214–222.
- Beaulieu, J. M., Sotnikova, T. D., Marion, S., Lefkowitz, R. J., Gainetdinov, R. R., and Caron, M. G. (2005). An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell* 122, 261–273.
- Benderska, N., Becker, K., Girault, J. A., Becker, C. M., Andreadis, A., and Stamm, S. (2010). DARPP-32 binds to tra2-beta1 and influences alternative splicing. *Biochim. Biophys. Acta.* 1799, 448–453.
- Bennett, B. D., and Wilson, C. J. (2000). "Synaptology and physiology of neostriatal neurones," in *Brain Dynamics and the Striatal Complex*, eds R. Miller and J. Wickens (London: H. A. Publishers), 111–140.
- Berger, S. L., Kouzarides, T., Shiekhattar, R., and Shilatifard, A. (2009). An operational definition of epigenetics. *Genes Dev.* 23, 781–783.
- Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamales, M., Herve, D., Valjent, E., and Girault, J. A. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J. Neurosci.* 28, 5671–5685.
- Bertran-Gonzalez, J., Hakansson, K., Borgkvist, A., Irinopoulou, T., Brami-Cherrier, K., Usiello, A., Greengard, P., Herve, D., Girault, J. A., Valjent, E., and Fisone, G. (2009). Histone H3 phosphorylation is under the opposite tonic control of dopamine D2 and adenosine A2A receptors in striatopallidal neurons. *Neuropsychopharmacology* 34, 1710–1720.
- Bertran-Gonzalez, J., Herve, D., Girault, J. A., and Valjent, E. (2010). What is the degree of segregation between striatonigral and striatopallidal projections? *Front. Neuroanat.* 4:136. doi: 10.3389/fnana.2010.00136
- Besnard, A., Galan-Rodriguez, B., Vanhoutte, P., and Caboche, J. (2010). Elk-1 a transcription factor with multiple facets in the brain. *Front. Neurosci.* 5:35. doi: 10.3389/fnins.2011.00035
- Bilbao, A., Parkitna, J. R., Engblom, D., Perreau-Lenz, S., Sanchis-Segura, C., Schneider, M., Konopka, W., Westphal, M., Breen, G., Desrivieres, S., Klugmann, M., Guindalini, C., Vallada, H., Laranjeira, R., de Fonseca, F. R., Schumann, G., Schutz, G., and Spanagel, R. (2008). Loss of the Ca2+/calmodulin-dependent protein kinase type IV in dopaminergic neurons enhances behavioral effects of cocaine. *Proc. Natl. Acad. Sci. U.S.A.* 105, 17549–17554.
- Black, I. B., Adler, J. E., Dreyfus, C. F., Friedman, W. F., LaGamma, E. F., and Roach, A. H. (1987). Biochemistry of information storage in the nervous system. *Science* 236, 1263–1268.
- Bollen, M., and Beullens, M. (2002). Signaling by protein phosphatases in the nucleus. *Trends Cell Biol.* 12, 138–145.
- Bollen, M., Peti, W., Ragusa, M. J., and Beullens, M. (2010). The extended PP1 toolkit: designed to create specificity. *Trends Biochem. Sci.* 35, 450–458.
- Borrelli, E., Nestler, E. J., Allis, C. D., and Sassone-Corsi, P. (2008). Decoding the epigenetic language of neuronal plasticity. *Neuron* 60, 961–974.
- Brami-Cherrier, K., Roze, E., Girault, J. A., Betuing, S., and Caboche, J. (2009). Role of the ERK/MSK1 signalling pathway in chromatin remodelling and brain responses to drugs of abuse. *J. Neurochem.* 108, 1323–1335.
- Brami-Cherrier, K., Valjent, E., Garcia, M., Pages, C., Hipskind, R. A., and Caboche, J. (2002). Dopamine induces a PI3-kinase-independent activation of Akt in striatal neurons: a new route to cAMP response element-binding protein phosphorylation. *J. Neurosci.* 22, 8911–8921.
- Brami-Cherrier, K., Valjent, E., Herve, D., Darragh, J., Corvol, J. C., Pages, C., Arthur, S. J., Girault, J. A., and Caboche, J. (2005). Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. *J. Neurosci.* 25, 11444–11454.
- Burack, W. R., and Shaw, A. S. (2005). Live cell imaging of ERK and MEK: simple binding equilibrium explains the regulated nucleocytoplasmic distribution of ERK. *J. Biol. Chem.* 280, 3832–3837.
- Chen, T., Brownawell, A. M., and Macara, I. G. (2004). Nucleocytoplasmic shuttling of JAZ, a new cargo protein for exportin-5. *Mol. Cell Biol.* 24, 6608–6619.
- Chuderland, D., Konson, A., and Seger, R. (2008). Identification and characterization of a general nuclear translocation signal in signaling proteins. *Mol. Cell* 31, 850–861.
- Cochran, B. H., Reffel, A. C., and Stiles, C. D. (1983). Molecular cloning of gene sequences regulated by platelet-derived growth factor. *Cell* 33, 939–947.
- Cohen, S., and Greenberg, M. E. (2008). Communication between the synapse and the nucleus in neuronal development, plasticity, and disease. *Annu. Rev. Cell Dev. Biol.* 24, 183–209.
- Corvol, J. C., Studler, J. M., Schonn, J. S., Girault, J. A., and Herve, D. (2001). Galpha(olf) is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. *J. Neurochem.* 76, 1585–1588.
- Creese, I., Sibley, D. R., Hamblin, M. W., and Leff, S. E. (1983). The classification of dopamine receptors: relationship to radioligand binding. *Annu. Rev. Neurosci.* 6, 43–71.
- Crosio, C., Heitz, E., Allis, C. D., Borrelli, E., and Sassone-Corsi, P. (2003). Chromatin remodeling and neuronal response: multiple signaling pathways induce specific histone H3 modifications and early gene expression in hippocampal neurons. *J. Cell. Sci.* 116, 4905–4914.
- Da Cruz e Silva, E. F., Fox, C. A., Ouimet, C. C., Gustafson, E., Watson, S. J., and Greengard, P. (1995). Differential expression of protein phosphatase 1 isoforms in mammalian brain. *J. Neurosci.* 15, 3375–3389.
- Day, J. J., and Sweatt, J. D. (2010). DNA methylation and memory formation. *Nat. Neurosci.* 13, 1319–1323.
- Deak, M., Clifton, A. D., Lucocq, L. M., and Alessi, D. R. (1998). Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. *EMBO J.* 17, 4426–4441.
- Deisseroth, K., and Tsien, R. W. (2002). Dynamic multiphosphorylation passwords for activity-dependent gene expression. *Neuron* 34, 179–182.
- Deng, J. V., Rodriguez, R. M., Hutchinson, A. N., Kim, I. H., Wetsel, W. C., and West, A. E. (2010). MeCP2 in the nucleus accumbens contributes to neural and behavioral responses to psychostimulants. *Nat. Neurosci.* 13, 1128–1136.
- Diaz, J., Lévesque, D., Lammers, C. H., Griffon, N., Martres, M.-P., Schwartz, J.-C., and Sokoloff, P. (1995). Phenotypic characterization of neurons expressing the dopamine D3 receptor in the rat brain. *Neuroscience* 65, 731–745.
- Di Chiara, G., Tanda, G., Cadoni, C., Acquas, E., Bassareo, V., and Carboni, E. (1998). Homologies and differences in the action of drugs of abuse and a conventional reinforcer (food) on dopamine transmission: an interpretative framework of the mechanism of drug dependence. *Adv. Pharmacol.* 42, 983–987.
- Eulalio, A., Nunes-Correia, I., Carvalho, A. L., Faro, C., Citovsky, V., Salas, J., Salas, M. L., Simoes, S., and de Lima, M. C. (2006). Nuclear export of African swine fever virus p37 protein occurs through two distinct pathways and is mediated by three independent signals. *J. Virol.* 80, 1393–1404.
- Fantozzi, D. A., Harootyan, A. T., Wen, W., Taylor, S. S., Feramisco, J. R., Tsien, R. Y., and Meinkoth, J. L. (1994). Thermostable inhibitor of cAMP-dependent protein kinase enhances the rate of export of the kinase catalytic subunit from the nucleus. *J. Biol. Chem.* 269, 2676–2686.
- Fasano, S., D'Antoni, A., Orban, P. C., Valjent, E., Putignano, E., Vara, H., Pizzorusso, T., Giustetto, M., Yoon, B., Soloway, P., Maldonado, R., Caboche, J., and Brambilla, R. (2009). Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) controls activation of extracellular signal-regulated kinase (ERK) signaling in the striatum and long-term behavioral responses to cocaine. *Biol. Psychiatry* 66, 758–768.
- Franklin, T. B., and Mansuy, I. M. (2010). The prevalence of epigenetic mechanisms in the regulation of cognitive functions and behaviour. *Curr. Opin. Neurobiol.* 20, 441–449.
- Fraser, P., and Bickmore, W. (2007). Nuclear organization of the genome and the potential for gene regulation. *Nature* 447, 413–417.
- Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J. Jr., and Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250, 1429–1432.
- Gervasi, N., Hepp, R., Tricoire, L., Zhang, J., Lambolez, B., Paupardin-Tritsch, D., and Vincent, P. (2007). Dynamics of protein kinase A signaling at the membrane, in the cytosol, and in the nucleus of neurons in mouse brain slices. *J. Neurosci.* 27, 2744–2750.
- Girault, J. A., Valjent, E., Caboche, J., and Herve, D. (2007). ERK2: a logical AND gate critical for drug-induced plasticity? *Curr. Opin. Pharmacol.* 7, 77–85.
- Goelet, P., Castellucci, V. F., Schacher, S., and Kandel, E. R. (1986). The long and the short of long-term memory—a molecular framework. *Nature* 322, 419–422.
- Goodman, R. H., and Smolik, S. (2000). CBP/p300 in cell growth, transformation, and development. *Genes Dev.* 14, 1553–1577.

- Grace, A. A. (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsiveness: a hypothesis for the etiology of schizophrenia. *Neuroscience* 41, 1–24.
- Greengard, P., Allen, P. B., and Nairn, A. C. (1999). Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* 23, 435–447.
- Groth, R. D., Weick, J. P., Bradley, K. C., Luoma, J. I., Aravamudan, B., Klug, J. R., Thomas, M. J., and Mermelstein, P. G. (2008). D1 dopamine receptor activation of NFAT-mediated striatal gene expression. *Eur. J. Neurosci.* 27, 31–42.
- Guo, J. U., Su, Y., Zhong, C., Ming, G. L., and Song, H. (2011). Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. *Cell* 145, 423–434.
- Hagiwara, M., Alberts, A., Brindle, P., Meinkoth, J., Feramisco, J., Deng, T., Karin, M., Shenolikar, S., and Montminy, M. (1992). Transcriptional attenuation following cAMP induction requires PP-1-mediated dephosphorylation of CREB. *Cell* 70, 105–113.
- Hanz, S., Perlson, E., Willis, D., Zheng, J. Q., Massarwa, R., Huerta, J. J., Koltzenburg, M., Kohler, M., van-Minnen, J., Twiss, J. L., and Fainzilber, M. (2003). Axoplasmic importins enable retrograde injury signaling in lesioned nerve. *Neuron* 40, 1095–1104.
- Harootyan, A. T., Adams, S. R., Wen, W., Meinkoth, J. L., Taylor, S. S., and Tsien, R. Y. (1993). Movement of the free catalytic subunit of cAMP-dependent protein kinase into and out of the nucleus can be explained by diffusion. *Mol. Biol. Cell* 4, 993–1002.
- Hasbi, A., Fan, T., Alijanian, M., Nguyen, T., Perreault, M. L., O'Dowd, B. F., and George, S. R. (2009). Calcium signaling cascade links dopamine D1-D2 receptor heteromer to striatal BDNF production and neuronal growth. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21377–21382.
- Heffron, D., and Mandell, J. W. (2005). Differential localization of MAPK-activated protein kinases RSK1 and MSK1 in mouse brain. *Brain Res. Mol. Brain Res.* 136, 134–141.
- Hemmings, H. C. Jr., Greengard, P., Tung, H. Y. L., and Cohen, P. (1984). DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. *Nature* 310, 503–505.
- Hempel, C. M., Vincent, P., Adams, S. R., Tsien, R. Y., and Selverston, A. I. (1996). Spatio-temporal dynamics of cyclic AMP signals in an intact neural circuit. *Nature* 384, 166–169.
- Henikoff, S. (2008). Nucleosome destabilization in the epigenetic regulation of gene expression. *Nat. Rev. Genet.* 9, 15–26.
- Herve, D., Levi-Strauss, M., Marey-Semper, I., Verney, C., Tassin, J. P., Glowinski, J., and Girault, J. A. (1993). G(olf) and Gs in rat basal ganglia: possible involvement of G(olf) in the coupling of dopamine D1 receptor with adenylyl cyclase. *J. Neurosci.* 13, 2237–2248.
- Hoelz, A., Debler, E. W., and Blobel, G. (2011). The structure of the nuclear pore complex. *Annu. Rev. Biochem.* 80, 613–643.
- Hollander, J. A., Im, H. I., Amelio, A. L., Kocerha, J., Bali, P., Lu, Q., Willoughby, D., Wahlestedt, C., Konkright, M. D., and Kenny, P. J. (2010). Striatal microRNA controls cocaine intake through CREB signalling. *Nature* 466, 197–202.
- Hsu, J. Y., Sun, Z. W., Li, X., Reuben, M., Tatchell, K., Bishop, D. K., Grushcow, J. M., Brame, C. J., Caldwell, J. A., Hunt, D. F., Lin, R., Smith, M. M., and Allis, C. D. (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell* 102, 279–291.
- Hur, E. M., and Zhou, F. Q. (2010). GSK3 signalling in neural development. *Nat. Rev. Neurosci.* 11, 539–551.
- Im, H. I., Hollander, J. A., Bali, P., and Kenny, P. J. (2010). MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. *Nat. Neurosci.* 13, 1120–1127.
- Impey, S., Obrietan, K., Wong, S. T., Poser, S., Yano, S., Wayman, G., Deloulme, J. C., Chan, G., and Storm, D. R. (1998). Cross talk between ERK and PKA is required for Ca²⁺ stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron* 21, 869–883.
- Jiang, Y., Jakovcevski, M., Bharadwaj, R., Connor, C., Schroeder, F. A., Lin, C. L., Straubhaar, J., Martin, G., and Akbarian, S. (2010). Setdb1 histone methyltransferase regulates mood-related behaviors and expression of the NMDA receptor subunit NR2B. *J. Neurosci.* 30, 7152–7167.
- Johannessen, M., and Moens, U. (2007). Multisite phosphorylation of the cAMP response element-binding protein (CREB) by a diversity of protein kinases. *Front. Biosci.* 12, 1814–1832.
- Johansen, K. M., and Johansen, J. (2006). Regulation of chromatin structure by histone H3S10 phosphorylation. *Chromosome Res.* 14, 393–404.
- Jordan, B. A., and Kreutz, M. R. (2009). Nucleocytoplasmic protein shuttling: the direct route in synapse-to-nucleus signaling. *Trends Neurosci.* 32, 392–401.
- Kaffman, A., and O'Shea, E. K. (1999). Regulation of nuclear localization: a key to a door. *Annu. Rev. Cell Dev. Biol.* 15, 291–339.
- Kebabian, J. W., and Calne, D. B. (1979). Multiple receptors for dopamine. *Nature* 277, 93–96.
- Khokhlatchev, A. V., Canagarajah, B., Wilsbacher, J., Robinson, M., Atkinson, M., Goldsmith, E., and Cobb, M. H. (1998). Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. *Cell* 93, 605–615.
- Koshibu, K., Graff, J., Beullens, M., Heitz, F. D., Berchtold, D., Russig, H., Farinelli, M., Bollen, M., and Mansuy, I. M. (2009). Protein phosphatase 1 regulates the histone code for long-term memory. *J. Neurosci.* 29, 13079–13089.
- Kreitzer, A. C. (2009). Physiology and pharmacology of striatal neurons. *Annu. Rev. Neurosci.* 32, 127–147.
- Kriaucionis, S., and Heintz, N. (2009). The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science* 324, 929–930.
- Kudo, N., Matsumori, N., Taoka, H., Fujiwara, D., Schreiner, E. P., Wolff, B., Yoshida, M., and Horinouchi, S. (1999). Leptomycin B inactivates CRM1/exportin 1 by covalent modification at a cysteine residue in the central conserved region. *Proc. Natl. Acad. Sci. U.S.A.* 96, 9112–9117.
- Kumar, A., Choi, K. H., Renthal, W., Tsankova, N. M., Theobald, D. E., Truong, H. T., Russo, S. J., Laplant, Q., Sasaki, T. S., Whistler, K. N., Neve, R. L., Self, D. W., and Nestler, E. J. (2005). Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* 48, 303–314.
- Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., and Jenuwein, T. (2001). Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* 410, 116–120.
- LaPlant, Q., and Nestler, E. J. (2011). CRACKing the histone code: cocaine's effects on chromatin structure and function. *Horm. Behav.* 59, 321–330.
- LaPlant, Q., Vialou, V., Covington, H. E., 3rd, Dumitriu, D., Feng, J., Warren, B. L., Maze, I., Dietz, D. M., Watts, E. L., Iniguez, S. D., Koo, J. W., Mouzon, E., Renthal, W., Hollis, F., Wang, H., Noonan, M. A., Ren, Y., Eisch, A. J., Bolanos, C. A., Kabbaj, M., Xiao, G., Neve, R. L., Hurd, Y. L., Oosting, R. S., Fan, G., Morrison, J. H., and Nestler, E. J. (2010). Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nat. Neurosci.* 13, 1137–1143.
- Lee, S. J., Matsuura, Y., Liu, S. M., and Stewart, M. (2005). Structural basis for nuclear import complex dissociation by RanGTP. *Nature* 435, 693–696.
- Levenson, J. M., and Sweatt, J. D. (2005). Epigenetic mechanisms in memory formation. *Nat. Rev. Neurosci.* 6, 108–118.
- Levine, A. A., Guan, Z., Barco, A., Xu, S., Kandel, E. R., and Schwartz, J. H. (2005). CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. *Proc. Natl. Acad. Sci. U.S.A.* 102, 19186–19191.
- Li, J., Guo, Y., Schroeder, F. A., Youngs, R. M., Schmidt, T. W., Ferris, C., Konradi, C., and Akbarian, S. (2004). Dopamine D2-like antagonists induce chromatin remodeling in striatal neurons through cyclic AMP-protein kinase A and NMDA receptor signaling. *J. Neurochem.* 90, 1117–1131.
- Lidke, D. S., Huang, F., Post, J. N., Rieger, B., Wilsbacher, J., Thomas, J. L., Pouyssegur, J., Jovin, T. M., and Lenormand, P. (2010). ERK nuclear translocation is dimerization-independent but controlled by the rate of phosphorylation. *J. Biol. Chem.* 285, 3092–3102.
- Lischka, P., Rosorius, O., Trommer, E., and Stamminger, T. (2001). A novel transferable nuclear export signal mediates CRM1-independent nucleocytoplasmic shuttling of the human cytomegalovirus transactivator protein pUL69. *EMBO J.* 20, 7271–7283.
- Martin, K. C., Michael, D., Rose, J. C., Barad, M., Casadio, A., Zhu, H. X., and Kandel, E. R. (1997). MAP kinase translocates into the nucleus of the presynaptic cell and is required for long-term facilitation in Aplysia. *Neuron* 18, 899–912.
- Matamales, M., Bertran-Gonzalez, J., Salomon, L., Degos, B., Deniau, J. M., Valjent, E., Herve, D., and Girault, J. A. (2009). Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. *PLoS ONE* 4, e4770. doi: 10.1371/journal.pone.0004770

- Matsushita, M., Tomizawa, K., Moriwaki, A., Li, S. T., Terada, H., and Matsui, H. (2001). A high-efficiency protein transduction system demonstrating the role of PKA in long-lasting long-term potentiation. *J. Neurosci.* 21, 6000–6007.
- Maze, I., Covington, H. E., 3rd, Dietz, D. M., LaPlant, Q., Renthal, W., Russo, S. J., Mechanic, M., Mouzon, E., Neve, R. L., Haggarty, S. J., Ren, Y., Sampath, S. C., Hurd, Y. L., Greengard, P., Tarakhovskiy, A., Schaefer, A., and Nestler, E. J. (2010). Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science* 327, 213–216.
- Maze, I., Feng, J., Wilkinson, M. B., Sun, H., Shen, L., and Nestler, E. J. (2011). Cocaine dynamically regulates heterochromatin and repetitive element unsilencing in nucleus accumbens. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3035–3040.
- Meffert, M. K., Chang, J. M., Wiltgen, B. J., Fanselow, M. S., and Baltimore, D. (2003). NF-kappa B functions in synaptic signaling and behavior. *Nat. Neurosci.* 6, 1072–1078.
- Meinkoth, J. L., Ji, Y., Taylor, S. S., and Feramisco, J. R. (1990). Dynamics of the distribution of cyclic AMP-dependent protein kinase in living cells. *Proc. Natl. Acad. Sci. U.S.A.* 87, 9595–9599.
- Miyamoto, E., Kuo, J. F., and Greengard, P. (1968). Adenosine 3',5'-monophosphate-dependent protein kinase from brain. *Science* 165, 63–65.
- Moorhead, G. B. G., Trinkle-Mulcahy, L., and Ulke-Lemée, A. (2007). Emerging roles of nuclear protein phosphatases. *Nat. Rev. Mol. Cell Biol.* 7, 235–244.
- Mosammaparast, N., and Pemberton, L. F. (2004). Karyopherins: from nuclear-transport mediators to nuclear-function regulators. *Trends Cell Biol.* 14, 547–556.
- Nardozi, J. D., Lott, K., and Cingolani, G. (2010). Phosphorylation meets nuclear import: a review. *Cell Commun. Signal* 8, 32.
- Nestler, E. J. (2008). Review. Transcriptional mechanisms of addiction: role of DeltaFosB. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 363, 3245–3255.
- Nicholas, A. P., Lubin, F. D., Hallett, P. J., Vattm, P., Ravenscroft, P., Bezaud, E., Zhou, S., Fox, S. H., Brochie, J. M., Sweatt, J. D., and Staadaert, D. G. (2008). Striatal histone modifications in models of levodopa-induced dyskinesia. *J. Neurochem.* 106, 486–494.
- Nicola, S. M., Surmeier, J., and Malenka, R. C. (2000). Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu. Rev. Neurosci.* 23, 185–215.
- Ouimet, C. C., da Cruz e Silva, E. F., and Greengard, P. (1995). The alpha and gamma 1 isoforms of protein phosphatase 1 are highly and specifically concentrated in dendritic spines. *Proc. Natl. Acad. Sci. U.S.A.* 92, 3396–3400.
- Ouimet, C. C., Miller, P. E., Hemmings, H. C. Jr., Walaas, S. I., and Greengard, P. (1984). DARPP-32, a dopamine- and adenosine 3',5'-monophosphate-regulated phosphoprotein enriched in dopamine-innervated brain regions. III Immunocytochemical localization. *J. Neurosci.* 4, 111–124.
- Paolillo, M., Feliciello, A., Porcellini, A., Garbi, C., Bifulco, M., Schinelli, S., Ventra, C., Stabile, E., Ricciardelli, G., Schettini, G., and Avvedimento, E. V. (1999). The type and the localization of cAMP-dependent protein kinase regulate transmission of cAMP signals to the nucleus in cortical and cerebellar granule cells. *J. Biol. Chem.* 274, 6546–6552.
- Pascoli, V., Besnard, A., Herve, D., Pages, C., Heck, N., Girault, J. A., Caboche, J., and Vanhoutte, P. (2011). Cyclic adenosine monophosphate-independent tyrosine phosphorylation of NR2B mediates cocaine-induced extracellular signal-regulated kinase activation. *Biol. Psychiatry* 69, 218–227.
- Patel, S. S., Belmont, B. J., Sante, J. M., and Rexach, M. F. (2007). Natively unfolded nucleoporins gate protein diffusion across the nuclear pore complex. *Cell* 129, 83–96.
- Patterson, S. L., Pittenger, C., Morozov, A., Martin, K. C., Scannell, H., Drake, C., and Kandel, E. R. (2001). Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. *Neuron* 32, 123–140.
- Pemberton, L. F., and Paschal, B. M. (2005). Mechanisms of receptor-mediated nuclear import and nuclear export. *Traffic* 6, 187–198.
- Petrij, F., Giles, R. H., Dauwerse, H. G., Saris, J. J., Hennekam, R. C., Masuno, M., Tommerup, N., van Ommen, G. J., Goodman, R. H., Peters, D. J., and Breuning, M. H. (1995). Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* 376, 348–351.
- Poon, I. K., and Jans, D. A. (2005). Regulation of nuclear transport: central role in development and transformation? *Traffic* 6, 173–186.
- Ptashne, M. (2007). On the use of the word 'epigenetic'. *Curr. Biol.* 17, R233–6.
- Ramon y Cajal, S. (1899). *Textura del Sistema Nervioso del Hombre y de los Vertebrados*. French translation, Maloine, Paris, 1955, I, 195–201.
- Renthal, W., Kumar, A., Xiao, G., Wilkinson, M., Covington, H. E., 3rd, Maze, I., Sikder, D., Robison, A. J., LaPlant, Q., Dietz, D. M., Russo, S. J., Vialou, V., Chakravarty, S., Kodadek, T. J., Stack, A., Kabbaj, M., and Nestler, E. J. (2009). Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. *Neuron* 62, 335–348.
- Ricci, A. (2010). Dynamic epigenetic regulation in neurons: enzymes, stimuli and signaling pathways. *Nat. Neurosci.* 13, 1330–1337.
- Santini, E., Alcacer, C., Cacciatore, S., Heiman, M., Herve, D., Greengard, P., Girault, J. A., Valjent, E., and Fisone, G. (2009). L-DOPA activates ERK signaling and phosphorylates histone H3 in the striatonigral medium spiny neurons of hemiparkinsonian mice. *J. Neurochem.* 108, 621–633.
- Santini, E., Valjent, E., Usiello, A., Carta, M., Borgkvist, A., Girault, J. A., Herve, D., Greengard, P., and Fisone, G. (2007). Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *J. Neurosci.* 27, 6995–7005.
- Sastri, M., Barraclough, D. M., Carmichael, P. T., and Taylor, S. S. (2005). A-kinase-interacting protein localizes protein kinase A in the nucleus. *Proc. Natl. Acad. Sci. U.S.A.* 102, 349–354.
- Sgambato, V., Pagès, C., Rogard, M., Besson, M. J., and Caboche, J. (1998). Extracellular signal-regulated kinase (ERK) controls immediate early gene induction on corticostriatal stimulation. *J. Neurosci.* 18, 8814–8825.
- Shaywitz, A. J., and Greenberg, M. E. (1999). CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu. Rev. Biochem.* 68, 821–861.
- Shilatfard, A. (2006). Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. *Annu. Rev. Biochem.* 75, 243–269.
- Stipanovich, A., Valjent, E., Matamales, M., Nishi, A., Ahn, J. H., Maroteaux, M., Bertran-Gonzalez, J., Brami-Cherrier, K., Enslen, H., Corbille, A. G., Filhol, O., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2008). A phosphatase cascade by which rewarding stimuli control nucleosomal response. *Nature* 453, 879–884.
- Svenningsson, P., Nishi, A., Fisone, G., Girault, J. A., Nairn, A. C., and Greengard, P. (2004). DARPP-32: an integrator of neurotransmission. *Annu. Rev. Pharmacol. Toxicol.* 44, 269–296.
- Taylor, S. S., Buechler, J. A., and Yonemoto, W. (1990). cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. *Annu. Rev. Biochem.* 59, 971–1005.
- Taylor, S. S., Kim, C., Cheng, C. Y., Brown, S. H., Wu, J., and Kannan, N. (2008). Signaling through cAMP and cAMP-dependent protein kinase: diverse strategies for drug design. *Biochim. Biophys. Acta* 1784, 16–26.
- Tepper, J. M., and Bolam, J. P. (2004). Functional diversity and specificity of neostriatal interneurons. *Curr. Opin. Neurobiol.* 14, 685–692.
- Terry, L. J., Shows, E. B., and Wente, S. R. (2007). Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. *Science* 318, 1412–1416.
- Thomas, G. M., and Haganir, R. L. (2004). MAPK cascade signalling and synaptic plasticity. *Nat. Rev. Neurosci.* 5, 173–183.
- Thompson, K. R., Otis, K. O., Chen, D. Y., Zhao, Y., O'Dell, T. J., and Martin, K. C. (2004). Synapse to nucleus signaling during long-term synaptic plasticity: a role for the classical active nuclear import pathway. *Neuron* 44, 997–1009.
- Toki, S., Kawasaki, H., Tashiro, N., Housman, D. E., and Graybiel, A. M. (2001). Guanine nucleotide exchange factors CalDAG-GEFI and CalDAG-GEFII are colocalized in striatal projection neurons. *J. Comp. Neurol.* 437, 398–407.
- Trifilieff, P., Lavour, J., Pascoli, V., Kappes, V., Brami-Cherrier, K., Pages, C., Mischeau, J., Caboche, J., and Vanhoutte, P. (2009). Endocytosis controls glutamate-induced nuclear accumulation of ERK. *Mol. Cell. Neurosci.* 41, 325–336.
- Tsankova, N., Renthal, W., Kumar, A., and Nestler, E. J. (2007). Epigenetic regulation in psychiatric disorders. *Nat. Rev. Neurosci.* 8, 355–367.
- Valjent, E., Bertran-Gonzalez, J., Herve, D., Fisone, G., and Girault, J. A. (2009). Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci.* 32, 538–547.

- Valjent, E., Corvol, J. C., Pages, C., Besson, M. J., Maldonado, R., and Caboche, J. (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J. Neurosci.* 20, 8701–8709.
- Valjent, E., Pages, C., Herve, D., Girault, J. A., and Caboche, J. (2004). Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. *Eur. J. Neurosci.* 19, 1826–1836.
- Valjent, E., Pages, C., Rogard, M., Besson, M. J., Maldonado, R., and Caboche, J. (2001). Delta 9-tetrahydrocannabinol-induced MAPK/ERK and Elk-1 activation in vivo depends on dopaminergic transmission. *Eur. J. Neurosci.* 14, 342–352.
- Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., Stipanovich, A., Caboche, J., Lombroso, P. J., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 102, 491–496.
- Vanhoutte, P., Barnier, J. V., Guibert, B., Pagès, C., Besson, M. J., Hip-skind, R. A., and Caboche, J. (1999). Glutamate induces phosphorylation of Elk-1 and CREB, along with c-fos activation, via an extracellular signal-regulated kinase-dependent pathway in brain slices. *Mol. Cell. Biol.* 19, 136–146.
- Walaas, S. I., Aswad, D. W., and Greengard, P. (1983). A dopamine- and cyclic AMP-regulated phosphoprotein enriched in dopamine-innervated brain regions. *Nature* 301, 69–71.
- Wang, B., Li, Z., Xu, L., Goggi, J., Yu, Y., and Zhou, J. (2004). Molecular cloning and characterization of rat karyopherin alpha 1 gene: structure and expression. *Gene* 331, 149–157.
- Wang, J. Q., Fibuch, E. E., and Mao, L. (2007). Regulation of mitogen-activated protein kinases by glutamate receptors. *J. Neurochem.* 100, 1–11.
- Wittmann, M., Queisser, G., Eder, A., Wiegert, J. S., Bengtson, C. P., Hellwig, A., Wittum, G., and Bading, H. (2009). Synaptic activity induces dramatic changes in the geometry of the cell nucleus: interplay between nuclear structure, histone H3 phosphorylation, and nuclear calcium signaling. *J. Neurosci.* 29, 14687–14700.
- Wong, W., and Scott, J. D. (2004). AKAP signalling complexes: focal points in space and time. *Nat. Rev. Mol. Cell Biol.* 5, 959–970.
- Xu, D., Farmer, A., and Chook, Y. M. (2010). Recognition of nuclear targeting signals by Karyopherin-beta proteins. *Curr. Opin. Struct. Biol.* 20, 782–790.
- Yoshida, M., and Precht, W. (1971). Monosynaptic inhibition of neurons of the substantia nigra by caudato-nigral fibers. *Brain Res.* 32, 225–228.
- Zhang, L., Lou, D., Jiao, H., Zhang, D., Wang, X., Xia, Y., Zhang, J., and Xu, M. (2004). Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. *J. Neurosci.* 24, 3344–3354.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 May 2011; paper pending published: 10 June 2011; accepted: 13 June 2011; published online: 06 July 2011.
Citation: Matamales M and Girault J-A (2011) Signaling from the cytoplasm to the nucleus in striatal medium-sized spiny neurons. *Front. Neuroanat.* 5:37. doi: 10.3389/fnana.2011.00037
Copyright © 2011 Matamales and Girault. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Beyond the dopamine receptor: regulation and roles of serine/threonine protein phosphatases

Sven Ivar Walaas¹, Hugh Carroll Hemmings Jr.^{2,3}, Paul Greengard⁴ and Angus Clark Nairn^{5*}

¹ Department of Biochemistry, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

² Department of Anesthesiology, Weill Cornell Medical College, New York, NY, USA

³ Department of Pharmacology, Weill Cornell Medical College, New York, NY, USA

⁴ Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY, USA

⁵ Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA

Edited by:

Emmanuel Valjent, Université Montpellier 1 & 2, France

Reviewed by:

Isabelle M. Mansuy, University of Zurich, Switzerland

Philippe Marin, University of Montpellier, France

*Correspondence:

Angus Clark Nairn, Department of Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven, CT 06508, USA.
e-mail: angus.nairn@yale.edu

Dopamine plays an important modulatory role in the central nervous system, helping to control critical aspects of motor function and reward learning. Alteration in normal dopaminergic neurotransmission underlies multiple neurological diseases including schizophrenia, Huntington's disease, and Parkinson's disease. Modulation of dopamine-regulated signaling pathways is also important in the addictive actions of most drugs of abuse. Our studies over the last 30 years have focused on the molecular actions of dopamine acting on medium spiny neurons, the predominant neurons of the neostriatum. Striatum-enriched phosphoproteins, particularly dopamine and adenosine 3':5'-monophosphate-regulated phosphoprotein of 32 kDa (DARPP-32), regulator of calmodulin signaling (RCS), and ARPP-16, mediate pleiotropic actions of dopamine. Notably, each of these proteins, either directly or indirectly, regulates the activity of one of the three major subclasses of serine/threonine protein phosphatases, PP1, PP2B, and PP2A, respectively. For example, phosphorylation of DARPP-32 at Thr34 by protein kinase A results in potent inhibition of PP1, leading to potentiation of dopaminergic signaling at multiple steps from the dopamine receptor to the nucleus. The discovery of DARPP-32 and its emergence as a critical molecular integrator of striatal signaling will be discussed, as will more recent studies that highlight novel roles for RCS and ARPP-16 in dopamine-regulated striatal signaling pathways.

Keywords: phosphorylation, protein phosphatase, protein kinase A, calcineurin, DARPP-32, ARPP-21, ARPP-16, RCS

INTRODUCTION

The neuronal circuitry of the mammalian basal ganglia (also referred to as caudate–putamen or neostriatum) is generally well understood. Major inputs in the form of glutamatergic excitatory monosynaptic inputs derive from the cerebral cortex and thalamus, with axospinous synapses terminating on the dendrites of the local cells, the vast majority of which are represented by the medium-sized spiny neurons (MSN, representing approx. 95% of total striatal neurons), which also represent the origin of the major efferent projections. These GABAergic inhibitory striatal neurons have both dense populations of local collaterals as well as efferent striatonigral and striatopallidal GABAergic fibers terminating on local neurons in either the substantia nigra pars reticulata (the direct efferent pathway) or on local neurons in the external globus pallidus (the indirect pathway). Two major modulatory systems regulate these circuits, the most important being the nigrostriatal dopaminergic (DA) fibers originating in the pars compacta of the substantia nigra, and a less predominant serotonergic pathway originating in the raphe nuclei (Anden et al., 1966; Steinbusch, 1981; Bjorklund and Lindvall, 1984). Local intrastriatal modulations are most prominently mediated through giant cholinergic cells present within the striatum itself, which represent approximately 2% of total cell number but have major axonal connections

that radiate throughout the neostriatal neuropil (Bolam et al., 1984; Zhou et al., 2002).

In addition to the classical neostriatal circuitry and cytology mentioned above, the nucleus accumbens, an adjacent brain region, was determined to represent a ventral part of the neostriatum in the 1970s. The nucleus accumbens has generally identical cellular populations and transmitters, with major glutamate inputs from hippocampus, amygdala, prefrontal cortex and thalamus, major afferent DA and serotonergic modulatory inputs, and efferent GABAergic outputs predominantly terminating in the ventral pallidum and rostral substantia nigra (Walaas and Fonnum, 1979, 1980).

Given the role of the basal ganglia in control of motor function and reward learning, and the disruption of dopaminergic neurotransmission in diseases including schizophrenia, Huntington's disease, and Parkinson's disease, a major effort has been made to identify the molecular mechanisms that mediate and modulate neurotransmission within this brain region. Here we review aspects of DA signaling mechanisms which operate postsynaptically in MSNs, and how they serve to integrate striatal inputs. A major influence on these studies has been the characterization of phosphoproteins regulated by DA and protein kinase A (PKA) in MSNs (Nairn et al., 1985; Greengard et al., 1999;

Svenningsson et al., 2004, 2005). In particular, the discovery of a family of substrates for PKA in MSNs has not only advanced our knowledge of dopamine-regulated signaling in the central nervous system, but has provided a framework for other signaling pathways in the nervous system, and in non-neuronal cells. Here we discuss the discovery of these proteins, and describe their roles as integrators of the multiple signaling pathways that converge on MSNs.

HISTORICAL OVERVIEW OF DA SIGNALING IN STRIATAL MSNs

Approximately 30 years ago, the existence of a DA-containing input to the caudate/putamen was generally accepted based on histofluorescence, neurochemical, and immunohistochemical studies (Bjorklund and Lindvall, 1984; Bjorklund and Dunnett, 2007; Iversen and Iversen, 2007). However, essentially nothing was known about the neurobiological mechanisms that transduced DA inputs into neurophysiological responses. Early studies demonstrated that stimulation of DA cells in the mesencephalon gave monosynaptic EPSPs in the caudate nucleus (Kitai et al., 1976), but the receptor mechanisms involved were essentially unknown. It was unlikely that DA represented a primary ionotropic neurotransmitter that directly regulated ion channel conductances. Rather, studies performed in the superior cervical ganglion (Kebabian and Greengard, 1971; Kebabian and Calne, 1979) indicated the presence in nervous tissue of DA receptors capable of generating cAMP, and more specific studies in rodent brain (Kebabian et al., 1972; Clement-Cormier et al., 1974) demonstrated that this DA receptor was indeed enriched in the neostriatum. However, the remaining cAMP cascade [cAMP-dependent protein kinase (PKA), protein substrates for this enzyme, as well as phosphoprotein phosphatases] was essentially unknown.

DISCOVERY OF THE PHOSPHOPROTEIN SUBSTRATES FOR PKA IN MSNs

A preliminary analysis of acid-soluble phosphoprotein substrates for PKA in different regions of rat brains, performed early in 1980, demonstrated that one set of widely distributed phosphoproteins (subsequently designated the synapsins) were present in all brain regions examined, but that a novel protein substrate for PKA of apparent molecular mass of 32 kDa also was present

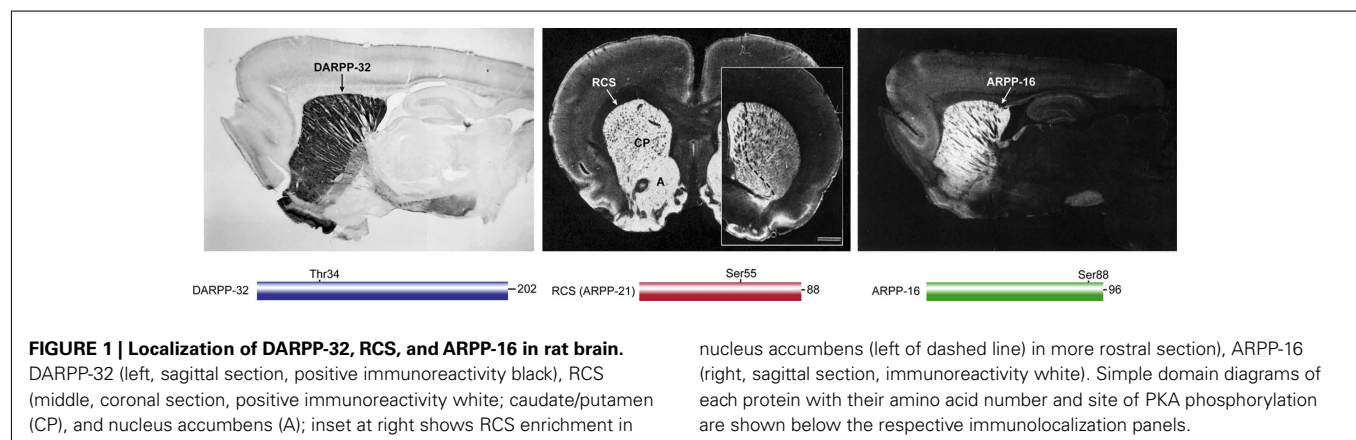
but only in dorsal and ventral striatal regions, and essentially absent from the adjacent lateral septum or the more distant hippocampus or neocortex (Walaas and Greengard, 1984). Analysis of brain slices from the neostriatum containing intact local MSNs demonstrated that incubation with DA induced a significant increase in the state of phosphorylation of this 32 kDa protein, which was given the name “dopamine and adenosine 3':5'-monophosphate-regulated phosphoprotein of 32 kDa” (DARPP-32; Walaas et al., 1983a). Subsequently, characterization of this phosphoprotein was pursued in a first set of neurochemical, protein purification, and immunohistochemical studies (Hemmings Jr. et al., 1984b; Ouimet et al., 1984; Walaas and Greengard, 1984).

The success of these early studies led to a more comprehensive analysis of the anatomy of brain protein phosphorylation systems, where it became clear that the major population of region-specific PKA substrate proteins were highly enriched in the basal ganglia, particularly in the neostriatum (Walaas et al., 1983b,c). Major striatal phosphoproteins present in addition to DARPP-32 had apparent molecular masses (as determined by SDS-polyacrylamide gel electrophoresis, SDS-PAGE) of 21 kDa (designated ARPP-21 or regulator of calmodulin signaling, RCS, see below), 39 kDa (Walaas and Greengard, 1993), 90 kDa (Walaas et al., 1989); now known to be Rap1GAP (McAvoy et al., 2009), and ARPP-16 (Horiuchi et al., 1990). Further studies therefore concentrated on striatum and DA, where anatomy, disease state, neuro-, and psychopharmacological properties were reasonably well known, but where the neurobiological and physiological responses to the effects of DA mediated through protein phosphorylation were relatively unknown. Largely based on their physical properties, DARPP-32, RCS, and ARPP-16 were relatively straightforward to purify from bovine striatum, and subsequent studies have revealed that each of these proteins is highly enriched in MSNs (Figure 1) and that in different ways they serve to mediate dopamine actions through regulation of serine/threonine protein phosphatases.

DARPP-32

BIOCHEMICAL PROPERTIES

Following its identification in rat striatum as an acid-soluble phosphoprotein (Walaas et al., 1983a), DARPP-32 was purified from



bovine caudate, based on its stability in acidic extraction conditions (Hemmings Jr. et al., 1984b). The bovine protein contains 202 amino acids with a predicted molecular weight of 22,614 (Williams et al., 1986; Kurihara et al., 1988; **Figure 2**). Physicochemical analysis indicated that the protein has little secondary structure and exists as an elongated monomer (Hemmings Jr. et al., 1984b). The N-terminal 40 amino acids of DARPP-32 are highly related to a corresponding region of protein phosphatase inhibitor-1, a protein originally described in skeletal muscle (Huang and Glinzmann, 1976). DARPP-32 and inhibitor-1 are phosphorylated by PKA at a conserved threonine residue (Thr34 in DARPP-32, Thr35 in inhibitor-1; note that amino acid numbering is for the mouse protein, unless otherwise indicated), with phosphorylation converting each protein into a potent inhibitor of PP1 (see further discussion below). A notable feature of the amino acid sequence of DARPP-32 is the presence of a highly acidic central region containing 24 glutamic or aspartic acid residues in a total of 32 residues. The precise function of this region is not known, although it contains a phosphorylation site for the CK2 protein kinase (Ser97), and a nuclear export sequence, that are important in control of the nucleo-cytoplasmic localization of DARPP-32 (see below). Comparison of the DARPP-32 amino acid sequence from different species indicates some heterogeneity at the C-terminus of the protein, a region that may be subject to variable phosphorylation (see below).

Fluorescence (Neyroz et al., 1993) and NMR (Lin et al., 2004; Dancheck et al., 2008; Marsh et al., 2010) spectroscopy have shown that, consistent with initial physicochemical studies, DARPP-32 has little secondary structure when studied in free solution. While the protein has all the features of a so-called “intrinsically disordered protein,” there is a propensity for some α -helical content between residues 22 and 29 located N-terminal to the PKA phosphorylation site, and between residues 92 and 109, the region containing Ser97 and the nuclear export signal sequence (Dancheck et al., 2008; Marsh et al., 2010). Moreover, either fluorescence anisotropy or NMR studies of DARPP-32 peptides that contained paramagnetic spin labels in various positions (Dancheck et al., 2008), indicated that the central region of the

protein (residues ~80–115) can exist as a compact core, while both the N-terminal domain including Thr34, and the C-terminus, are much more mobile. Phosphorylation by either PKA or CK2 has no marked effect on the secondary or tertiary structure of the protein.

Intrinsically disordered proteins have the ability to make extensive interactions with their interacting partners. Such disordered proteins allow multiple structural states that facilitate interactions with different binding sites at once, an important property of network hub proteins that interact with multiple binding sites and integrate signals from various pathways (Mittag et al., 2010). However, upon binding to targets like PP1, elements of DARPP-32 may assume a more stable structure as has been found for inhibitor-2 and spinophilin, two other regulators of PP1 (Ragusa et al., 2010; Dancheck et al., 2011). As will be discussed below, the major target for DARPP-32 is PP1. DARPP-32 also interacts with the multiple kinases and phosphatases that regulate its phosphorylation, including PKA which it can also regulate. DARPP-32 is imported into and exported from the nucleus (see below), and a recent study has identified DARPP-32 as being able to interact with the tra2-beta1 splicing factor (Benderska et al., 2010). Therefore, DARPP-32 may have other direct protein binding partners. The intrinsically disordered feature of DARPP-32 may be critical for these various types of interaction.

EXPRESSION PROFILE

A number of detailed immunocytochemical studies have indicated that DARPP-32 is localized primarily in brain regions enriched in dopaminergic nerve terminals (Ouimet et al., 1984, 1998; Foster et al., 1987, 1988; Ouimet and Greengard, 1990). Thus, DARPP-32 is highly expressed within the caudatoputamen, nucleus accumbens, olfactory tubercle, bed nucleus of the stria terminalis, and portions of the amygdaloid complex. DARPP-32 is a cytosolic protein and immunoreactivity is present throughout neuronal cell bodies and dendrites (Ouimet et al., 1984, 1998; Ouimet and Greengard, 1990; Glausier et al., 2010). In brain regions known to receive projections from these nuclei, nerve terminals are strongly immunoreactive for DARPP-32. These target areas include the

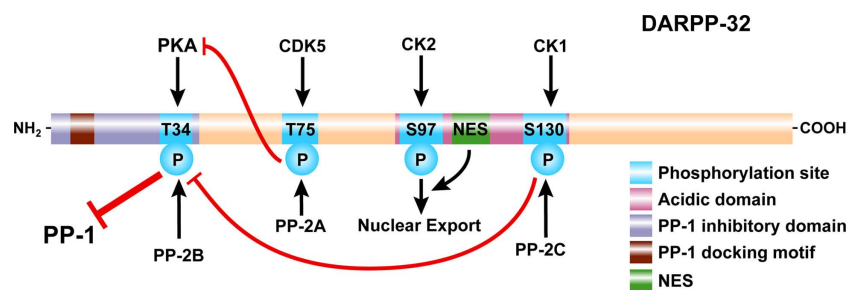


FIGURE 2 | Domain organization of DARPP-32. DARPP-32 is phosphorylated at Thr34 by PKA (and PKG, not shown), at Thr75 by Cdk5, at Ser97 by CK2, and at Ser130 by CK1. Thr34 is preferentially dephosphorylated by PP2B (calcineurin); Thr75 is preferentially dephosphorylated by PP2A; Ser97 is also preferentially dephosphorylated by PP2A (not shown); Ser130 is dephosphorylated by PP2C. Phosphorylation of Thr34 converts DARPP-32 into a potent inhibitor of PP1. A PP1 docking motif and phosphorylation of Thr34 are required for binding and inhibition of PP1. Phosphorylation of Thr75

converts DARPP-32 into an inhibitor of PKA, reducing its ability to phosphorylate DARPP-32 and other substrates. Phosphorylation of Ser130 increases phosphorylation of Thr34 through inhibition of PP2B and potentiates dopaminergic signaling via the cAMP/PKA/DARPP-32/PP-1 pathway. In contrast, phosphorylation of Thr75 acts to inhibit dopaminergic signaling via this pathway. Phosphorylation of Ser97 in conjunction with a nuclear export signal (NES) act to export DARPP-32 from the nucleus and maintain the cytoplasmic localization of the protein observed under basal conditions.

globus pallidus, ventral pallidum, entopeduncular nucleus, and the pars reticulata of the substantia nigra (Walaas and Ouimet, 1989). No immunoreactivity is detected in neuronal cell bodies or dendrites of dopaminergic neurons. In a phylogenetic survey, DARPP-32 was identified in dopaminergic brain regions from representative members of the amniote vertebrate classes (birds and reptiles), while none was identified in dopaminergic brain regions from representative members of the anamniote vertebrate classes (bony fishes and amphibians) or in nervous tissue from representative members of several invertebrate classes (Hemmings Jr. and Greengard, 1986).

In non-striatal regions, many neurons are weakly immunoreactive for DARPP-32 and some of these are found in areas that apparently lack a dopaminergic input (Ouimet et al., 1984, 1992; Glausier et al., 2010). For example, weakly labeled neuronal cell bodies and dendrites are found throughout the neocortex, primarily in layer VI, and in the Purkinje neurons of the cerebellum. DARPP-32 immunoreactivity is also present in certain glial cells, especially in the median eminence, arcuate nucleus, and medial habenula.

DARPP-32 EXPRESSION IN NON-NEURONAL CELLS/TISSUES

While most work has focused on the function of DARPP-32 in neurons, especially in the neostriatum, DARPP-32 is also expressed in non-neuronal cells and tissues. DARPP-32 has been identified at low levels in several peripheral tissues, including choroid plexus, parathyroid cells, adrenal chromaffin cells, posterior pituitary gland, pineal gland, and superior cervical sympathetic ganglion (Hemmings Jr. and Greengard, 1986). Several studies have elucidated a function for DARPP-32 in the kidney, where it plays a role in regulation of the Na^+/K^+ -ATPase by dopamine (Meister et al., 1989; Eklof et al., 2001). DARPP-32 is expressed at high levels in the renal medulla, specifically in the thick ascending limb of Henle. DARPP-32 has also been found in brown adipose tissue from pigs (Meister et al., 1988) and cow (Stralfors et al., 1989), where it likely has the same function as inhibitor-1 which is expressed instead of DARPP-32 in adipose tissue from other mammals. In addition to the brain, DARPP-32 is also expressed in the ciliary epithelium of the eye (Stone et al., 1986), neurohypophysis, parathyroid gland (Meister et al., 1991), choroid plexus, and peripheral nervous tissues such as superior cervical ganglion and adrenal gland, most of which are associated with dopamine signaling (Hemmings Jr. and Greengard, 1986).

Unexpectedly, DARPP-32 expression has been found to be associated with a number of different cancers including gastric, adenocarcinoma, esophageal, and breast (El-Rifai et al., 2002; Ebihara et al., 2004; Varis et al., 2004). The precise role of DARPP-32 is unclear with some studies suggesting a causal role in cell proliferation, a protective role, or a role in drug resistance (Hansen et al., 2006, 2009; Hong et al., 2007). An interesting component of these studies has been the discovery of the expression of a truncated form of DARPP-32 (termed t-DARPP-32) which is missing the amino acids encoded by exon 1 (the region that contains Thr34 and inhibits phosphatase PP1 activity), and which contains an alternatively spliced exon followed by amino acids encoded by exons 2–7. t-DARPP-32 is expressed in cancer cells where it may mediate resistance to anti-cancer drugs (Belkhir

et al., 2008a,b; Gu et al., 2009; Hamel et al., 2010; Vangamudi et al., 2010).

DARPP-32 PHOSPHORYLATED AT Thr34 BY PKA REGULATES PP1

Biochemical and subsequent structure–function studies have shown that residues 5–40 of DARPP-32, in conjunction with phosphorylation of Thr34 by PKA, comprise a domain that binds to and inhibits the activity of PP1, a serine/threonine protein phosphatase that plays a major role in dephosphorylation in eukaryotic cells (Hemmings Jr. et al., 1984a, 1990; Huang et al., 1999). There are at least two sub-domains within residues 5–40 that are critical for enabling phospho-Thr34-DARPP-32 to function as a potent “pseudosubstrate-like” inhibitor of PP1 (Figure 2). A short basic/hydrophobic sequence between residues 7 and 11 (KKIQF) in DARPP-32 represents a conserved PP1 docking motif. While the exact sequence of the motif is not conserved, the basic and hydrophobic features are found in as many as 200 proteins that interact with PP1 at a conserved site in a mutually exclusive manner (Bollen et al., 2010). X-ray crystallography studies have shown that the PP1 docking motif interacts with the surface of PP1 at a position on the back of the enzyme (relative to the metal-containing active site positioned at front center; Goldberg et al., 1995; Egloff et al., 1997). Amino acids in PP1 that interact with the basic/hydrophobic docking motif are distinct from PP2A and PP2B (calcineurin), two related serine/threonine protein phosphatases that have very similar overall structure to PP1 (Watanabe et al., 2001).

Phosphorylation of Thr34 by PKA (and also PKG) converts the protein into a potent inhibitor of PP1 with an IC_{50} of ~ 1 nM (Hemmings Jr. et al., 1984a; Huang et al., 1999). Phospho-Thr34-DARPP-32 is specific for PP1, and is unable to inhibit the closely related enzymes PP2A and PP2B, or the structurally distinct PP2C. While dephospho-DARPP-32 exhibits virtually no inhibitory activity (at least $\sim 10^6$ -fold less), the docking motif can interact with PP1 in the absence or presence of phosphorylation of Thr34 indicating the importance of cooperative two-site interaction to the high inhibitory potency (Desdouits et al., 1995a; Huang et al., 1999). DARPP-32 levels in MSNs have been estimated to be ~ 50 μM or higher (Greengard et al., 1999), and while the striatum also expresses high levels of the three PP1 isoforms found in mammalian tissues (da Cruz e Silva et al., 1995), the total concentration is likely significantly less than that of DARPP-32. Phosphorylation of Thr34 is basally low and can be stimulated ~ 3 - to 5-fold in response to activation of PKA (see below). Thus, even small increases in DARPP-32 phosphorylation are expected to result in substantial inhibition of PP1.

While the exact structural details of how phospho-Thr34-DARPP-32 inhibits PP1 activity are not yet known, biochemical and modeling studies suggest that once docked via residues 7–11, phospho-Thr34 is positioned close to or in the active site, but in a conformation that is not easily dephosphorylated (Goldberg et al., 1995; Huang et al., 1997, 1999). It is possible that four arginine residues that precede Thr34, and are important for phosphorylation by PKA, interact electrostatically with residues that contribute to the acidic groove of PP1, one of three grooves (acidic, hydrophobic, and C-terminal) that emanate from the active site (Goldberg et al., 1995). However, site-directed mutagenesis has

failed to demonstrate any significant role of the acidic side-chains within the acidic groove (Goldberg et al., 1995; Huang et al., 1997).

DARPP-32 PHOSPHORYLATED AT Thr75 BY Cdk5 REGULATES PKA

Thr75 of DARPP-32 is phosphorylated by Cdk5, a cyclin-dependent kinase family member, which is basally active in post-mitotic neurons through association with its non-cyclin cofactor, p35 (Bibb et al., 1999). As a result, Thr75 is basally phosphorylated to a relatively high level in striatal neurons (~ 0.25 mol/mol; Bibb et al., 1999). Phosphorylation of Thr75 by Cdk5 has a major inhibitory effect on the phosphorylation of Thr34 by PKA. Phosphorylation of Thr75 inhibits the phosphorylation of exogenous substrates such as ARPP-16 and RCS, *in vitro*, supporting the hypothesis that phospho-Thr75-DARPP-32 acts generally as a PKA inhibitor. It is also possible that some of the effects of phospho-Thr75-DARPP-32 on PKA-mediated Thr34 phosphorylation result from an intramolecular effect that renders Thr34 a poorer substrate for PKA (Figure 2). As discussed in more detail below, phosphorylation of Thr75 acts in a negative feedback manner to limit the phosphorylation of Thr34 by PKA. However, this negative feedback can be relieved indirectly by PKA-regulated dephosphorylation of Thr75 through a process that involves activation of PP2A by PKA (Nishi et al., 2000). Thus in many instances in intact cell preparations, there is a reciprocal relationship between the states of phosphorylation of Thr34 and Thr75.

PHOSPHORYLATION OF DARPP-32 BY CK1 AND CK2

Biochemical studies *in vitro* have shown that Ser97 of DARPP-32 is phosphorylated by CK2, while Ser130 is phosphorylated by CK1, and that both of these sites are phosphorylated to high stoichiometry *in vivo* (Girault et al., 1989; Desdouits et al., 1995b,c). Earlier studies *in vitro* indicated that phosphorylation of DARPP-32 at Ser97 increased the efficiency of phosphorylation of Thr34 by PKA (Girault et al., 1989). However, it is not clear if this is a major mechanism *in vivo*, since more recent studies have shown convincingly that phosphorylation of Ser97 by CK2 is required for nuclear export of DARPP-32, a process that ensures that under basal conditions, DARPP-32 is localized to the cytoplasm (Stipanovich et al., 2008; Figure 2; see further discussion below).

Studies both *in vitro* and *in vivo* have shown that phosphorylation of Ser130 by CK1 decreases the efficiency of dephosphorylation of Thr34 by calcineurin (PP2B), an effective phosphatase in dephosphorylating Thr34 (Hemmings Jr. et al., 1984a; King et al., 1984). Thus phosphorylation of Ser130 helps to maintain Thr34 phosphorylation levels, and thereby to potentiate dopamine/D1/PKA signaling (Figure 2; see below for further discussion).

In addition to Thr34, Thr75, Ser97, and Ser130, a mass spectrometric analysis has identified phosphorylation of either Ser45 or Ser46 and Ser192 in mouse striatum *in vivo* (Jin et al., 2005). Ser45/46 are probably phosphorylated by CK2 (Girault et al., 1989), although the function is not known. Ser192 is close to the C-terminus of mouse DARPP-32 and the serine and surrounding residues are not conserved in other species. The relevant kinase or the function of this phosphorylation site is also unknown.

DEPHOSPHORYLATION OF DARPP-32

Characterization of the phosphatases involved in the dephosphorylation of the various sites in DARPP-32 have also been important in revealing mechanisms that control DARPP-32 function (Svenningsson et al., 2004). Thr34 is efficiently dephosphorylated by the Ca^{2+} -dependent phosphatase calcineurin (PP2B), which enables glutamate to negatively regulate the inhibition of PP1, and antagonize the effects of dopamine (Nishi et al., 1997, 2005). Different heterotrimeric forms of PP2A play important roles in regulation of DARPP-32 dephosphorylation (Nishi et al., 2000, 2002). PP2A containing the B56 δ subunit can be activated by phosphorylation by PKA (Ahn et al., 2007a) leading to dephosphorylation of both Thr75 and Ser97. Dephosphorylation of Thr75 relieves Cdk5-mediated inhibition of PKA and acts as an important positive feedback to stimulate Thr34 phosphorylation and inhibition of PP1 (Nishi et al., 2000). Dephosphorylation of Ser97 by PP2A plays an important role in controlling the nuclear export of DARPP-32 (Stipanovich et al., 2008). Ca^{2+} -dependent activation of the PR72-containing heterotrimer of PP2A also plays an important role in controlling dephosphorylation of Thr75 (Ahn et al., 2007b), and possibly also Thr34 (Nishi et al., 1999, 2002) and Ser97. Finally, Ser130 dephosphorylation appears to be exclusively controlled by PP2C, where it acts as part of a phosphatase cascade whereby the level of phosphorylation of Ser130 regulates the ability of calcineurin to dephosphorylate Thr34, and hence control the level of inhibition of PP1 (Desdouits et al., 1995b,c).

REGULATION OF DARPP-32 PHOSPHORYLATION

A major effort has been made to investigate the regulation of the various phosphorylation sites in DARPP-32 in order to reveal down-stream signaling pathways of DA. An important component of these studies was the availability of phospho-specific antibodies to the four main phosphorylation sites in DARPP-32: Thr34, Thr75, Ser97, and Ser137. While initial focus was on the phosphorylation of Thr34, the site that directly influences PP1 activity, a large amount of information is now known about the phosphorylation of the other sites, especially Thr75 and Ser97. Extensive studies have shown the regulation of DARPP-32 phosphorylation by neurotransmitters including dopamine, serotonin, glutamate, and GABA, as well as antipsychotic drugs and drugs of abuse (reviewed in detail by Svenningsson et al., 2004; Svenningsson et al., 2005, and in an accompanying article, Nishi et al.). Thus a key feature of DARPP-32 is to integrate the signals from diverse neurotransmitter inputs, ultimately to control the activity of PP1 and thereby its diverse down-stream targets (see below).

An important aspect of DARPP-32 regulation is the fact that the protein is expressed in all MSNs at the same level, yet it has become increasingly clear that direct and indirect pathway MSNs are functionally distinct in terms of the expression patterns of large numbers of proteins, including different DA receptor subtypes, the latter controlling opposing types of intracellular signals (Heiman et al., 2008; Valjent et al., 2009; Surmeier et al., 2010). Here we will review some recent studies of functional distinctions in DARPP-32 phosphorylation in the two principal sub-populations of striatal MSNs.

Cell-type specific analysis of signal transduction in MSNs has been difficult to address due to the anatomical and morphological similarities of these cells. Traditional biochemical studies have the limitation that they examine a mixed population of cells and results represent only an average of signaling events. To overcome these limitations and to study DARPP-32 phosphorylation selectively in striatonigral (D1-MSNs) and striatopallidal (D2-MSNs) neurons, BAC transgenic mice were generated that express both C-terminal Flag-tagged DARPP-32 under the control of the D1R promoter and C-terminal Myc-tagged DARPP-32 under the control of the D2R promoter (Bateup et al., 2008). Immunofluorescence studies confirmed the non-overlapping distribution of the two forms of tagged DARPP-32 in the D1 and D2-MSNs. Using the Flag and Myc tags, DARPP-32 from either D1R or D2R-expressing neurons was then selectively immunoprecipitated and phosphorylation in whole animals and brain slices analyzed in a cell-type specific manner.

Cocaine blocks the dopamine transporter thereby increasing the availability of dopamine at the synapse. Using the tagged DARPP-32 mice, acute cocaine treatment was found to increase Thr34-DARPP-32 phosphorylation and decrease Thr75-DARPP-32 phosphorylation in D1-MSNs while Thr34-DARPP-32 phosphorylation was decreased and Thr75-DARPP-32 phosphorylation was increased in D2-MSNs. Adenosine A_{2A} receptors are highly expressed in the striatum and selectively localized to striatopallidal D2-MSNs. Acute treatment with the A_{2A}R antagonist caffeine increased Thr75-DARPP-32 phosphorylation in D2-MSNs with little effect on Thr34-DARPP-32 phosphorylation. Together, these results uncover important biochemical differences between D1-MSNs and D2-MSNs and demonstrate the significant advantages of using a cell-type targeted approach. It is otherwise difficult to identify opposing changes in phosphorylation in D1- and D2-MSNs which would cancel each other out in whole tissue homogenates.

DOWN-STREAM TARGETS FOR DARPP-32/PP1

As a result of the pleiotropic actions of PP1 on many cellular substrates, DARPP-32 is able to control diverse down-stream targets that range from ion channels and ligand-gated neurotransmitter receptors, to intracellular signaling cascades that control gene transcription. These in turn are coupled to long-term alterations in synaptic plasticity, and ultimately to control of behavior (see detailed reviews in Svenningsson et al., 2004, 2005). Initial studies of DARPP-32 function relied on biochemical approaches, but the availability of DARPP-32 knockout mice (Fienberg et al., 1998), and more recently “knockin” mice in which single phosphorylation sites were mutated to alanine (Svenningsson et al., 2003; Valjent et al., 2005; Zachariou et al., 2006; Zhang et al., 2006; Borgkvist et al., 2007; Stipanovich et al., 2008; Bertran-Gonzalez et al., 2009), has directly established a critical role for DARPP-32 in neuronal function. DARPP-32 is required for normal responses to dopamine, as well as for the actions of drugs that influence dopamine function, including antipsychotic drugs and many drugs of abuse. DARPP-32 is also required for the actions of other neurotransmitters such as serotonin (Svenningsson et al., 2002a,b, 2003) or adenosine (Lindskog et al., 2002).

As an example of these studies, an important role for DARPP-32 has been found in the regulation of activity of the extracellular signal-regulated kinase (ERK), an enzyme that is critical for long-term synaptic plasticity in MSNs (Valjent et al., 2005). Many drugs of abuse exert their addictive effects by increasing extracellular DA in the nucleus accumbens, where they likely alter the plasticity of corticostriatal glutamatergic transmission. In particular, psychostimulant drugs and other drugs of abuse activate ERK in D1-MSNs in dorsal and ventral striatum, through the combined action of glutamate NMDA and D1-dopamine receptors. Notably, activation of ERK by various drugs, including D-amphetamine, cocaine, nicotine, morphine, or Δ^9 -tetrahydrocannabinol, was attenuated in DARPP-32 null mice. Moreover, the effects of D-amphetamine or cocaine on ERK activation in the striatum were prevented in knockin mice in which Thr34 was mutated to alanine. Regulation by DARPP-32 was found to occur at multiple levels, both upstream of ERK and at the level of striatal-enriched tyrosine phosphatase (STEP). Altered behavioral responses to psychostimulants were also prevented in the DARPP-32 mutant mice. Thus, activation of ERK, via a multi-level control of protein phosphatases, functions to integrate the coincident action of dopamine and glutamate converging on MSNs, and is critical for long-lasting effects of drugs of abuse.

While most studies have focused on the biochemical actions of DARPP-32 and behavioral consequences in the dorsal and ventral striatum, DARPP-32 also plays a role in other brain regions including cortex, hippocampus, and hypothalamus. For example, in hippocampus and cortex, DARPP-32 phosphorylation is regulated by serotonin, and biochemical and behavioral studies with DARPP-32 knockout mice implicate DARPP-32 in the actions of serotonin and anti-depressant drugs (Svenningsson et al., 2002a,b). In hypothalamus, Thr34 of DARPP-32 is phosphorylated in response to progesterone, and the actions of progesterone on sexual receptivity were attenuated in DARPP-32 knockout mice (Mani et al., 2000). Recent genetic studies also implicate DARPP-32 in prefrontal cortical processes linked to general intelligence, learning, and cognition (Frank et al., 2007, 2009; Kolata et al., 2010; Doll et al., 2011; Frank and Fossella, 2011). Other studies have shown a role for DARPP-32 in non-neuronal cells and tissues (see above).

As discussed above, DARPP-32 is found in all MSNs, where its phosphorylation is differentially controlled by the selective expression of different neurotransmitter receptors, including D1 and D2 dopamine receptors (Svenningsson et al., 2004). To address the specific role of DARPP-32 in different sub-populations of MSNs, studies have been carried out recently to selectively knockout the protein in either striatonigral (D1-MSNs) or striatopallidal (D2-MSNs) medium spiny neurons (Bateup et al., 2010). Loss of DARPP-32 in D1-MSNs decreased both basal and cocaine-induced locomotion and also abolished dyskinetic behaviors in response to L-DOPA, a drug used to treat Parkinson's disease. Loss of DARPP-32 in D2-MSNs increased locomotor activity and strongly reduced the cataleptic response to the antipsychotic drug haloperidol. Interestingly, LTP was disrupted in both D1- and D2-MSNs. These results highlight the selective contributions of DARPP-32 in different MSN populations, and reinforce the need for highly specific control of gene expression when carrying out

functional analysis of proteins in the striatum or other parts of the central nervous system.

REGULATION OF NUCLEAR TRAFFICKING OF DARPP-32

As discussed above, signaling pathways important for the transcriptional effects of dopamine involve PKA and ERK, and clearly also involve DARPP-32. However, the precise mechanisms of the transfer of information from the cytoplasm to the nucleus of striatal neurons are still poorly characterized. Recent studies have found that phosphorylation/dephosphorylation of Ser97 of DARPP-32 is crucial for control of the nucleo-cytoplasmic distribution of DARPP-32 (Stipanovich et al., 2008; see also accompanying article by Girault et al., for further details). As mentioned above, previous studies of DARPP-32 had suggested it to be largely a cytoplasmic protein, regulating the phosphorylation state of cytoplasmic or plasma membrane-associated targets. However, some earlier studies hinted that the protein might also be present in the nucleus (Ouimet and Greengard, 1990). Immunohistochemical studies led to the observation that Thr34-phospho-DARPP-32 immunoreactivity was strong in the nuclei of mice treated acutely with cocaine, D-amphetamine, or morphine, and this was confirmed with an antibody that recognized total DARPP-32. Subsequent biochemical studies of DARPP-32 in culture and using mice bearing a Ser97Ala point mutation identified a nuclear export motif adjacent to Ser97 that was controlled by phosphorylation of Ser97 (Figure 2). Ser97 of DARPP-32 is phosphorylated to high levels under typical basal conditions *in vivo*. Nuclear accumulation was found to be mediated through a signaling cascade involving dopamine D1 receptors and cAMP-dependent activation of a specific heterotrimeric form of protein phosphatase PP2A that acts to dephosphorylate DARPP-32 at Ser97, leading to inhibition of its nuclear export. Other studies have clarified the mechanism of activation of PP2A by PKA, which involves phosphorylation of the B56 δ subunit of the PP2A heterotrimer (Ahn et al., 2007a).

Further studies by Stipanovich et al. (2008) showed that translocation of the active phospho-Thr34-DARPP-32 to the nucleus was accompanied by inhibition of nuclear PP1 and marked stimulation of the phosphorylation level of Ser10 of histone H3, a key component of the nucleosomal response. Notably, drugs of abuse, as well as food reinforcement learning, promote nuclear accumulation of DARPP-32, but this did not occur in mice expressing Ser97Ala-DARPP-32, which remained preferentially nuclear. Moreover, behavioral studies in mice indicated that mutation of Ser97 profoundly altered a number of responses to drugs of abuse, emphasizing the functional importance of this signaling cascade.

MODELING OF DARPP-32/PP1 SIGNAL TRANSDUCTION

The extensive studies of DARPP-32-dependent signaling have provided significant insight into modes of signal transduction machinery used by neurons to provide a coordinated set of appropriate physiological responses to multiple diverse stimuli including neurotransmitters or drugs of abuse. In a broader context, a large proportion of studies in the biological sciences are now devoted to elucidating such signaling pathways, which are far more complex than had been anticipated and for which it is often impossible to intuitively gauge the relative importance of the different

components involved. As a result, alternative approaches including mathematical modeling are necessary to understand the functions of these complex signaling pathways. In this regard, several recent studies have been carried out that use the DARPP-32/PP1 signaling network as a model (Fernandez et al., 2006; Barbano et al., 2007; Lindskog, 2008; Nakano et al., 2010). While the models employ different components and use different approaches, a number of common features emerge. As more details emerge of the DARPP-32 network, these approaches are likely to become more important, and be predictive of the *in vivo* state of striatal neuron signaling.

In one study, a mathematical tool was developed that can elucidate biological network properties by analyzing global features of the network dynamics without relying on detailed information about the concentrations of signaling components or rate constants for the reaction pathways that link these components (Barbano et al., 2007). The conclusions from this study were that the network topology appears to serve to stabilize the net state of DARPP-32 phosphorylation in response to variation of the input levels of the neurotransmitters dopamine and glutamate, despite significant perturbation to the concentrations and levels of activity of the intermediate chemical species. A notable component of the stability of the DARPP-32 network was the positive- and negative-feedback modulatory phosphorylation pathways of DARPP-32 involving CK1, CK2, and Cdk5.

Two other studies modeled overlapping components of the DARPP-32 network, but attempted to estimate component concentrations and rate constants based on experimental data (Fernandez et al., 2006; Lindskog, 2008). In particular the study by Fernandez and colleagues presented a comprehensive number of parameters, and incorporated three of the four phosphorylation sites in DARPP-32 (Thr34, Thr75, and Ser130), whereas the Lindskog model was more limited in the number of components and incorporated only Thr34 and Thr75. Notably, the Fernandez model confirmed an important role of Cdk5 and Thr75 phosphorylation, but suggested less of a role for PKA-dependent activation of PP2A. The model also analyzed the pattern of inputs from calcium and cAMP, and investigated the effects of mutation of the key phosphorylation sites *in silico*. The Lindskog model also emphasized the important role of phosphorylation of Thr75 by Cdk5, its role in inhibiting PKA and the dephosphorylation of Thr75 by PP2A following activation of PKA.

In a more recent study, elements of the DARPP-32 network have been incorporated into a model of synaptic plasticity in a dendritic spine from a MSN (Nakano et al., 2010). The model includes inputs from NMDA, AMPA and mGluR glutamate receptors, dopamine D1 receptors, and voltage-gated Ca²⁺ channels, with the output being AMPA receptor trafficking to the post-synaptic membrane. This model included DARPP-32 phosphorylated at Thr34, Thr75, and Ser130, and also relied largely on experimentally derived parameters. In contrast to the Fernandez et al. (2006) study, the study by Nakano and colleagues demonstrated a robust bi-stable behavior for the PKA/PP2A/Thr75 element of the model, and suggested that this could play an important role in the switch between long-term potentiation and long-term depression in striatal neurons. Together these models provide viable hypotheses for experimental testing.

DARPP-32 AND SCHIZOPHRENIA/DISEASE

The critical role of DARPP-32 in integrating dopaminergic, serotonergic, and glutamatergic signaling in dopaminergic neurons has suggested the possible involvement of DARPP-32 in the pathophysiology of neuropsychiatric diseases. A potential role for DARPP-32 signaling in psychogenesis was demonstrated using a mouse model of schizophrenia and mutations affecting DARPP-32 signaling. Phosphorylation of DARPP-32 by and behavioral responses to diverse psychotomimetics were strongly attenuated in mice with genetic deletion of DARPP-32 or with specific phospho-null mutations in the phosphorylation sites Thr34, Thr75, and Ser130 (Svenningsson et al., 2003). This study showed that phosphorylation of DARPP-32 at multiple sites involved in its regulation of PP1 and PKA signaling is increased by psychotomimetic drugs that target dopaminergic (amphetamine), serotonergic (LSD), and glutamatergic (ketamine) signaling, and that phosphorylation of DARPP-32 is crucial to their behavioral actions.

In human studies, biochemical and neuropathological evidence has implicated altered DARPP-32 function in both the positive (psychotic) and negative (cognitive dysfunction) symptoms of schizophrenia. DARPP-32 protein levels were reduced in post-mortem samples of dorsolateral prefrontal cortex obtained from 14 schizophrenic subjects compared to matched controls (Albert et al., 2002). This brain region that has been implicated in the prefrontal cognitive-affective dysfunction observed in schizophrenia, and signaling in this area is subject to dopaminergic and serotonergic modulation. Control experiments showed that this effect was specific for DARPP-32 compared to two synaptic phosphoproteins and was not secondary to neuroleptic drug use. An independent study reported reduction in DARPP-32 protein expression detected by immunoblotting and immunocytochemistry in post-mortem dorsolateral prefrontal cortex samples from schizophrenic and bipolar patients compared to controls (Ishikawa et al., 2007). Reduced DARPP-32-immunoreactive cell density has been found in postmortem samples of the superior temporal gyrus from schizophrenic patients, a brain region associated with structural and functional abnormalities in schizophrenia (Kunii et al., 2011). In a small flow cytometric analysis of DARPP-32 expression in peripheral immune cells, reduced levels of DARPP-32 were detected in CD4+ T lymphocytes and CD56+ NK cells isolated from schizophrenic patients compared to controls (Torres et al., 2009). In contrast, analysis by real time PCR of the expression of 17 genes associated with dopamine signaling in dorsolateral prefrontal cortex samples found increased DARPP-32 expression in patients with schizophrenia or bipolar disorder compared to matched controls (Zhan et al., 2011).

Analysis of DARPP-32 mRNA expression by *in situ* hybridization found no change in postmortem dorsolateral prefrontal or anterior cingulate cortex (Baracska et al., 2006) or thalamus (Clinton et al., 2005) samples from schizophrenic patients compared to controls. This suggests that changes in protein expression are translational or post-translation rather than transcriptional. However a small underpowered study reported preliminary evidence for reduced DARPP-32 mRNA expression and differences in single nucleotide polymorphisms (SNPs) in postmortem prefrontal cortex samples between schizophrenic patients who died by

suicide compared to those who died from other causes (Feldcamp et al., 2008). Studies in rodents have confirmed that neuroleptic medications do not affect DARPP-32 protein or mRNA levels (Greibb et al., 1990; Baracska et al., 2006; Souza et al., 2008).

A systematic search for mutations in the DARPP-32 gene (*PP1R1B*) in Chinese schizophrenic patients identified a number of coding and non-coding mutations. However follow-up SNP and haplotype studies of additional schizophrenic patients and controls showed no association of any of the variants with the disease (Li et al., 2006). An analysis of five different SNPs in the *PP1R1B* gene of 520 Chinese schizophrenic patients and 320 controls (Hu et al., 2007), as well as a similar analysis of four SNPs in 384 Japanese schizophrenic subjects and 384 controls (Yoshimi et al., 2008), found no association of any of the variants with the disease. However a traditional genetic approach involving sequencing of the *PP1R1B* gene in postmortem human brain from white and African American subjects identified common non-coding single nucleotide variants, mostly within a seven SNP haplotype, that were associated with differences in mRNA expression, and neostriatal volume, activation and functional connectivity with the prefrontal cortex (Meyer-Lindenberg et al., 2007). Moreover, the haplotype was associated with a risk for schizophrenia in a preliminary family based association study. Interestingly, the *PP1R1B* gene is located at 17q12, near a region implicated in the risk for development of schizophrenia (Lewis et al., 2003).

In summary, although there are some inconsistent findings, studies have reported reductions in DARPP-32 protein expression in human brain regions associated with schizophrenic pathology. This change is not correlated with reduced mRNA expression or specific genetic mutations, and is not associated with neuroleptic treatment. It is most likely due to effects of the disease on protein translation or post-translational modification of DARPP-32, or to selective neuron loss. Further studies will be required to determine the mechanisms involved, the functional implications, and the potential for therapeutic intervention.

ARPP-21 (RCS)

BIOCHEMICAL PROPERTIES

Regulator of calmodulin signaling (RCS) is a small, heat-stable, acidic protein of 88 residues, with a calculated molecular mass of 9561 for the bovine protein (Hemmings Jr. and Greengard, 1989; Williams et al., 1989). RCS is phosphorylated by PKA at a single serine residue (Ser55, **Figure 1**; Hemmings Jr. et al., 1989). To date no other phosphorylation sites have been identified. Studies using striatal slices have found that Ser55 of RCS is phosphorylated in response to activation of PKA by D1 receptor agonists, while activation of D2 receptors leads to decreased phosphorylation (Tsou et al., 1993; Caporaso et al., 2000). Moreover, exposure of mice to the psychostimulants methamphetamine or cocaine also increased Ser55 phosphorylation (Caporaso et al., 2000). *In vitro*, phospho-RCS is effectively dephosphorylated by PP1 and PP2A, but not by PP2B or PP2C (Hemmings Jr. and Greengard, 1989), but studies using DARPP-32 knockout mice suggest little involvement of PP1 *in vivo* (Caporaso et al., 2000).

The protein is predicted to have little secondary or tertiary structure, and like DARPP-32 and ARPP-16, is likely to be an intrinsically disordered protein. The amino acid sequence gives

no insight into its potential function, and RCS has no similarity to any other protein except for a thymocyte-specific variant, termed thymus-specific cyclic AMP-regulated phosphoprotein (TARPP), that is alternatively spliced from the RCS gene (Kisielow et al., 2001). TARPP protein is highly expressed in immature thymocytes, where it may have a role in thymocyte development. However, TARPP is expressed at very low levels in brain.

EXPRESSION PROFILE

Extensive immunocytochemical analysis has shown that within the basal ganglia, DARPP-32, RCS, and ARPP-16 are all highly expressed in the MSNs that are the targets for mesolimbic and nigrostriatal dopamine systems (Ouimet et al., 1984, 1989; Girault et al., 1990; **Figure 1**). While DARPP-32, RCS, and ARPP-16 are all enriched in these brain regions, the precise localizations of these proteins are not identical. Studies in rat have revealed that RCS immunostaining is more intense in the nucleus accumbens than in the caudatoputamen (Ouimet et al., 1989). RCS, like DARPP-32 and ARPP-16, is a cytoplasmic protein that does not appear to be enriched in any particular sub-cellular compartment. Within the caudatoputamen, RCS immunoreactivity is strongest in the medial portion of rostral sections of the nucleus accumbens. In contrast to DARPP-32, for which most MSNs in the striatum were immunoreactive, RCS staining was not present in all MSNs. The distribution of DARPP-32 and RCS are also distinct in cortical regions, including the frontal cortex where RCS is found in layers I and II, whereas DARPP-32 is more concentrated in deeper layers. Thus, RCS and DARPP-32 may subservise overlapping but distinct roles in signal transduction processes that are specific to one or another population of neurons present within different regions of the basal ganglia.

REGULATION OF CALMODULIN BY RCS

A yeast two-hybrid system, used to study potential protein-protein interactions, identified the ubiquitous Ca^{2+} -binding protein calmodulin (CaM) as a binding partner for ARPP-21 (Rakhilin et al., 2004). The interaction between ARPP-21 and CaM was confirmed using a variety of biochemical approaches. Notably, phosphorylation of ARPP-21 by PKA resulted in increased affinity of the protein for CaM. Phosphorylated, but not dephosphorylated, ARPP-21 effectively inhibited the activities of the CaM-dependent protein phosphatase, calcineurin, and a CaM kinase (CaMKI). The inhibitory activity of phospho-RCS appears to result from sequestration of CaM, thereby preventing CaM binding to these targets. Although CaM is expressed in very high concentrations in most cell types (possibly $>100 \mu\text{M}$), the level of CaM is significantly lower than that of all of its targets. Thus the amount of free CaM is limited and phosphorylation of RCS would be expected to influence CaM-dependent signaling by regulating its availability. Based on these properties, ARPP-21 was renamed RCS.

As mentioned above, in MSNs, calcineurin plays a critical role in dephosphorylation of Thr34 of DARPP-32. For example, in striatal slices D2-receptor agonists activate calcineurin leading to dephosphorylation of Thr34 of DARPP-32 (Nishi et al., 1999). Consistent with the role of RCS to suppress calcineurin activity, the effect of the D2-receptor agonist, quinpirole, on Thr34 dephosphorylation,

was greater in striatal neurons from RCS knockout mice (Rakhilin et al., 2004).

Previous studies had found that calcineurin is a potent regulator of L-type Ca^{2+} channels in MSNs (Hernandez-Lopez et al., 2000). Mobilization of intracellular Ca^{2+} stores by activation of either D2 dopaminergic or M1 muscarinic receptors leads to activation of calcineurin and suppression of L-type Ca^{2+} channel currents. Following on from this earlier work, studies using neurons from RCS knockout mice showed that the protein plays an important role in modulation of L-type Ca^{2+} current by D2 dopaminergic or by M1 muscarinic receptors through its PKA-mediated suppression of calcineurin (Rakhilin et al., 2004).

ROLE OF RCS IN TRANSCRIPTIONAL CONTROL BY MEF2

The myocyte enhancer factor 2 (MEF2) transcription factor has been found to play an important role in the behavioral responses to repeated cocaine administration, and to be also involved in the effects of this psychostimulant on the dendritic spine morphology of MSNs in nucleus accumbens and dorsal striatum (Flavell et al., 2006; Shalizi et al., 2006). MEF2 activity is stimulated by glutamatergic synaptic activity, which increases Ca^{2+} influx via L-type voltage-sensitive Ca^{2+} channels (LT-VSCCs) and activation of Ca^{2+} /calmodulin (Ca^{2+} /CaM)-dependent signaling pathways. Ca^{2+} /CaM then stimulates calcineurin to dephosphorylate MEF2, including two inhibitory Cdk5 sites, to promote MEF2 activation. Recent studies showed that cocaine suppresses striatal MEF2 activity in part through a mechanism involving cAMP, RCS, and calcineurin (Pulipparacharuvil et al., 2008). Reduced MEF2 activity in the nucleus accumbens *in vivo* was found to be required for the cocaine-induced increases in dendritic spine density. Surprisingly, increased MEF2 activity in the accumbens, which blocked the cocaine-induced increase in dendritic spine density, enhanced sensitized behavioral responses to cocaine. Together, these findings implicate MEF2 as a key regulator of structural synapse plasticity and sensitized responses to cocaine and suggest that reducing MEF2 activity (and increasing spine density) in the nucleus accumbens may be a compensatory mechanism to limit long-lasting maladaptive behavioral responses to cocaine. The fact that RCS plays a role in the regulation of MEF2 dephosphorylation suggests that it likely is involved in the processes whereby psychostimulants can increase spine density in MSNs.

FUNCTIONAL ROLE OF RCS IN ANXIETY AND MOTIVATION

Regulator of calmodulin signaling expression is high in the dorsal striatum, nucleus accumbens, and amygdala, suggesting that the protein is involved in limbic-striatal function. RCS knockout mice have been recently created and examined in terms of behavioral models dependent on these brain areas (Davis et al., in revision). While RCS knockout mice showed normal acquisition of a food-motivated instrumental response, they exhibited a lower breakpoint when tested on responding on a progressive ratio schedule of reinforcement. Moreover, RCS knockout mice displayed decreased exploration in both the open arms of an elevated plus maze and in the center region of an open field, suggesting an enhanced anxiety response. Notably, biochemical studies revealed a reduction in the levels of DARPP-32 and of the

August 2011 | Volume 5 | Article 50 | 89

a slightly different amino acid sequence. With respect to ENSA, a similar situation occurs. The correct spliced gene is found on Chr1 and a likely pseudogene is found on Chr14 (in human).

Comparison of the various related amino acid sequences reveals that ARPP-16 represents the core structure, with the other isoforms containing additional and distinct N- or C-termini. ENSA differs slightly from ARPP-16 within the core structure (16 out of 96 amino acids are changed; Dulubova et al., 2001). In addition, homologs of ARPP-16/19 have been identified in *Drosophila*, *Schistosoma mansoni*, *Caenorhabditis elegans*, and yeast genomes. Two regions are conserved in all species, the first of which contains a motif of eight residues, KYFDSGDY, that includes a novel phosphorylation site (see further discussion below) and the second of which encompasses the site phosphorylated by PKA in mammalian species and presumably in other organisms. PKA phosphorylates ARPP-16 at Ser88 (Ser104 in ARPP-19). In mouse striatal slices, the phosphorylation of both ARPP-16 and ENSA isoforms was found to be increased following stimulation of adenylyl cyclase by forskolin (Dulubova et al., 2001). Treatment of striatal slices with a specific D1-agonist (SKF81297) also increased phosphorylation of ARPP-16 and ENSA. In contrast, treatment with a specific D2-agonist (quinpirole) decreased both the basal level and the D1-stimulated phosphorylation of both ARPP-16 and ENSA.

EXPRESSION PROFILE

Given the criteria for the protein screen that led to its discovery, enrichment of ARPP-16 in the basal ganglia was already known. Subsequent work by immunoblotting of specific brain regions and peripheral organs in addition to immunocytochemistry and *in situ* hybridization studies confirmed that ARPP-16 is found mainly in the striatonigral neurons in the dorsal striatum, as well as the nucleus accumbens, amygdala, and frontal cortex (Girault et al., 1990; Brene et al., 1994). Isoform-specific antibodies have been used to show that both ARPP-19 and ENSA isoforms are present in rat brain cytosol, and comigrate within the 19-kDa region. However, ENSA is much more abundant in rodent striatum compared to ARPP-19. In contrast, ARPP-19 was found to be the major isoform in all cell lines analyzed. Since the core ARPP-16 sequence is common to all the related isoforms, there is not an antibody available that only recognizes ARPP-16. However, as expected from earlier work, ARPP-16 was detected by immunoblotting only in brain with high enrichment in the striatum. The phylogenetic distribution of ARPP-16 has also been described (Girault et al., 1990). Interestingly, by immunoblotting, ARPP-16 was found in the striatum (or paleostriatum in non-mammalian eukaryotes) and frontal cortex of monkey, rat, mouse, cow, rabbit, and canary; whereas, it was not present in pigeon, amphibians, fish, or *Aplysia*. Immunoblotting from mouse tissue samples taken at various points in development revealed that ARPP-16 is present at its highest levels from 3 to 8 postnatal weeks when expression plateaus, whereas ARPP-19/ENSA are found at their highest level at embryonic day 16 and decline with development (Girault et al., 1990).

The molecular mechanism involved in the alternative splicing of ARPP-16 in striatal neurons is not presently known. Hyman and colleagues found that ania-6, a novel cyclin that associates

with RNA polymerase II, is induced by dopamine or cocaine in the striatum (Berke et al., 2001; Nairn and Greengard, 2001). Two splice forms of ania-6 are differentially regulated in striatal neurons in response to glutamate, dopamine, and BDNF (Sgambato et al., 2003). The regulatory pathways included both ERK and CaM kinase(s). Therefore, it is possible that regulation of expression of ARPP-16, as well as other alternatively spliced genes in striatal neurons, may be sensitive to modulation of these different intracellular pathways.

POTENTIAL FUNCTIONS OF ARPP-16

As with DARPP-32 and RCS, the initial analysis of the amino acid sequence of ARPP-16 did not reveal any obvious function. One study showed a reduction in total protein level of ARPP-19 in the temporal lobe from postmortem samples of individuals with Down's syndrome as well as reduced ARPP-19 in the cerebellum of patients with Alzheimer's disease (Kim et al., 2001). Several studies have identified possible functions for both ARPP-19 and ENSA. A role for ARPP-19 in nerve growth factor-dependent stabilization of the mRNA for growth-associated protein-43 (GAP-43) was suggested (Irwin et al., 2002). However, the specific interaction of ARPP-16 or ARPP-19 with GAP-43 mRNA has not been confirmed by follow-up studies (Andrade and Nairn, unpublished results). A role for ARPP-19, as well as ENSA, in interacting with α -synuclein has also been suggested (Woods et al., 2007; Boettcher et al., 2008). While this interaction appears to be possible *in vitro*, its physiological relevance remains to be established.

ENSA was originally named after studies that identified it as a possible endogenous regulator of the K_{ATP} -coupled sulfonylurea receptor, which is targeted by the class of antidiabetic drugs, the sulfonylureas (Virsolvy-Vergine et al., 1992, 1996; Peyrollier et al., 1996). Further studies found that recombinant ENSA was able to compete with [^3H]-glibenclamide (a sulfonylurea) in receptor binding assays as well as to increase insulin release in MIN6 cells, a pancreatic β -cell line (Heron et al., 1998). However, it is unlikely that ENSA, a cytosolic protein, could fulfill such a role *in vivo* (Gros et al., 2002).

REGULATION OF THE SERINE/THREONINE PROTEIN PHOSPHATASE PP2A BY ARPP-16 FAMILY MEMBERS

The homolog of ARPP-19 in *S. cerevisiae*, YNL157W, has an unknown function, although reduced fitness in minimal media has been shown in null strains (Giaever et al., 2002). No significant phenotype was observed with deletion of the ARPP-19 homolog, K10C3.2, in *C. elegans* (Rogers et al., 2008). However, mutant ENSA in *Drosophila* oocytes exhibit marked meiotic maturation (Von Stetina et al., 2008). Moreover, mice null for ARPP-16/19 exhibit embryonic lethality (Horiuchi and Nairn, unpublished), suggestive of a critical function for the protein(s).

Two recent studies of mitosis in *Xenopus* oocytes have unexpectedly revealed a critical functional role for ARPP-19 and ENSA in control of the G2/M phase of the cell cycle (Gharbi-Ayachi et al., 2010; Mochida et al., 2010). Both of these studies were focused on indentifying substrates for a kinase, termed Great-wall (Gwl), that was known to provide a link between Cdk1, the cyclin-dependent controller of mitosis, and the inhibition of protein phosphatase 2A (PP2A; Virshup and Kaldis, 2010). Through

activation by Cdk1, Gwl was thought to regulate PP2A as part of a positive feed-back mechanism designed to increase the phosphorylation of mitotic substrates for Cdk1. Using proteomic screens for Gwl substrates, one study identified ARPP-19 (Gharbi-Ayachi et al., 2010), while the other identified ENSA (Mochida et al., 2010), as the major targets for Gwl in oocytes. Gwl phosphorylates the serine residue in the conserved KYFDSGDY domain (Ser62 in ARPP-19, Ser67 in ENSA, Ser46 in ARPP-16), resulting in the binding of ARPP-19 or ENSA to PP2A, and subsequent inhibition of PP2A activity.

A similar role for ARPP-19 or ENSA in regulation of mitosis in mammalian cells is implicated by preliminary studies in HeLa cells (Gharbi-Ayachi et al., 2010), and by other studies which have revealed a critical role for the mammalian homolog of Gwl, termed microtubule-associated serine/threonine kinase-like (for MASTL) in cell cycle control (Voets and Wolthuis, 2010). Notably, biochemical studies of ARPP-16 have found that it can interact directly with the core AC dimer of PP2A (the A scaffolding and C catalytic subunits; Andrade et al., in preparation). Additional studies have found that Ser46 of ARPP-16 is phosphorylated to very high levels under basal conditions in striatal tissue. It is likely that the MAST3 isoform, which is related to MASTL and Gwl, phosphorylates ARPP-19 at Ser46 since this isoform is highly expressed in striatum (Garland et al., 2008). Moreover, *in vitro*, ARPP-16 phosphorylated by MAST kinase inhibits a number of different PP2A heterotrimeric complexes. Finally, phosphorylation of several PP2A substrates is increased in striatal tissue from conditional knockout mice in which ARPP-16 has been deleted in forebrain, including Thr75 of DARPP-32 (see Figure 3). These studies suggest that while MAST/Gwl/ARPP-16/19/ENSA signaling serves to regulate PP2A in post-mitotic neurons as well as at mitosis, the way this process is used is distinct, with PP2A being basally inhibited in MSNs, while PP2A is transiently inhibited by ARPP-19/ENSA during mitosis. MAST/ARPP-16 signaling in MSNs is also distinct from the directionality of regulation of DARPP-32 and RCS, where stimulation of PKA converts them from inactive proteins, into inhibitors of PP1, or PP2B (via CaM sequestration), respectively. The precise role of phosphorylation of ARPP-16 by PKA in terms of regulation of PP2A remains to be determined.

SUMMARY AND FUTURE DIRECTIONS

The studies of DARPP-32, RCS, and ARPP-16 over the last three decades has provided a wealth of understanding of the intracellular signaling pathways that mediate the effects of dopamine in striatal neurons. Perhaps the most striking feature of the function of these proteins is that they all act in one way or another to control the activity of three of the four major subclasses of serine/threonine protein phosphatases. Presumably, in MSNs this is a reflection of the temporal and possibly spatial requirements for intracellular signaling responding to dopamine and other neurotransmitter inputs that coordinate with dopamine action. As perhaps best exemplified by the ability of DARPP-32 to control the activation of ERK-dependent signaling at multiple levels first in the cytosol and then in the nucleus, the DARPP-32/PP1 pathway seems designed to be able to couple weak or strong inputs to specific cellular responses. In this respect, DARPP-32/PP1 may be an

important component of a logical “AND gate” that serves to couple the actions of drugs abuse to coincident activation of DA and glutamate receptors and subsequently to regulation of adaptive changes in ERK signaling in the nucleus (Girault et al., 2007).

It is of interest that while DARPP-32, RCS, and ARPP-16 all act to control protein phosphatase activity they work in different ways. Both DARPP-32 and RCS are inactive until phosphorylated by PKA. DARPP-32 is a very potent direct pseudosubstrate inhibitor of PP1, while RCS works indirectly to regulate calcineurin. Phospho-ARPP-16 appears to act basally as an inhibitor of PP2A, and PKA likely modulates this action. Common to all three proteins are that they are intrinsically disordered proteins, a property that allows for great flexibility in terms of how they can interact with their targets. At least in the case of DARPP-32 and ARPP-16, they are regulated by multiple protein kinases, and thus act as regulatory hubs to integrate the actions of various neurotransmitters, and to allow for both positive and negative feedback processes to act on the regulation of the phosphatase targets.

While extensive immunocytochemical analysis has shown that all three proteins are highly expressed in MSNs, their precise localizations are not identical. For example, RCS is more highly expressed in nucleus accumbens in more rostral regions. Thus, while these three PKA targets likely function in an integrated manner in MSNs to coordinate dopaminergic signaling, they may also contribute to distinct actions of dopamine in subpopulations MSNs. While detailed studies have only been carried out for DARPP-32, it appears that all three proteins are expressed in both D1- and D2-MSNs. As a consequence, their regulation by cAMP/PKA signaling is likely to be distinct in these two major populations of striatal neurons. In D1-MSNs, increased cAMP would act to increase phosphorylation by PKA, while in D2-MSNs activation of D2 receptors would act to decrease phosphorylation by PKA. Extensive studies of DARPP-32 have identified a complex network of both positive and negative feedback processes that involve regulation of multiple sites by various kinases and phosphatases. The flexibility of these complex signaling processes may be required to allow the divergent regulation of DARPP-32 in D1- and D2-MSNs. It is also likely that DARPP-32, RCS, and ARPP-16 are themselves part of a more complex interacting signaling system, since both RCS and ARPP-16 can influence DARPP-32 phosphorylation at different sites (see Figure 3).

Despite the wealth of information concerning the regulation and function of DARPP-32, RCS, and ARPP-16, there are still outstanding questions. There is no detailed structural information about how the proteins interact with their targets, and additional X-ray crystallography and NMR studies are required. While there is some knowledge concerning the function of PP1 in the nucleus, the precise targets for nuclear DARPP-32 remain to be identified. To date most of the functional analysis of DARPP-32, RCS, and ARPP-16 has been restricted to their actions in MSNs of striatum, but it is likely that even at lower levels of expression in other brain regions, they play important roles in neuronal signaling.

ACKNOWLEDGMENTS

We would like to thank the many colleagues and collaborators who over the last 30 years have contributed to the studies of basal

ganglia phosphoproteins. In particular, Charles Ojomet, Atsuko Horiuchi, Jean-Antoine Girault, Akinori Nishi, Gretchen Snyder, Per Svenningsson, Allen Fienberg, James Bibb, Sergey Rakhilin, Maya Davies, Erika Andrade, Veronica Musante, James Surmeier, and Anita Aperia have made important discoveries related to the

elucidation of the functions of DARPP-32, RCS and, ARPP-16. We would also like to acknowledge the generous support from the National Institute of Mental Health, the National Institute of Drug Addiction, and NARSAD, who have supported the work discussed here for many years.

REFERENCES

- Ahn, J. H., McAvoy, T., Rakhilin, S. V., Nishi, A., Greengard, P., and Nairn, A. C. (2007a). Protein kinase A activates protein phosphatase 2A by phosphorylation of the B56delta subunit. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2979–2984.
- Ahn, J. H., Sung, J. Y., McAvoy, T., Nishi, A., Janssens, V., Goris, J., Greengard, P., and Nairn, A. C. (2007b). The B'/PR72 subunit mediates Ca²⁺-dependent dephosphorylation of DARPP-32 by protein phosphatase 2A. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9876–9881.
- Albert, K. A., Hemmings, H. C. Jr., Adamo, A. I., Potkin, S. G., Akbarian, S., Sandman, C. A., Cotman, C. W., Bunney, W. E. Jr., and Greengard, P. (2002). Evidence for decreased DARPP-32 in the prefrontal cortex of patients with schizophrenia. *Arch. Gen. Psychiatry* 59, 705–712.
- Anden, N. E., Fuxe, K., Hamberger, B., and Hokfelt, T. (1966). A quantitative study on the nigro-neostriatal dopamine neuron system in the rat. *Acta Physiol. Scand.* 306–312.
- Baracska, K. L., Haroutunian, V., and Meador-Woodruff, J. H. (2006). Dopamine receptor signaling molecules are altered in elderly schizophrenic cortex. *Synapse* 60, 271–279.
- Barbano, P. E., Spivak, M., Flajolet, M., Nairn, A. C., Greengard, P., and Greengard, L. (2007). A mathematical tool for exploring the dynamics of biological networks. *Proc. Natl. Acad. Sci. U.S.A.* 104, 19169–19174.
- Bateup, H. S., Santini, E., Shen, W., Birnbaum, S., Valjent, E., Surmeier, D. J., Fisone, G., Nestler, E. J., and Greengard, P. (2010). Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14845–14850.
- Bateup, H. S., Svenningsson, P., Kuroiwa, M., Gong, S., Nishi, A., Heintz, N., and Greengard, P. (2008). Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. *Nat. Neurosci.* 11, 932–939.
- Belkhir, A., Dar, A. A., Peng, D. F., Razvi, M. H., Rinehart, C., Arteaga, C. L., and El-Rifai, W. (2008a). Expression of t-DARPP mediates trastuzumab resistance in breast cancer cells. *Clin. Cancer Res.* 14, 4564–4571.
- Belkhir, A., Dar, A. A., Zaika, A., Kelley, M., and El-Rifai, W. (2008b). t-Darpp promotes cancer cell survival by up-regulation of Bcl2 through Akt-dependent mechanism. *Cancer Res.* 68, 395–403.
- Benderska, N., Becker, K., Girault, J. A., Becker, C. M., Andreadis, A., and Stamm, S. (2010). DARPP-32 binds to tra2-beta1 and influences alternative splicing. *Biochim. Biophys. Acta* 1799, 448–453.
- Berke, J. D., Sgambato, V., Zhu, P. P., Lavoie, B., Vincent, M., Krause, M., and Hyman, S. E. (2001). Dopamine and glutamate induce distinct striatal splice forms of Ania-6, an RNA polymerase II-associated cyclin. *Neuron* 32, 277–287.
- Bertran-Gonzalez, J., Hakansson, K., Borgkvist, A., Irinopoulou, T., Brami-Cherrier, K., Usiello, A., Greengard, P., Herve, D., Girault, J. A., Valjent, E., and Fisone, G. (2009). Histone H3 phosphorylation is under the opposite tonic control of dopamine D2 and adenosine A2A receptors in striatopallidal neurons. *Neuropsychopharmacology* 34, 1710–1720.
- Bibb, J. A., Snyder, G. L., Nishi, A., Yan, Z., Meijer, L., Fienberg, A. A., Tsai, L. H., Kwon, Y. T., Girault, J. A., Czernik, A. J., Huganir, R. L., Hemmings, H. C. Jr., Nairn, A. C., and Greengard, P. (1999). Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature* 402, 669–671.
- Bjorklund, A., and Dunnett, S. B. (2007). Fifty years of dopamine research. *Trends Neurosci.* 30, 185–187.
- Bjorklund, A., and Lindvall, O. (1984). "Dopamine-containing systems in the CNS," in *Classical Transmitters in the CNS*, Part I, eds A. Bjorklund and T. Hokfelt (Amsterdam: Elsevier), 55–122.
- Boettcher, J. M., Hartman, K. L., Lador, D. T., Qi, Z., Woods, W. S., George, J. M., and Rienstra, C. M. (2008). Membrane-induced folding of the cAMP-regulated phosphoprotein endosulfine-alpha. *Biochemistry* 47, 12357–12364.
- Bolam, J. P., Wainer, B. H., and Smith, A. D. (1984). Characterization of cholinergic neurons in the rat neostriatum. A combination of choline acetyltransferase immunocytochemistry, Golgi-impregnation and electron microscopy. *Neuroscience* 12, 711–718.
- Bollen, M., Peti, W., Ragusa, M. J., and Beullens, M. (2010). The extended PP1 toolkit: designed to create specificity. *Trends Biochem. Sci.* 35, 450–458.
- Borgkvist, A., Usiello, A., Greengard, P., and Fisone, G. (2007). Activation of the cAMP/PKA/DARPP-32 signaling pathway is required for morphine psychomotor stimulation but not for morphine reward. *Neuropsychopharmacology* 32, 1995–2003.
- Brene, S., Lindefors, N., Ehrlich, M., Taubes, T., Horiuchi, A., Kopp, J., Hall, H., Sedvall, G., Greengard, P., and Persson, H. (1994). Expression of mRNAs encoding ARPP-16/19, ARPP-21, and DARPP-32 in human brain tissue. *J. Neurosci.* 14, 985–998.
- Caporaso, G. L., Bibb, J. A., Snyder, G. L., Valle, C., Rakhilin, S., Fienberg, A. A., Hemmings, H. C., Nairn, A. C., and Greengard, P. (2000). Drugs of abuse modulate the phosphorylation of ARPP-21, a cyclic AMP-regulated phosphoprotein enriched in the basal ganglia. *Neuropharmacology* 39, 1637–1644.
- Clement-Cormier, Y. C., Kebejian, J. W., Petzold, G. L., and Greengard, P. (1974). Dopamine-sensitive adenylyl cyclase in mammalian brain: a possible site of action of antipsychotic drugs. *Proc. Natl. Acad. Sci. U.S.A.* 71, 1113–1117.
- Clinton, S. M., Ibrahim, H. M., Frey, K. A., Davis, K. L., Haroutunian, V., and Meador-Woodruff, J. H. (2005). Dopaminergic abnormalities in select thalamic nuclei in schizophrenia: involvement of the intracellular signal integrating proteins calycon and spinophilin. *Am. J. Psychiatry* 162, 1859–1871.
- da Cruz e Silva, E. F., Fox, C. A., Ojomet, C. C., Gustafson, E., Watson, S. J., and Greengard, P. (1995). Differential expression of protein phosphatase 1 isoforms in mammalian brain. *J. Neurosci.* 15, 3375–3389.
- Dancheck, B., Nairn, A. C., and Peti, W. (2008). Detailed structural characterization of unbound protein phosphatase 1 inhibitors. *Biochemistry* 47, 12346–12356.
- Dancheck, B., Ragusa, M. J., Allaire, M., Nairn, A. C., Page, R., and Peti, W. (2011). Molecular investigations of the structure and function of the protein phosphatase 1-spinophilin-inhibitor 2 heterotrimeric complex. *Biochemistry* 50, 1238–1246.
- Desdouts, F., Cheetham, J. J., Huang, H. B., Kwon, Y. G., da Cruz e Silva, E. F., Deneffe, P., Ehrlich, M. E., Nairn, A. C., Greengard, P., and Girault, J. A. (1995a). Mechanism of inhibition of protein phosphatase 1 by DARPP-32: studies with recombinant DARPP-32 and synthetic peptides. *Biochem. Biophys. Res. Commun.* 206, 652–658.
- Desdouts, F., Cohen, D., Nairn, A. C., Greengard, P., and Girault, J. A. (1995b). Phosphorylation of DARPP-32, a dopamine- and cAMP-regulated phosphoprotein, by casein kinase I in vitro and in vivo. *J. Biol. Chem.* 270, 8772–8778.
- Desdouts, F., Siciliano, J. C., Greengard, P., and Girault, J. A. (1995c). Dopamine- and cAMP-regulated phosphoprotein DARPP-32: phosphorylation of Ser-137 by casein kinase I inhibits dephosphorylation of Thr-34 by calcineurin. *Proc. Natl. Acad. Sci. U.S.A.* 92, 2682–2685.
- Doll, B. B., Hutchison, K. E., and Frank, M. J. (2011). Dopaminergic genes predict individual differences in susceptibility to confirmation bias. *J. Neurosci.* 31, 6188–6198.
- Dulubova, I., Horiuchi, A., Snyder, G. L., Girault, J. A., Czernik, A. J., Shao, L., Ramabhadran, R., Greengard, P., and Nairn, A. C. (2001). ARPP-16/ARPP-19: a highly conserved family of cAMP-regulated phosphoproteins. *J. Neurochem.* 77, 229–238.
- Ebihara, Y., Miyamoto, M., Fukunaga, A., Kato, K., Shichinohe, T., Kawarada, Y., Kurokawa, T., Cho, Y., Murakami, S., Uehara, H., Kaneko, H., Hashimoto, H., Murakami, Y., Itoh, T., Okushiba, S., Kondo, S., and Katoh, H. (2004). DARPP-32 expression arises after a phase of dysplasia in esophageal squamous cell carcinoma. *Br. J. Cancer* 91, 119–123.

- Egloff, M. P., Johnson, D. F., Moorhead, G., Cohen, P. T., Cohen, P., and Barford, D. (1997). Structural basis for the recognition of regulatory subunits by the catalytic subunit of protein phosphatase 1. *EMBO J.* 16, 1876–1887.
- Eklöf, A. C., Holtback, U., Svennilson, J., Fienberg, A., Greengard, P., and Aperia, A. (2001). Increased blood pressure and loss of anp-induced natriuresis in mice lacking DARPP-32 gene. *Clin. Exp. Hypertens.* 23, 449–460.
- El-Rifai, W., Smith, M. F. Jr., Li, G., Beckler, A., Carl, V. S., Montgomery, E., Knuutila, S., Moskaluk, C. A., Frier, H. F. Jr., and Powell, S. M. (2002). Gastric cancers overexpress DARPP-32 and a novel isoform, t-DARPP. *Cancer Res.* 62, 4061–4064.
- Feldcamp, L. A., Souza, R. P., Romano-Silva, M., Kennedy, J. L., and Wong, A. H. (2008). Reduced prefrontal cortex DARPP-32 mRNA in completed suicide victims with schizophrenia. *Schizophr. Res.* 103, 192–200.
- Fernandez, E., Schiappa, R., Girault, J. A., and Le, N. N. (2006). DARPP-32 is a robust integrator of dopamine and glutamate signals. *PLoS Comput. Biol.* 2, e176. doi: 10.1371/journal.pcbi.0020176
- Fienberg, A. A., Hiroi, N., Mermelstein, P. G., Song, W., Snyder, G. L., Nishi, A., Cheramy, A., O'Callaghan, J. P., Miller, D. B., Cole, D. G., Corbett, R., Haile, C. N., Cooper, D. C., Onn, S. P., Grace, A. A., Ouimet, C. C., White, F. J., Hyman, S. E., Surmeier, D. J., Girault, J., Nestler, E. J., and Greengard, P. (1998). DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. *Science* 281, 838–842.
- Flavell, S. W., Cowan, C. W., Kim, T. K., Greer, P. L., Lin, Y., Paradis, S., Griffith, E. C., Hu, L. S., Chen, C., and Greenberg, M. E. (2006). Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. *Science* 311, 1008–1012.
- Foster, G. A., Schultzberg, M., Hokfelt, T., Goldstein, M., Hemmings, H. C. Jr., Ouimet, C. C., Walaas, S. I., and Greengard, P. (1987). Development of a dopamine- and cyclic adenosine 3':5'-monophosphate-regulated phosphoprotein (DARPP-32) in the prenatal rat central nervous system, and its relationship to the arrival of presumptive dopaminergic innervation. *J. Neurosci.* 7, 1994–2018.
- Foster, G. A., Schultzberg, M., Hokfelt, T., Goldstein, M., Hemmings, H. C. Jr., Ouimet, C. C., Walaas, S. I., and Greengard, P. (1988). Ontogeny of the dopamine and cyclic adenosine 3':5'-monophosphate-regulated phosphoprotein (DARPP-32) in the pre- and postnatal mouse central nervous system. *Int. J. Dev. Neurosci.* 6, 367–386.
- Frank, M. J., Doll, B. B., Oas-Terpstra, J., and Moreno, F. (2009). Prefrontal and striatal dopaminergic genes predict individual differences in exploration and exploitation. *Nat. Neurosci.* 12, 1062–1068.
- Frank, M. J., and Fossella, J. A. (2011). Neurogenetics and pharmacology of learning, motivation, and cognition. *Neuropsychopharmacology* 36, 133–152.
- Frank, M. J., Moustafa, A. A., Haughey, H. M., Curran, T., and Hutchison, K. E. (2007). Genetic triple dissociation reveals multiple roles for dopamine in reinforcement learning. *Proc. Natl. Acad. Sci. U.S.A.* 104, 16311–16316.
- Garland, P., Quraishi, S., French, P., and O'Connor, V. (2008). Expression of the MAST family of serine/threonine kinases. *Brain Res.* 1195, 12–19.
- Gharbi-Ayachi, A., Labbe, J. C., Burgess, A., Vigneron, S., Strub, J. M., Brioudes, E., Van-Dorselaer, A., Castro, A., and Lorca, T. (2010). The substrate of Greatwall kinase, Arpp19, controls mitosis by inhibiting protein phosphatase 2A. *Science* 330, 1673–1677.
- Giaever, G., Chu, A. M., Ni, L., Connelly, C., Riles, L., Veronneau, S., Dow, S., Lucau-Danila, A., Anderson, K., Andre, B., Arkin, A. P., Astromoff, A., El-Bakkoury, M., Bangham, R., Benito, R., Brachat, S., Campanaro, S., Curtiss, M., Davis, K., Deutschbauer, A., Entian, K. D., Flaherty, P., Foury, F., Garfinkel, D. J., Gerstein, M., Gotte, D., Guldener, U., Hegemann, J. H., Hempel, S., Herman, Z., Jaramillo, D. F., Kelly, D. E., Kelly, S. L., Kotter, P., LaBonte, D., Lamb, D. C., Lan, N., Liang, H., Liao, H., Liu, L., Luo, C., Lussier, M., Mao, R., Menard, P., Ooi, S. L., Revuelta, J. L., Roberts, C. J., Rose, M., Ross-Macdonald, P., Scherens, B., Schimmack, G., Shafer, B., Shoemaker, D. D., Sookhai-Mahadeo, S., Storms, R. K., Strathern, J. N., Valle, G., Voet, M., Volckaert, G., Wang, C. Y., Ward, T. R., Wilhelmy, J., Winzler, E. A., Yang, Y., Yen, G., Youngman, E., Yu, K., Bussey, H., Boeke, J. D., Snyder, M., Philippsen, P., Davis, R. W., and Johnston, M. (2002). Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 418, 387–391.
- Girault, J. A., Hemmings, H. C. Jr., Williams, K. R., Nairn, A. C., and Greengard, P. (1989). Phosphorylation of DARPP-32, a dopamine- and cAMP-regulated phosphoprotein, by casein kinase II. *J. Biol. Chem.* 264, 21748–21759.
- Girault, J. A., Horiuchi, A., Gustafson, E. L., Rosen, N. L., and Greengard, P. (1990). Differential expression of ARPP-16 and ARPP-19, two highly related cAMP-regulated phosphoproteins, one of which is specifically associated with dopamine-innervated brain regions. *J. Neurosci.* 10, 1124–1133.
- Girault, J. A., Valjent, E., Caboche, J., and Herve, D. (2007). ERK2: a logical AND gate critical for drug-induced plasticity? *Curr. Opin. Pharmacol.* 7, 77–85.
- Glausier, J. R., Maddox, M., Hemmings, H. C. Jr., Nairn, A. C., Greengard, P., and Muly, E. C. (2010). Localization of dopamine- and cAMP-regulated phosphoprotein-32 and inhibitor-1 in area 9 of *Macaca mulatta* prefrontal cortex. *Neuroscience* 167, 428–438.
- Goldberg, J., Huang, H. B., Kwon, Y. G., Greengard, P., Nairn, A. C., and Kuriyan, J. (1995). Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1. *Nature* 376, 745–753.
- Grebb, J. A., Girault, J. A., Ehrlich, M., and Greengard, P. (1990). Chronic treatment of rats with SCH-23390 or raclopride does not affect the concentrations of DARPP-32 or its mRNA in dopamine-innervated brain regions. *J. Neurochem.* 55, 204–207.
- Greengard, P., Allen, P. B., and Nairn, A. C. (1999). Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* 23, 435–447.
- Gros, L., Breant, B., Duchene, B., Leroy, C., Fauconnier, G., Bataille, D., and Virsolvy, A. (2002). Localization of alpha-endosulphine in pancreatic somatostatin delta cells and expression during rat pancreas development. *Diabetologia* 45, 703–710.
- Gu, L., Waliany, S., and Kane, S. E. (2009). Darpp-32 and its truncated variant t-Darpp have antagonistic effects on breast cancer cell growth and herceptin resistance. *PLoS ONE* 4, e6220. doi: 10.1371/journal.pone.0006220
- Hamel, S., Bouchard, A., Ferrario, C., Hassan, S., Aguilar-Mahecha, A., Buchanan, M., Quenneville, L., Miller, W., and Basik, M. (2010). Both t-Darpp and DARPP-32 can cause resistance to trastuzumab in breast cancer cells and are frequently expressed in primary breast cancers. *Breast Cancer Res. Treat.* 120, 47–57.
- Hansen, C., Greengard, P., Nairn, A. C., Andersson, T., and Vogel, W. F. (2006). Phosphorylation of DARPP-32 regulates breast cancer cell migration downstream of the receptor tyrosine kinase DDR1. *Exp. Cell Res.* 312, 4011–4018.
- Hansen, C., Howlin, J., Tengholm, A., Dyachok, O., Vogel, W. F., Nairn, A. C., Greengard, P., and Andersson, T. (2009). Wnt-5a-induced phosphorylation of DARPP-32 inhibits breast cancer cell migration in a CREB-dependent manner. *J. Biol. Chem.* 284, 27533–27543.
- Heiman, M., Schaefer, A., Gong, S., Peterson, J. D., Day, M., Ramsey, K. E., Suarez-Farinas, M., Schwarz, C., Stephan, D. A., Surmeier, D. J., Greengard, P., and Heintz, N. (2008). A translational profiling approach for the molecular characterization of CNS cell types. *Cell* 135, 738–748.
- Hemmings, H. C. Jr., Girault, J. A., Williams, K. R., LoPresti, M. B., and Greengard, P. (1989). ARPP-21, a cyclic AMP-regulated phosphoprotein (Mr = 21,000) enriched in dopamine-innervated brain regions. Amino acid sequence of the site phosphorylated by cyclic AMP in intact cells and kinetic studies of its phosphorylation in vitro. *J. Biol. Chem.* 264, 7726–7733.
- Hemmings, H. C. Jr., and Greengard, P. (1986). DARPP-32, a dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein: regional, tissue, and phylogenetic distribution. *J. Neurosci.* 6, 1469–1481.
- Hemmings, H. C. Jr., and Greengard, P. (1989). ARPP-21, a cyclic AMP-regulated phosphoprotein enriched in dopamine-innervated brain regions. I. Purification and characterization of the protein from bovine caudate nucleus. *J. Neurosci.* 9, 851–864.
- Hemmings, H. C. Jr., Greengard, P., Tung, H. Y., and Cohen, P. (1984a). DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. *Nature* 310, 503–505.
- Hemmings, H. C. Jr., Nairn, A. C., Aswad, D. W., and Greengard, P. (1984b). DARPP-32,

- a dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein enriched in dopamine-innervated brain regions. II. Purification and characterization of the phosphoprotein from bovine caudate nucleus. *J. Neurosci.* 4, 99–110.
- Hemmings, H. C. Jr., Nairn, A. C., Elliott, J. I., and Greengard, P. (1990). Synthetic peptide analogs of DARPP-32 (Mr 32,000 dopamine- and cAMP-regulated phosphoprotein), an inhibitor of protein phosphatase-1. Phosphorylation, dephosphorylation, and inhibitory activity. *J. Biol. Chem.* 265, 20369–20376.
- Hernandez-Lopez, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H., and Surmeier, D. J. (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca²⁺ currents and excitability via a novel PLC[β 1]-IP3-calcineurin-signaling cascade. *J. Neurosci.* 20, 8987–8995.
- Heron, L., Virsolvy, A., Peyrollier, K., Gribble, F. M., Le, C. A., Ashcroft, F. M., and Bataille, D. (1998). Human alpha-endosulfine, a possible regulator of sulfonylurea-sensitive KATP channel: molecular cloning, expression and biological properties. *Proc. Natl. Acad. Sci. U.S.A.* 95, 8387–8391.
- Hong, L., Wang, J., Zhao, Y., Han, Z., Zhou, X., Guo, W., Zhang, X., Jin, H., Wu, K., Ding, J., and Fan, D. (2007). DARPP-32 mediates multidrug resistance of gastric cancer through regulation of P-gp and ZNRD1. *Cancer Invest.* 25, 699–705.
- Horiuchi, A., Williams, K. R., Kurihara, T., Nairn, A. C., and Greengard, P. (1990). Purification and cDNA cloning of ARPP-16, a cAMP-regulated phosphoprotein enriched in basal ganglia, and of a related phosphoprotein, ARPP-19. *J. Biol. Chem.* 265, 9476–9484.
- Hu, J. X., Yu, L., Shi, Y. Y., Zhao, X. Z., Meng, J. W., He, G., Xu, Y. F., Feng, G. Y., and He, L. (2007). An association study between PPP1R1B gene and schizophrenia in the Chinese population. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31, 1303–1306.
- Huang, F. L., and Glimsman, W. (1976). A second heat-stable protein inhibitor of phosphorylase phosphatase from rabbit muscle. *FEBS Lett.* 62, 326–329.
- Huang, H. B., Chen, Y. C., Horiuchi, A., Tsai, L. H., Liu, H. T., Chyan, C. L., Hsieh, M. J., Liu, C. K., Lin, F. M., Greengard, P., Nairn, A. C., Shiao, M. S., and Lin, T. H. (2001). Backbone 1H, 15N, and 13C resonance assignments of ARPP-19. *J. Biomol. NMR* 19, 383–384.
- Huang, H. B., Horiuchi, A., Goldberg, J., Greengard, P., and Nairn, A. C. (1997). Site-directed mutagenesis of amino acid residues of protein phosphatase 1 involved in catalysis and inhibitor binding. *Proc. Natl. Acad. Sci. U.S.A.* 94, 3530–3535.
- Huang, H. B., Horiuchi, A., Watanabe, T., Shih, S. R., Tsay, H. J., Li, H. C., Greengard, P., and Nairn, A. C. (1999). Characterization of the inhibition of protein phosphatase-1 by DARPP-32 and inhibitor-2. *J. Biol. Chem.* 274, 7870–7878.
- Irwin, N., Chao, S., Goritschenko, L., Horiuchi, A., Greengard, P., Nairn, A. C., and Benowitz, L. I. (2002). Nerve growth factor controls GAP-43 mRNA stability via the phosphoprotein ARPP-19. *Proc. Natl. Acad. Sci. U.S.A.* 99, 12427–12431.
- Ishikawa, M., Mizukami, K., Iwakiri, M., and Asada, T. (2007). Immunohistochemical and immunoblot analysis of Dopamine and cyclic AMP-regulated phosphoprotein, relative molecular mass 32,000 (DARPP-32) in the prefrontal cortex of subjects with schizophrenia and bipolar disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31, 1177–1181.
- Iversen, S. D., and Iversen, L. L. (2007). Dopamine: 50 years in perspective. *Trends Neurosci.* 30, 188–193.
- Jin, M., Bateup, H., Padovan, J. C., Greengard, P., Nairn, A. C., and Chait, B. T. (2005). Quantitative analysis of protein phosphorylation in mouse brain by hypothesis-driven multistage mass spectrometry. *Anal. Chem.* 77, 7845–7851.
- Kebabian, J. W., and Calne, D. B. (1979). Multiple receptors for dopamine. *Nature* 277, 93–96.
- Kebabian, J. W., and Greengard, P. (1971). Dopamine-sensitive adenylyl cyclase: possible role in synaptic transmission. *Science* 174, 1346–1349.
- Kebabian, J. W., Petzold, G. L., and Greengard, P. (1972). Dopamine-sensitive adenylyl cyclase in caudate nucleus of rat brain, and its similarity to the “dopamine receptor”. *Proc. Natl. Acad. Sci. U.S.A.* 69, 2145–2149.
- Kim, S. H., Nairn, A. C., Cairns, N., and Lubec, G. (2001). Decreased levels of ARPP-19 and PKA in brains of Down syndrome and Alzheimer's disease. *J. Neural Transm. Suppl.* 61, 263–272.
- King, M. M., Huang, C. Y., Chock, P. B., Nairn, A. C., Hemmings, H. C. Jr., Chan, K. F., and Greengard, P. (1984). Mammalian brain phosphoproteins as substrates for calcineurin. *J. Biol. Chem.* 259, 8080–8083.
- Kisielow, J., Nairn, A. C., and Karjalainen, K. (2001). TARPP, a novel protein that accompanies TCR gene rearrangement and thymocyte education. *Eur. J. Immunol.* 31, 1141–1149.
- Kitai, S. T., Sugimori, M., and Kocsis, J. D. (1976). Excitatory nature of dopamine in the nigro-caudate pathway. *Exp. Brain Res.* 24, 351–363.
- Kolata, S., Light, K., Wass, C. D., Colas-Zelin, D., Roy, D., and Matzel, L. D. (2010). A dopaminergic gene cluster in the prefrontal cortex predicts performance indicative of general intelligence in genetically heterogeneous mice. *PLoS ONE* 5, e14036. doi: 10.1371/journal.pone.0014036
- Kunii, Y., Yabe, H., Wada, A., Yang, Q., Nishiura, K., and Niwa, S. I. (2011). Altered DARPP-32 expression in the superior temporal gyrus in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 1139–1143.
- Kurihara, T., Lewis, R. M., Eisler, J., and Greengard, P. (1988). Cloning of cDNA for DARPP-32, a dopamine- and cyclic AMP-regulated neuronal phosphoprotein. *J. Neurosci.* 8, 508–517.
- Lewis, C. M., Levinson, D. F., Wise, L. H., DeLisi, L. E., Straub, R. E., Hovatta, I., Williams, N. M., Schwab, S. G., Pulver, A. E., Faraone, S. V., Brzustowicz, L. M., Kaufmann, C. A., Garver, D. L., Gurling, H. M., Lindholm, E., Coon, H., Moises, H. W., Byerley, W., Shaw, S. H., Mesen, A., Sherrington, R., O'Neill, F. A., Walsh, D., Kendler, K. S., Ekelund, J., Paunio, T., Lonnqvist, J., Peltonen, L., O'Donovan, M. C., Owen, M. J., Wildenauer, D. B., Maier, W., Nestadt, G., Blouin, J. L., Antonarakis, S. E., Mowry, B. J., Silverman, J. M., Crowe, R. R., Cloninger, C. R., Tsuang, M. T., Malaspina, D., Harkavy-Friedman, J. M., Svrtak, D. M., Bassett, A. S., Holcomb, J., Kalsi, G., McQuillan, A., Brynjolfsson, J., Sigmundsson, T., Petursson, H., Jazin, E., Zoega, T., and Helgason, T. (2003). Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. *Am. J. Hum. Genet.* 73, 34–48.
- Li, C. H., Liao, H. M., Hung, T. W., and Chen, C. H. (2006). Mutation analysis of DARPP-32 as a candidate gene for schizophrenia. *Schizophr. Res.* 87, 1–5.
- Lin, T. H., Huang, Y. C., Chin, M. L., Chen, Y. C., Jeng, H. H., Lin, F. M., Shiao, M. S., Horiuchi, A., Greengard, P., Nairn, A. C., and Huang, H. B. (2004). 1H, 15N, and 13C resonance assignments of DARPP-32 (dopamine and cAMP-regulated phosphoprotein, Mr. 32,000) – a protein inhibitor of protein phosphatase-1. *J. Biomol. NMR* 28, 413–414.
- Lindskog, M. (2008). Modelling of DARPP-32 regulation to understand intracellular signaling in psychiatric disease. *Pharmacopsychiatry* 41(Suppl. 1), S99–S104.
- Lindskog, M., Svenningsson, P., Pozzi, L., Kim, Y., Fienberg, A. A., Bibb, J. A., Fredholm, B. B., Nairn, A. C., Greengard, P., and Fisone, G. (2002). Involvement of DARPP-32 phosphorylation in the stimulant action of caffeine. *Nature* 418, 774–778.
- Mani, S. K., Fienberg, A. A., O'Callaghan, J. P., Snyder, G. L., Allen, P. B., Dash, P. K., Moore, A. N., Mitchell, A. J., Bibb, J., Greengard, P., and O'Malley, B. W. (2000). Requirement for DARPP-32 in progesterone-facilitated sexual receptivity in female rats and mice. *Science* 287, 1053–1056.
- Marsh, J. A., Dancheck, B., Ragusa, M. J., Allaire, M., Forman-Kay, J. D., and Peti, W. (2010). Structural diversity in free and bound states of intrinsically disordered protein phosphatase 1 regulators. *Structure* 18, 1094–1103.
- McAvoy, T., Zhou, M. M., Greengard, P., and Nairn, A. C. (2009). Phosphorylation of Rap1GAP, a striatally enriched protein, by protein kinase A controls Rap1 activity and dendritic spine morphology. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3531–3536.
- Meister, B., Askergren, J., Tunevall, G., Hemmings, H. C. Jr., and Greengard, P. (1991). Identification of a dopamine- and 3':5'-cyclic adenosine monophosphate-regulated phosphoprotein of 32 kD (DARPP-32) in parathyroid hormone-producing cells of the human parathyroid gland. *J. Endocrinol. Invest.* 14, 655–661.
- Meister, B., Fried, G., Hokfelt, T., Hemmings, H. C. Jr., and Greengard, P. (1988). Immunohistochemical evidence for the existence of a dopamine- and cyclic AMP-regulated phosphoprotein (DARPP-32) in brown adipose tissue of pigs. *Proc. Natl. Acad. Sci. U.S.A.* 85, 8713–8716.

- Meister, B., Fryckstedt, J., Schalling, M., Cortes, R., Hokfelt, T., Aperia, A., Hemmings, H. C. Jr., Nairn, A. C., Ehrlich, M., and Greengard, P. (1989). Dopamine- and cAMP-regulated phosphoprotein (DARPP-32) and dopamine DA1 agonist-sensitive Na⁺, K⁺-ATPase in renal tubule cells. *Proc. Natl. Acad. Sci. U.S.A.* 86, 8068–8072.
- Meyer-Lindenberg, A., Straub, R. E., Lipska, B. K., Verchinski, B. A., Goldberg, T., Callicott, J. H., Egan, M. F., Huffaker, S. S., Mattay, V. S., Kolachana, B., Kleinman, J. E., and Weinberger, D. R. (2007). Genetic evidence implicating DARPP-32 in human frontostriatal structure, function, and cognition. *J. Clin. Invest.* 117, 672–682.
- Mittag, T., Kay, L. E., and Forman-Kay, J. D. (2010). Protein dynamics and conformational disorder in molecular recognition. *J. Mol. Recognit.* 23, 105–116.
- Mochida, S., Maslen, S. L., Skehel, M., and Hunt, T. (2010). Greatwall phosphorylates an inhibitor of protein phosphatase 2A that is essential for mitosis. *Science* 330, 1670–1673.
- Nairn, A. C., and Greengard, P. (2001). A novel cyclin provides a link between dopamine and RNA processing. *Neuron* 32, 174–176.
- Nairn, A. C., Hemmings, H. C. Jr., and Greengard, P. (1985). Protein kinases in the brain. *Annu. Rev. Biochem.* 54, 931–976.
- Nakano, T., Doi, T., Yoshimoto, J., and Doya, K. (2010). A kinetic model of dopamine- and calcium-dependent striatal synaptic plasticity. *PLoS Comput. Biol.* 6, e1000670. doi: 10.1371/journal.pcbi.1000670
- Neyroz, P., Desdoutis, F., Benfenati, F., Knutson, J. R., Greengard, P., and Girault, J. A. (1993). Study of the conformation of DARPP-32, a dopamine- and cAMP-regulated phosphoprotein, by fluorescence spectroscopy. *J. Biol. Chem.* 268, 24022–24031.
- Nishi, A., Bibb, J. A., Matsuyama, S., Hamada, M., Higashi, H., Nairn, A. C., and Greengard, P. (2002). Regulation of DARPP-32 dephosphorylation at PKA- and Cdk5-sites by NMDA and AMPA receptors: distinct roles of calcineurin and protein phosphatase-2A. *J. Neurochem.* 81, 832–841.
- Nishi, A., Bibb, J. A., Snyder, G. L., Higashi, H., Nairn, A. C., and Greengard, P. (2000). Amplification of dopaminergic signaling by a positive feedback loop. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12840–12845.
- Nishi, A., Snyder, G. L., and Greengard, P. (1997). Bidirectional regulation of DARPP-32 phosphorylation by dopamine. *J. Neurosci.* 17, 8147–8155.
- Nishi, A., Snyder, G. L., Nairn, A. C., and Greengard, P. (1999). Role of calcineurin and protein phosphatase-2A in the regulation of DARPP-32 dephosphorylation in neostriatal neurons. *J. Neurochem.* 72, 2015–2021.
- Nishi, A., Watanabe, Y., Higashi, H., Tanaka, M., Nairn, A. C., and Greengard, P. (2005). Glutamate regulation of DARPP-32 phosphorylation in neostriatal neurons involves activation of multiple signaling cascades. *Proc. Natl. Acad. Sci. U.S.A.* 102, 1199–1204.
- Ouimet, C. C., and Greengard, P. (1990). Distribution of DARPP-32 in the basal ganglia: an electron microscopic study. *J. Neurocytol.* 19, 39–52.
- Ouimet, C. C., Hemmings, H. C. Jr., and Greengard, P. (1989). ARPP-21, a cyclic AMP-regulated phosphoprotein enriched in dopamine-innervated brain regions. II. Immunocytochemical localization in rat brain. *J. Neurosci.* 9, 865–875.
- Ouimet, C. C., LaMantia, A. S., Goldman-Rakic, P., Rakic, P., and Greengard, P. (1992). Immunocytochemical localization of DARPP-32, a dopamine and cyclic-AMP-regulated phosphoprotein, in the primate brain. *J. Comp. Neurol.* 323, 209–218.
- Ouimet, C. C., Langley-Gullion, K. C., and Greengard, P. (1998). Quantitative immunocytochemistry of DARPP-32-expressing neurons in the rat caudatoputamen. *Brain Res.* 808, 8–12.
- Ouimet, C. C., Miller, P. E., Hemmings, H. C. Jr., Walaas, S. I., and Greengard, P. (1984). DARPP-32, a dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein enriched in dopamine-innervated brain regions. III. Immunocytochemical localization. *J. Neurosci.* 4, 111–124.
- Peyrollier, K., Heron, L., Virsolvy-Vergine, A., Le, C. A., and Bataille, D. (1996). Alpha endosulfine is a novel molecule, structurally related to a family of phosphoproteins. *Biochem. Biophys. Res. Commun.* 223, 583–586.
- Pulipparacharuvil, S., Renthal, W., Hale, C. F., Taniguchi, M., Xiao, G., Kumar, A., Russo, S. J., Sikder, D., Dewey, C. M., Davis, M. M., Greengard, P., Nairn, A. C., Nestler, E. J., and Cowan, C. W. (2008). Cocaine regulates MEF2 to control synaptic and behavioral plasticity. *Neuron* 59, 621–633.
- Ragusa, M. J., Dancheck, B., Critton, D. A., Nairn, A. C., Page, R., and Peti, W. (2010). Spinophilin directs protein phosphatase 1 specificity by blocking substrate binding sites. *Nat. Struct. Mol. Biol.* 17, 459–464.
- Rakhilin, S. V., Olson, P. A., Nishi, A., Starkova, N. N., Fienberg, A. A., Nairn, A. C., Surmeier, D. J., and Greengard, P. (2004). A network of control mediated by regulator of calcium/calmodulin-dependent signaling. *Science* 306, 698–701.
- Rogers, A., Antoshechkin, I., Bieri, T., Blasari, D., Bastiani, C., Canaran, P., Chan, J., Chen, W. J., Davis, P., Fernandes, J., Fiedler, T. J., Han, M., Harris, T. W., Kishore, R., Lee, R., McKay, S., Muller, H. M., Nakamura, C., Ozersky, P., Petcherski, A., Schindelman, G., Schwarz, E. M., Spooner, W., Tuli, M. A., Van, A. K., Wang, D., Wang, X., Williams, G., Yook, K., Durbin, R., Stein, L. D., Spieth, J., and Sternberg, P. W. (2008). WormBase 2007. *Nucleic Acids Res.* 36, D612–D617.
- Sgambato, V., Minassian, R., Nairn, A. C., and Hyman, S. E. (2003). Regulation of ania-6 splice variants by distinct signaling pathways in striatal neurons. *J. Neurochem.* 86, 153–164.
- Shalizi, A., Gaudilliere, B., Yuan, Z., Stegmuller, J., Shirogane, T., Ge, Q., Tan, Y., Schulman, B., Harper, J. W., and Bonni, A. (2006). A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. *Science* 311, 1012–1017.
- Souza, B. R., Motta, B. S., Rosa, D. V., Torres, K. C., Castro, A. A., Comim, C. M., Sampaio, A. M., Lima, F. F., Jeromin, A., Quevedo, J., and Romano-Silva, M. A. (2008). DARPP-32 and NCS-1 expression is not altered in brains of rats treated with typical or atypical antipsychotics. *Neurochem. Res.* 33, 533–538.
- Steinbusch, H. W. (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* 6, 557–618.
- Stipanovich, A., Valjent, E., Matamalas, M., Nishi, A., Ahn, J. H., Maroteaux, M., Bertran-Gonzalez, J., Brami-Cherrier, K., Enslen, H., Corbille, A. G., Filhol, O., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2008). A phosphatase cascade by which rewarding stimuli control nucleosomal response. *Nature* 453, 879–884.
- Stone, R. A., Laties, A. M., Hemmings, H. C. Jr., Ouimet, C. C., and Greengard, P. (1986). DARPP-32 in the ciliary epithelium of the eye: a neurotransmitter-regulated phosphoprotein of brain localizes to secretory cells. *J. Histochem. Cytochem.* 34, 1465–1468.
- Stralfors, P., Hemmings, H. C. Jr., and Greengard, P. (1989). Inhibitors of protein phosphatase-1. Inhibitor-1 of bovine adipose tissue and a dopamine- and cAMP-regulated phosphoprotein of bovine brain are identical. *Eur. J. Biochem.* 180, 143–148.
- Surmeier, D. J., Shen, W., Day, M., Gertler, T., Chan, S., Tian, X., and Plotkin, J. L. (2010). The role of dopamine in modulating the structure and function of striatal circuits. *Prog. Brain Res.* 183, 149–167.
- Svenningsson, P., Nairn, A. C., and Greengard, P. (2005). DARPP-32 mediates the actions of multiple drugs of abuse. *AAPS J.* 7, E353–E360.
- Svenningsson, P., Nishi, A., Fisone, G., Girault, J. A., Nairn, A. C., and Greengard, P. (2004). DARPP-32: an integrator of neurotransmission. *Annu. Rev. Pharmacol. Toxicol.* 44, 269–296.
- Svenningsson, P., Tzavara, E. T., Caruthers, R., Rachleff, I., Wattler, S., Nehls, M., McKinzie, D. L., Fienberg, A. A., Nomikos, G. G., and Greengard, P. (2003). Diverse psychotomimetics act through a common signaling pathway. *Science* 302, 1412–1415.
- Svenningsson, P., Tzavara, E. T., Liu, F., Fienberg, A. A., Nomikos, G. G., and Greengard, P. (2002a). DARPP-32 mediates serotonergic neurotransmission in the forebrain. *Proc. Natl. Acad. Sci. U.S.A.* 99, 3188–3193.
- Svenningsson, P., Tzavara, E. T., Witkin, J. M., Fienberg, A. A., Nomikos, G. G., and Greengard, P. (2002b). Involvement of striatal and extrastriatal DARPP-32 in biochemical and behavioral effects of fluoxetine (Prozac). *Proc. Natl. Acad. Sci. U.S.A.* 99, 3182–3187.
- Torres, K. C., Souza, B. R., Miranda, D. M., Nicolato, R., Neves, F. S., Barros, A. G., Dutra, W. O., Gollob, K. J., Correa, H., and Romano-Silva, M. A. (2009). The leukocytes expressing DARPP-32 are reduced in patients with schizophrenia and bipolar disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 214–219.

- Tsou, K., Girault, J. A., and Greengard, P. (1993). Dopamine D1 agonist SKF 38393 increases the state of phosphorylation of ARPP-21 in substantia nigra. *J. Neurochem.* 60, 1043–1046.
- Valjent, E., Bertran-Gonzalez, J., Herve, D., Fisone, G., and Girault, J. A. (2009). Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci.* 32, 538–547.
- Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., Stipanovich, A., Caboche, J., Lombroso, P. J., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 102, 491–496.
- Vangamudi, B., Peng, D. F., Cai, Q., El-Rifai, W., Zheng, W., and Belkhir, A. (2010). t-DARPP regulates phosphatidylinositol-3-kinase-dependent cell growth in breast cancer. *Mol. Cancer* 9, 240.
- Varis, A., Zaika, A., Puolakkainen, P., Nagy, B., Madrigal, I., Kokkola, A., Vayrynen, A., Karkkainen, P., Moskaluk, C., El-Rifai, W., and Knuutila, S. (2004). Coamplified and overexpressed genes at ERBB2 locus in gastric cancer. *Int. J. Cancer* 109, 548–553.
- Virshup, D. M., and Kaldis, P. (2010). Cell biology. Enforcing the Greatwall in mitosis. *Science* 330, 1638–1639.
- Virsolvy-Vergine, A., Leray, H., Kuroki, S., Lupo, B., Dufour, M., and Bataille, D. (1992). Endosulfine, an endogenous peptidic ligand for the sulfonylurea receptor: purification and partial characterization from ovine brain. *Proc. Natl. Acad. Sci. U.S.A.* 89, 6629–6633.
- Virsolvy-Vergine, A., Salazar, G., Sillard, R., Denoroy, L., Mutt, V., and Bataille, D. (1996). Endosulfine, endogenous ligand for the sulphonylurea receptor: isolation from porcine brain and partial structural determination of the alpha form. *Diabetologia* 39, 135–141.
- Voets, E., and Wolthuis, R. M. (2010). MASTL is the human orthologue of Greatwall kinase that facilitates mitotic entry, anaphase and cytokinesis. *Cell Cycle* 9, 3591–3601.
- Von Stetina, J. R., Tranchesi, S., Dey, S. K., Lee, L. A., Cha, B., and Drummond-Barbosa, D. (2008). alpha-Endosulfine is a conserved protein required for oocyte meiotic maturation in *Drosophila*. *Development* 135, 3697–3706.
- Walaas, I., and Fonnum, F. (1979). The effects of surgical and chemical lesions on neurotransmitter candidates in the nucleus accumbens of the rat. *Neuroscience* 4, 209–216.
- Walaas, I., and Fonnum, F. (1980). Biochemical evidence for gamma-aminobutyrate containing fibres from the nucleus accumbens to the substantia nigra and ventral tegmental area in the rat. *Neuroscience* 5, 63–72.
- Walaas, S. I., Aswad, D. W., and Greengard, P. (1983a). A dopamine- and cyclic AMP-regulated phosphoprotein enriched in dopamine-innervated brain regions. *Nature* 301, 69–71.
- Walaas, S. I., Nairn, A. C., and Greengard, P. (1983b). Regional distribution of calcium- and cyclic adenosine 3':5'-monophosphate-regulated protein phosphorylation systems in mammalian brain. I. Particulate systems. *J. Neurosci.* 3, 291–301.
- Walaas, S. I., Nairn, A. C., and Greengard, P. (1983c). Regional distribution of calcium- and cyclic adenosine 3':5'-monophosphate-regulated protein phosphorylation systems in mammalian brain. II. Soluble systems. *J. Neurosci.* 3, 302–311.
- Walaas, S. I., Cala, S., and Greengard, P. (1989). Localization of ARPP-90, a major 90 kiloDalton basal ganglion-enriched substrate for cyclic AMP-dependent protein kinase, in striatonigral neurons in the rat brain. *Brain Res. Mol. Brain Res.* 5, 149–157.
- Walaas, S. I., and Greengard, P. (1984). DARPP-32, a dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein enriched in dopamine-innervated brain regions. I. Regional and cellular distribution in the rat brain. *J. Neurosci.* 4, 84–98.
- Walaas, S. I., and Greengard, P. (1993). ARPP-39, a membrane-associated substrate for cyclic AMP-dependent protein kinase present in neostriatal neurons. *J. Mol. Neurosci.* 4, 73–88.
- Walaas, S. I., and Ouimet, C. C. (1989). The ventral striatopallidal complex: an immunocytochemical analysis of medium-sized striatal neurons and striatopallidal fibers in the basal forebrain of the rat. *Neuroscience* 28, 663–672.
- Watanabe, T., Huang, H. B., Horiuchi, A., da Cruze Silva, E. F., Hsieh-Wilson, L., Allen, P. B., Shenolikar, S., Greengard, P., and Nairn, A. C. (2001). Protein phosphatase 1 regulation by inhibitors and targeting subunits. *Proc. Natl. Acad. Sci. U.S.A.* 98, 3080–3085.
- Williams, K. R., Hemmings, H. C. Jr., LoPresti, M. B., and Greengard, P. (1989). ARPP-21, a cyclic AMP-regulated phosphoprotein enriched in dopamine-innervated brain regions. I. Amino acid sequence of ARPP-21B from bovine caudate nucleus. *J. Neurosci.* 9, 3631–3637.
- Williams, K. R., Hemmings, H. C. Jr., LoPresti, M. B., Konigsberg, W. H., and Greengard, P. (1986). DARPP-32, a dopamine- and cyclic AMP-regulated neuronal phosphoprotein. Primary structure and homology with protein phosphatase inhibitor-1. *J. Biol. Chem.* 261, 1890–1903.
- Woods, W. S., Boettcher, J. M., Zhou, D. H., Kloepper, K. D., Hartman, K. L., Lador, D. T., Qi, Z., Rienstra, C. M., and George, J. M. (2007). Conformation-specific binding of alpha-synuclein to novel protein partners detected by phage display and NMR spectroscopy. *J. Biol. Chem.* 282, 34555–34567.
- Yoshimi, A., Takahashi, N., Saito, S., Ito, Y., Aleksic, B., Usui, H., Kawamura, Y., Waki, Y., Yoshikawa, T., Kato, T., Iwata, N., Inada, T., Noda, Y., and Ozaki, N. (2008). Genetic analysis of the gene coding for DARPP-32 (PPP1R1B) in Japanese patients with schizophrenia or bipolar disorder. *Schizophr. Res.* 100, 334–341.
- Zachariou, V., Sgambato-Faure, V., Sasaki, T., Svenningsson, P., Berton, O., Fienberg, A. A., Nairn, A. C., Greengard, P., and Nestler, E. J. (2006). Phosphorylation of DARPP-32 at Threonine-34 is required for cocaine action. *Neuropsychopharmacology* 31, 555–562.
- Zhan, L., Kerr, J. R., Lafuente, M. J., Maclean, A., Chibalina, M. V., Liu, B., Burke, B., Bevan, S., and Nasir, J. (2011). Altered expression and coregulation of dopamine signalling genes in schizophrenia and bipolar disorder. *Neuropathol. Appl. Neurobiol.* 37, 206–219.
- Zhang, Y., Svenningsson, P., Picetti, R., Schlussman, S. D., Nairn, A. C., Ho, A., Greengard, P., and Kreek, M. J. (2006). Cocaine self-administration in mice is inversely related to phosphorylation at Thr34 (protein kinase A site) and Ser130 (kinase CK1 site) of DARPP-32. *J. Neurosci.* 26, 2645–2651.
- Zhou, F. M., Wilson, C. J., and Dani, J. A. (2002). Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J. Neurobiol.* 53, 590–605.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 May 2011; paper pending published: 13 June 2011; accepted: 23 July 2011; published online: 26 August 2011.
Citation: Walaas SI, Hemmings HC Jr., Greengard P and Nairn AC (2011) Beyond the dopamine receptor: regulation and roles of serine/threonine protein phosphatases. *Front. Neuroanat.* 5:50. doi: 10.3389/fnana.2011.00050
Copyright © 2011 Walaas, Hemmings, Greengard and Nairn. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



The role of striatal-enriched protein tyrosine phosphatase (STEP) in cognition

Christopher James Fitzpatrick¹ and Paul J. Lombroso^{2,3,4*}

¹ Department of Psychiatry, School of Medicine, University of Michigan, Ann Arbor, MI, USA

² Child Study Center, School of Medicine, Yale University, New Haven, CT, USA

³ Department of Psychiatry, School of Medicine, Yale University, New Haven, CT, USA

⁴ Department of Neurobiology, School of Medicine, Yale University, New Haven, CT, USA

Edited by:

Emmanuel Valjent, Université
Montpellier 1 & 2, France

Reviewed by:

Joël Bockaert, INSERM, France
Peter Vanhoutte, Centre National de la
Recherche Scientifique, France

*Correspondence:

Paul J. Lombroso, Laboratory of
Molecular Neurobiology, 230 South
Frontage Road, New Haven, CT
06520, USA.
e-mail: paul.lombroso@yale.edu

Striatal-enriched protein tyrosine phosphatase (STEP) has recently been implicated in several neuropsychiatric disorders with significant cognitive impairments, including Alzheimer's disease, schizophrenia, and fragile X syndrome. A model has emerged by which STEP normally opposes the development of synaptic strengthening and that disruption in STEP activity leads to aberrant synaptic function. We review the mechanisms by which STEP contributes to the etiology of these and other neuropsychiatric disorders. These findings suggest that disruptions in STEP activity may be a common mechanism for cognitive impairments in diverse illnesses.

Keywords: STEP, protein tyrosine phosphatase, cognition, Alzheimer's disease, fragile X syndrome, Huntington's disease, schizophrenia, excitotoxicity

INTRODUCTION

Although the genetic basis of cognition is poorly understood, there is a general agreement that normal cognitive function relies on environmental, genetic, and molecular interactions (Heyes and Huber, 2000). These interactions mediate the association between specific behaviors, rewards, and punishments, enabling individuals to respond effectively to their environment. Cognitive impairments present in numerous neuropsychiatric disorders, including Alzheimer's disease (AD; Folstein and Whitehouse, 1983), schizophrenia (SZ; Gold and Harvey, 1993), fragile X syndrome (FXS; Smith, 1993), Huntington's disease (HD; Bourne et al., 2006), hypoxic-ischemic brain injury (Anderson and Arciniegas, 2010), and alcohol use disorders (Bates et al., 2002).

Striatal-enriched protein tyrosine phosphatase (STEP) has emerged as a critical enzyme in the signaling pathways that appear to contribute to the cognitive impairments in each of these neuropsychiatric disorders. STEP is a brain-specific phosphatase that is highly expressed within the striatum (Lombroso et al., 1991) as well as the cortex, hippocampus, and amygdala (Lombroso et al., 1993; Boulanger et al., 1995). After transcription of the *Ptpn5* gene, alternative splicing produces mRNAs that encode four isoforms (STEP₂₀, STEP₃₈, STEP₄₆, and STEP₆₁); however, only STEP₄₆ and STEP₆₁ contain an active phosphatase domain (Sharma et al., 1995; Bult et al., 1996, 1997; Figure 1). STEP₄₆ is expressed primarily in the cytoplasm whereas STEP₆₁ is targeted to the postsynaptic density, extrasynaptic sites, and the endoplasmic reticulum (Lombroso et al., 1993; Boulanger et al., 1995; Goebel-Goody et al., 2009).

STEP₄₆ and STEP₆₁ activity are regulated by the phosphorylation of a serine (Ser) residue (Ser⁴⁹ and Ser²²¹, respectively) within a kinase-interacting motif (KIM), a binding site for all STEP substrates. Phosphorylation at this site sterically prevents STEP from associating with all of its substrates. Stimulation of glutamate

N-methyl-D-aspartate receptors (NMDARs) dephosphorylates and activates STEP through a calcineurin/PP1 pathway (Paul et al., 2003; Valjent et al., 2005). When activated, STEP dephosphorylates tyrosine (Tyr) residues on its substrates, causing their inactivation, or in the case of glutamate receptors, promoting their internalization from synaptosomal surface membranes (Figure 2; only NMDAR internalization shown for clarity).

Substrates of STEP include the GluN2B (previously called NR2B) subunit of the NMDAR (Braithwaite et al., 2006), the GluA2 (previously called GluR2) subunit of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA; Zhang et al., 2008), as well as the kinases Fyn (Nguyen et al., 2002) and Pyk2 (Xu et al., 2010), and the mitogen-activated protein kinases (MAPKs) extracellular-regulated kinase 1/2 (ERK1/2) and p38 (Munoz et al., 2003; Paul et al., 2003; Chen et al., 2009). Stimulation of dopamine D1 receptors (D1Rs), or blockade of dopamine D2 receptors (D2Rs), activates cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA; Stoof and Keibabian, 1981), which phosphorylates and prevents STEP₄₆ and STEP₆₁ from interacting with their substrates (Paul et al., 2000). D1R stimulation can also activate a cAMP-independent cascade in which Fyn phosphorylates GluN2B, potentiates NMDAR activity, and activates ERK (Pascoli et al., 2011). These actions complement the actions of cAMP-dependent STEP inactivation, because inactivation of STEP activates Fyn and ERK, and leads to NMDAR internalization.

Synaptic plasticity refers to a change in synaptic efficacy between neurons, which is fundamental to learning, memory, and cognition (Silva, 2003). This dynamic process requires synaptic efficacy to strengthen (long-term potentiation; LTP) or weaken (long-term depression; LTD) in response to developmental and environmental signals. Long-term potentiation is the most widely supported molecular model of memory (Bliss and Collingridge,

1993). It has recently become clear that LTP and LTD are not discrete phenomena, but rather complimentary processes with complex modulation that allow experiences to continuously reshape neural function (Malenka and Bear, 2004). As is discussed further below, STEP is currently believed to modulate both LTP and LTD through its ability to regulate internalization of NMDARs and AMPARs.

Glutamatergic transmission is essential for both LTP and LTD. While LTP is facilitated by the coordinated actions of NMDARs and AMPARs, metabotropic glutamate receptor (mGluR) activity

is important to the development of LTD. Moreover, glutamatergic signaling is often modulated by dopaminergic (Centonze et al., 2001; Calabresi et al., 2007), β -adrenergic (Vanhoose and Winder, 2003), and nicotinic cholinergic stimulation (Dajas-Bailador and Wonnacott, 2004), as well as by other neuromodulators (Jin et al., 2005; Mony et al., 2009).

Striatal-enriched protein tyrosine phosphatase has recently been implicated in the pathophysiology of AD, schizophrenia, FXS, HD, hypoxic-ischemic brain injury, and other neuropsychiatric disorders. This important discovery suggests that several

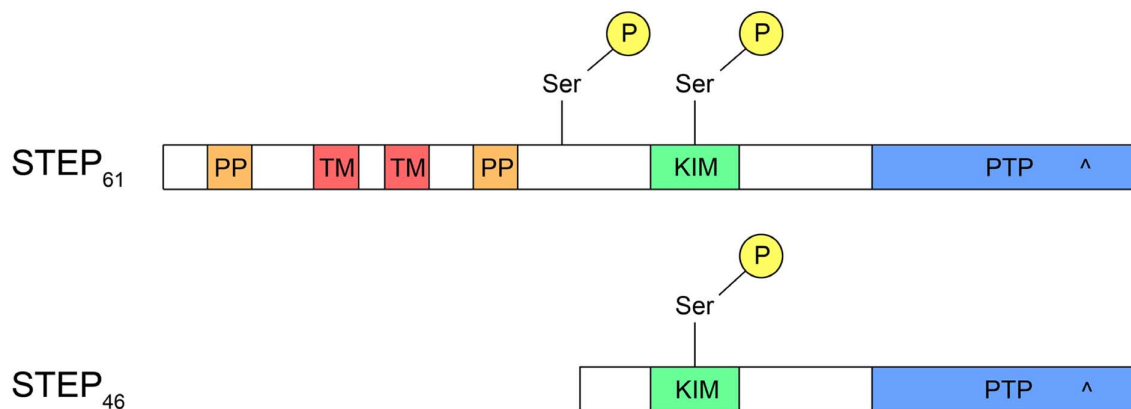


FIGURE 1 | Structure of STEP. Both STEP₄₆ and STEP₆₁ contain a kinase-interacting motif (KIM) domain, necessary for binding to all substrates, and a protein tyrosine phosphatase (PTP) domain with a catalytic site (*). In addition, STEP₆₁ has two polyproline (PP) domains, the first of which interacts with Fyn, and two transmembrane (TM) domains that target STEP₆₁ to the

endoplasmic reticulum or postsynaptic density. Whereas STEP₆₁ has two serine (Ser) phosphorylation sites, STEP₄₆ contains only one within the KIM domain. The additional phosphorylation site on STEP₆₁ may facilitate proteolytic cleavage of STEP₆₁ to produce an inactive STEP₃₃ fragment that is unable to bind substrates due to cleavage within the KIM domain.

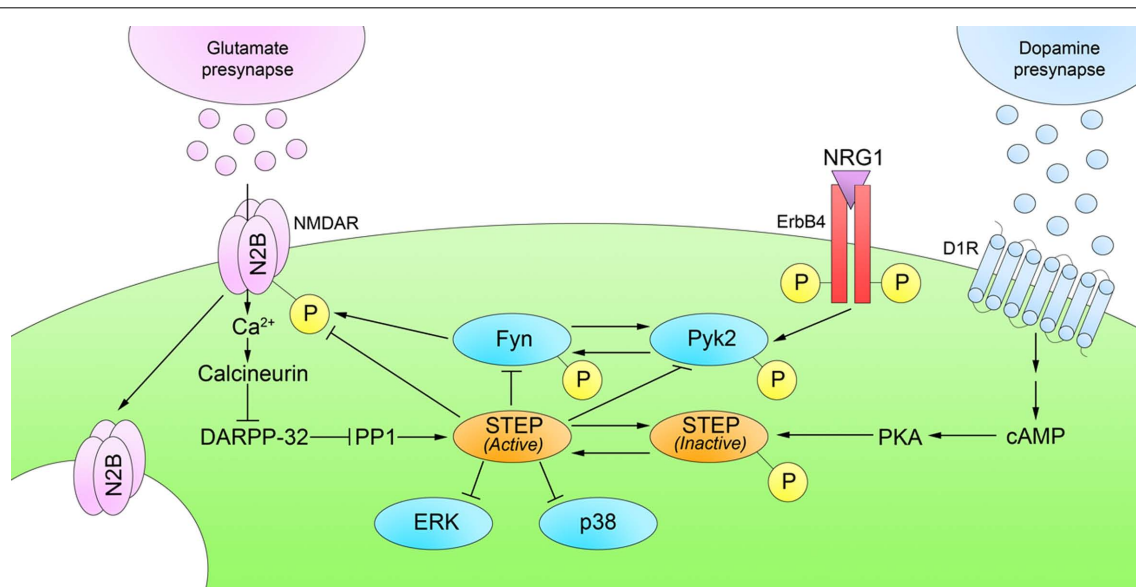


FIGURE 2 | Regulation of STEP phosphorylation. D1R stimulation phosphorylates and inactivates STEP through a cAMP/PKA pathway. Glutamatergic stimulation of NMDARs reverses this process by dephosphorylation and activation of STEP through a calcineurin/PP1 pathway. Active STEP dephosphorylates regulatory tyrosines within STEP substrates.

STEP dephosphorylation of Fyn, Pyk2, ERK1/2, and p38 leads to their inactivation; however, STEP dephosphorylation of GluN2B and GluA2 subunits results in internalization of NMDARs and AMPARs, respectively, from synaptosomal surface membranes. Only NMDARs are shown in the figure for clarity.

cognitive disorders that were previously regarded as distinct illnesses may share a common molecular pathway. Other proteins and STEP-independent mechanisms surely contribute to cognitive impairments, but this review focuses on the role of STEP in the pathogenesis of these neuropsychiatric disorders.

ALZHEIMER'S DISEASE: AMYLOID BETA LEADS TO INCREASED STEP₆₁ ACTIVITY

Alzheimer's disease is a common form of dementia that causes progressive cognitive impairment, most notably in memory and semantic-lexical language (Huff et al., 1987). Cognitive impairments are accompanied by behavioral alterations such as agitation, dysphoria, and apathy (Mega et al., 1996). The German psychiatrist Alois Alzheimer was the first to describe the hallmark pathogenic signs of the disease in brain autopsies: neurofibrillary tangles and plaques. This observation promoted the hypothesis that AD progression is mediated by these neuroanatomical abnormalities (Hardy and Higgins, 1992). In the 1980s, tau was identified as the main constituent of neurofibrillary tangles (Goedert et al., 1989) and amyloid β (A β) proteins as the main constituent of plaques (Tanzi et al., 1987). These findings led to the hypothesis that hyperphosphorylated tau protein (Alonso et al., 1996) and insoluble aggregates of A β peptide formed from mutated amyloid precursor protein (Cai et al., 1993; Suzuki et al., 1994) were a possible mechanism underlying the pathology of AD.

The focus shifted in 1991 from extracellular deposits to a disruption in synaptic function when Terry et al. (1991) showed that cortical synaptic density correlated more strongly with cognitive impairments than plaque density. In support of the synaptic hypothesis of AD, tau has been shown to reduce anterograde

transport even before it aggregates in neurofibrillary tangles (Mandelkowitz et al., 2003). Moreover, soluble A β oligomers impair synaptic function before neuronal loss occurs (Lue et al., 1999). These oligomers directly bind to and chronically activate nicotinic acetylcholine receptors (nAChRs), causing sustained increases in intracellular Ca^{2+} concentrations (Dougherty et al., 2003). In addition, these oligomers reduce NMDAR-dependent synaptic transmission and Ca^{2+} influx, resulting in the loss of synapses in rat hippocampal neurons (Cullen et al., 1996; Shankar et al., 2007). Finally, exogenous application of A β to neuronal cell cultures and tissue slices alters spine architecture and blocks LTP, and *in vivo* infusions impair cognitive function in rodents (Walsh et al., 2002; Lacor et al., 2007; Shankar et al., 2007, 2008).

The underlying mechanisms of A β -induced reductions in synaptic function remain a focus of intense research. Snyder et al. (2005) directly tested whether A β disrupts surface expression of NMDARs. They demonstrated that A β binds $\alpha 7$ nAChRs, leading to Ca^{2+} influx and activation of a calcineurin/PP1 pathway. PP1 dephosphorylates and activates STEP, which inhibits Fyn and promotes internalization of NMDARs and AMPARs from synaptosomal membrane surfaces (Figure 3). Chin et al. (2005) also found increased levels of STEP protein and decreased Fyn activity in the dentate gyrus of a transgenic mouse model of AD (the J20 mouse line).

Recently, Kurup et al. (2010) demonstrated that a second pathway increases STEP₆₁ activity in AD brains. This is due to a disruption in the normal degradation of STEP₆₁ as a result of an A β -mediated inhibition of the proteasome. Because STEP₆₁ is normally ubiquitinated and degraded by the ubiquitin proteasome pathway, inhibition of the proteasome results in substantial

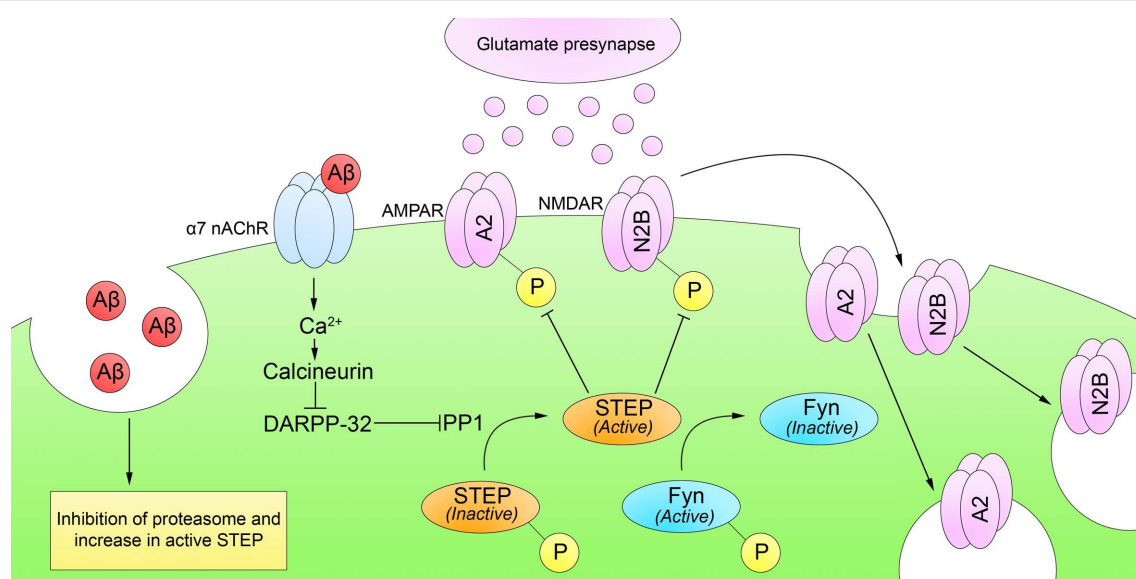


FIGURE 3 | Striatum-enriched protein tyrosine phosphatase and Alzheimer's disease. Cognitive impairments in AD appear before the formation of A β plaques, suggesting that soluble A β may affect synaptic function. Soluble A β increases both STEP levels and phosphorylation. First, soluble A β inhibits STEP degradation by an A β -mediated inhibition of the ubiquitin proteasome pathway. Second, soluble A β directly binds and activates

$\alpha 7$ nAChRs, leading to Ca^{2+} influx and activation of calcineurin. Calcineurin/PP1 then dephosphorylates and activates STEP. Active STEP promotes the dephosphorylation of Fyn, GluA2, and GluN2B (and other substrates not depicted). Dephosphorylation of Fyn inactivates it whereas dephosphorylation of GluN2B and GluA2 leads to NMDAR and AMPAR internalization from synaptosomal surface membranes and disruption of synaptic function.

increases in STEP₆₁ in brain samples from both human AD and mouse models.

If increased STEP₆₁ levels contribute to the pathophysiology of AD, then reducing them might reverse its biochemical and cognitive deficits. Zhang et al. (2010) tested this hypothesis in a mouse model of the disease by genetically decreasing STEP levels and demonstrating restored cognitive function in 6-month-old AD mice null for STEP. Importantly, cognitive impairments improved despite a continued elevation of A β and phosphorylated tau. It remains to be determined whether older AD mice (i.e., 12-months old) with a similar genetic reduction in STEP also have improved cognitive functioning. It should be emphasized that STEP is one of over 400 proteins within neuronal spines, and that STEP-independent pathways certainly contribute to the pathogenesis of AD as well as the other disorders discussed in subsequent sections.

SCHIZOPHRENIA: STEP CONTRIBUTES TO THE BENEFICIAL EFFECTS OF NEUROLEPTICS

Schizophrenia is characterized by cognitive and emotional deterioration, with symptoms typically emerging during young adulthood. Positive symptoms include delusions, disordered thought and speech, and hallucinations. Negative symptoms, which are deficits in emotional and mental processes, include blunted affect, poverty of speech, anhedonia, and asociality. In addition to positive and negative symptoms, cognitive impairments including deficits in executive functioning predate the onset of the above symptoms and persist when these subside.

Schizophrenia was considered untreatable until the introduction of the first neuroleptic, chlorpromazine, in 1952. The mechanism of action of these “typical” antipsychotics is unknown, despite their efficacy in the treatment of SZ. The dopamine hypothesis of SZ emerged from the discovery that typical antipsychotics, such as chlorpromazine, block D2Rs, and their clinical potency is related to the affinity of antipsychotic medications for this receptor (Seeman et al., 1975; Creese et al., 1976).

Although dopamine levels are increased in the striatum (Breier et al., 1997) and decreased in the frontal cortex (Dolan et al., 1995), recent evidence suggests that the pathophysiology of SZ includes deficits in other neurotransmitter systems, in particular glutamatergic hypofunction (Jentsch and Roth, 1999). This is due in part to the fact that the dopamine hypothesis of SZ does not appear to explain the SZ-like symptoms induced by non-competitive NMDAR antagonists, such as phencyclidine and ketamine. Further support for the glutamate hypothesis comes from research in animal models and human subjects. Mice expressing 5% of the normal levels of GluN1 display SZ-like behaviors, which are attenuated by treatment with the antipsychotics haloperidol and clozapine (Mohn et al., 1999). In human patients, Pilowsky et al. (2006) used *in vivo* positron emission tomography to demonstrate reductions in hippocampal NMDAR binding in medication-free but not antipsychotic-treated schizophrenics. Moreover, postmortem investigation of schizophrenic brains reveals that GluN1 mRNA levels are reduced by 30% in the superior temporal cortex of cognitively impaired schizophrenics, a deficit that is correlated with antemortem severity of cognitive impairment (Humphries et al.,

1996). Finally, the administration of glycine and D-serine, potentiators of NMDAR function, improves symptoms in medicated schizophrenics (Heresco-Levy et al., 1999; Kantrowitz et al., 2010).

Recent studies suggest that STEP plays a role in mediating the beneficial effects of neuroleptics. Both typical and atypical antipsychotics regulate cAMP-dependent PKA activity in the striatum and medial prefrontal cortex (Turalba et al., 2004). Recently, Carty et al. (2010) showed that both acute and subchronic administration of haloperidol, clozapine, and risperidone result in a PKA-mediated phosphorylation of STEP (Figure 4). As discussed above, this prevents STEP from interacting with its substrates. In addition, neuroleptic-mediated activation of PKA results in the phosphorylation and activation of DARPP-32. DARPP-32 is an indirect inhibitor of STEP through its inhibition of PP1, which dephosphorylates and inactivates STEP. Consequently, both PKA activation and PP1 inactivation contribute to decreased STEP activity.

DARPP-32 also activates STEP substrates through STEP-independent pathways. For example, ERK is activated by DARPP-32 through the inhibition of PP1, leading to activation of upstream modulators of ERK activity, such as mitogen-activated protein kinase kinase (MEK; Valjent et al., 2005). These results suggest that neuroleptics exert their beneficial effects at least in part through D2R/PKA-mediated phosphorylation and inactivation of STEP, promoting Tyr phosphorylation of STEP substrates that include GluN2B-containing NMDARs, which now traffic to synaptosomal surface membranes.

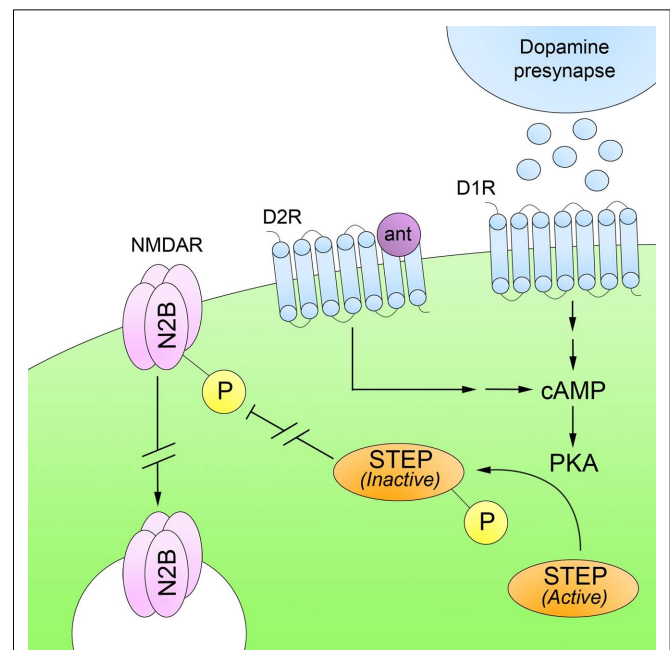


FIGURE 4 | Striatal-enriched protein tyrosine phosphatase and schizophrenia. Deficits in glutamatergic neurotransmission are believed to underlie the pathophysiology of SZ. Administration of typical and atypical antipsychotics, which are D2R antagonists (ant), increase PKA-mediated phosphorylation of STEP. Inactivation of STEP results in increased trafficking of NMDARs to synaptosomal surface membranes. This suggests that the beneficial effects of neuroleptics are mediated, at least in part, through restoration of NMDAR levels at synaptic sites.

FRAGILE X SYNDROME: THE FMRP PROTEIN NEGATIVELY REPRESSES STEP

Fragile X syndrome is an X-linked, monogenic neurological disorder and the leading cause of inherited mental retardation. It is characterized by hyperactivity, auditory hypersensitivity and audiogenic seizures, developmental delays, emotional dysfunction, and cognitive impairment (Visoosak et al., 2005; Koukoui and Chaudhuri, 2007). Common cognitive impairments in FXS include impaired quantitative skills, short-term memory, spatial visualization, and visual-motor coordination (Freund and Reiss, 1991).

Fragile X syndrome results from an expanded 5'-CCG repeat region in the first exon of the *fragile X mental retardation 1* (*Fmr1*) gene (Xq27.3). The increased number of cytosines are methylated and interfere with the transcription of *Fmr1* mRNA once the expansion exceeds 200 repeats (Oberle et al., 1991). The protein encoded by *Fmr1*, fragile X mental retardation protein (FMRP), is a cytosolic RNA-binding protein that associates with polyribosomes and the RNA-silencing complex, functioning as a negative repressor of translation (Laggerbauer et al., 2001; Li et al., 2001; Garber et al., 2006). Because FMRP and polyribosomes are present in dendritic spines, it was suggested that FMRP modulates synaptic activity through its ability to repress local translation of mRNAs (Weiler and Greenough, 1999). Synaptic activity was proposed to remove FMRP inhibitory effects, resulting in local translation of mRNAs (Bear et al., 2004). In this way, RNA-binding proteins, such as FMRP, influence synaptic plasticity by controlling activity-dependent regulation of mRNA localization and translation (Ule and Darnell, 2006).

In support of this hypothesis, FMRP is quickly synthesized and localized to dendrites after stimulation of group I mGluRs (Todd et al., 2003; Antar et al., 2004). Activation of mGluRs stimulates protein synthesis-dependent LTD in dendrites (Huber et al., 2000, 2001) and results in a rapid loss of synaptic NMDARs and AMPARs (Snyder et al., 2001). *Fmr1* KO mice display increased protein synthesis (Qin et al., 2005), enhanced hippocampal LTD (Huber et al., 2002), as well as abnormal synaptic morphology, including immature development of dendritic spines (Irwin et al., 2000), delayed spine turnover and stabilization (Cruz-Martin et al., 2010), increased spine density (Comery et al., 1997), and a decreased number of functional synapses (Pfeiffer and Huber, 2007).

It is believed that FMRP regulates mGluR-dependent LTD by tightly controlling protein synthesis and that decreased FMRP expression elevates synaptic protein levels, which exaggerate mGluR-dependent LTD and disrupt synaptic morphology (Bear et al., 2004; Ronesi and Huber, 2008). In *Fmr1* KO mice, mGluR-dependent LTD no longer requires new protein synthesis, suggesting that elevated levels of synaptic proteins, which would normally be inhibited by FMRP, enable LTD to persist without *de novo* protein synthesis (Nosyreva and Huber, 2006). For example, *Fmr1* KO mice exhibit decreased surface expression of NMDARs (Eadie et al., 2010) and GluA1-containing AMPARs (Li et al., 2002; Suvrathan et al., 2010), which is most likely a consequence of uninhibited mGluR signaling (Nakamoto et al., 2007). Although it is known that *Fmr1* KO exaggerates mGluR-dependent LTD by decreasing synaptic glutamate receptor expression, it is not known how this occurs.

Zhang et al. (2008) demonstrated that STEP translation is increased in the mouse hippocampus upon mGluR5 stimulation, resulting in AMPAR internalization through Tyr dephosphorylation of the GluA2 subunit, although the exact Tyr residue is not yet known (Figure 5). More recently, it was shown that STEP mRNA associates with FMRP and that STEP protein levels are elevated in *Fmr1* KO mice (Goebel-Goody et al., 2010). In addition, progeny were generated by crossing *Fmr1* and STEP KO mice to produce mice with lowered STEP levels despite the continued presence of the *Fmr1* mutation. These mice display fewer audiogenic seizures and less spatial anxiety than *Fmr1* KO mice. These results suggest that some of the deficits in animal models of FXS result from the failure of FMRP to suppress STEP translation upon mGluR stimulation, allowing increased STEP levels to inappropriately internalize NMDARs and AMPARs.

THE ROLE OF STEP IN OTHER NEUROPSYCHIATRIC DISORDERS

HUNTINGTON'S DISEASE

Huntington's disease is an autosomal dominant neurodegenerative disease caused by abnormal expansion of a CAG codon in exon 1 of the *huntingtin* (*HTT*) gene (4p16.3). The expansion results in a mutant HTT (mHTT) protein that contains excessive polyglutamine residues (The Huntington's Disease Collaborative Research Group, 1993). Projection neurons, which are the majority of striatal neurons, are specifically affected in HD (Reiner et al., 1988). HD symptoms such as chorea, a movement disorder, and cognitive impairments have been attributed to progressive excitotoxicity of these projection neurons resulting from NMDAR activation, which destroys striatal innervation of other brain regions (DiFiglia, 1990; Fan and Raymond, 2007). Cognitive impairments common to HD patients are loss of initiative, poor perseverance, impaired judgment, and emotional blunting

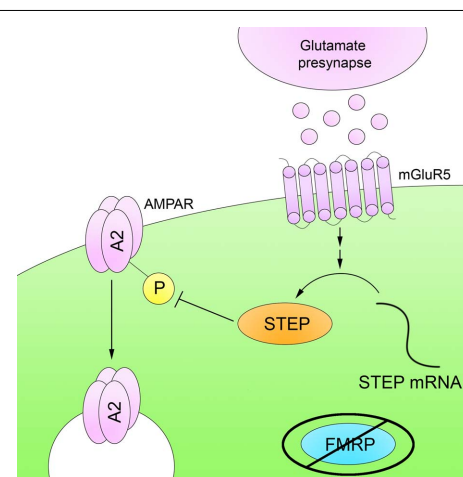


FIGURE 5 | Striatal-enriched protein tyrosine phosphatase and fragile X syndrome. FMRP is an RNA-binding protein that represses mRNA translation in dendrites. FMRP is absent in FXS, and as a result there is a disruption in mRNA translation. STEP mRNA associates with FMRP, and STEP protein levels are increased in *Fmr1* KO mice. The higher levels of active STEP protein are thought to disrupt synaptic function by dephosphorylation of STEP substrates.

(Craufurd et al., 2001). In addition, attention, executive function, language comprehension, and visuospatial immediate memory are impaired as the disease progresses (Bachoud-Levi et al., 2001).

Although mHTT is present at birth in affected individuals, symptoms typically begin in middle age. Transgenic lines of mice expressing mHTT are resistant to striatal toxicity induced by quinolinic acid, an NMDAR agonist, suggesting that compensatory mechanisms in the brain protect against the toxic effect of mHTT (Hansson et al., 1999). Similar mechanisms in humans might explain why HD is a late-onset disorder.

Several lines of evidence suggest that in animal models of HD, mHTT disrupts PKA and calcineurin activity, both important regulators of STEP activity. Dopamine D1-like receptor binding in R6/2 mice is significantly reduced at 8 weeks whereas D1R mRNA is reduced by 4 weeks, well before the onset of symptoms (Cha et al., 1998). Moreover, cAMP synthesis declines in *Hdh*^{Q111} mice at an early age (10 weeks) and results in decreased PKA activity and cAMP-responsive element-mediated gene transcription (Gines et al., 2003). In support of decreased D1R and PKA activity, proteins in the D1R/PKA pathway, such as DARPP-32, are also downregulated in medium spiny neurons of the striatum of transgenic HD mice (Bibb et al., 2000). In addition, calcineurin activity is decreased in R6/1 and Tet/HD94 mice (Xifro et al., 2009), which may be the result of an abnormal protein interaction between mHTT and calmodulin (Bao et al., 1996), which is required to activate calcineurin.

Saavedra et al. (2011) recently demonstrated reduced STEP levels in the striatum and cortex and increased phosphorylated STEP in the striatum, cortex, and hippocampus of several animal models of HD, including R6/1, R6/2, and Tet/HD94. Both of these events would decrease the ability of STEP to dephosphorylate its substrates. Intrastriatal injections of quinolinic acid resulted in elevated phosphorylated STEP levels in R6/1 mice and sustained ERK2 signaling, suggesting that mHTT downregulates STEP, thereby inactivating it and promoting a compensatory activation of ERK2 and resistance to mHTT-induced excitotoxicity. Furthermore, intrastriatal injection of TAT-STEP, a substrate-trapping form of STEP, increases quinolinic acid-induced cell death in R6/1 mice whereas mHTT transgene shutdown reestablishes STEP expression in Tet/HD94 mice. Moreover, mHTT activates ERK1/2, promotes cell survival, and protects against mHTT-induced excitotoxicity in PC12 and striatal cultures (Apostol et al., 2006).

Resistance to NMDA toxicity, however, is correlated with the appearance of nuclear inclusions and behavioral deficits in mice transgenic for exon 1 of *HTT* (Hansson et al., 2001). In the YAC128 mouse model of HD, cognitive dysfunction precedes both neuropathological alterations and motor dysfunction (Van Raamsdonk et al., 2005). Moreover, impaired spatial cognition and synaptic plasticity in CA1 and the dentate gyrus occurs before the manifestation of an overt phenotype in R6/2 mice (Murphy et al., 2000). Therefore, while resistance to excitotoxicity delays neuronal loss in the early stages of HD, it also contributes to the development of synaptic dysfunction that likely contributes to the observed cognitive impairment in HD. In summary, STEP is downregulated in HD and promotes resistance to mHTT-induced excitotoxicity, most likely due in part to STEP inactivation and more sustained ERK2 activation.

ALCOHOL-INDUCED COGNITIVE IMPAIRMENT

Alcohol-induced cognitive impairment is well-documented in alcohol use disorders, and it is believed that inhibition of NMDAR function, especially in the hippocampus, partly contributes to the ability of alcohol to disrupt memory and learning (White et al., 2000). Numerous kinases (PKA, protein kinase C, calcium-/calmodulin-dependent protein kinase II, cyclin-dependent protein kinase 5) and phosphatases (calcineurin) regulate the phosphorylation state of Ser and Tyr residues on NMDARs and contribute to the behavioral effects of alcohol (Ron, 2004). Previously, it was demonstrated that ethanol reduces Tyr phosphorylation of GluN2A/GluN2B subunits of NMDARs (Alvestad et al., 2003) and treatment with tyrosine phosphatase inhibitors reduces the ability of ethanol to inhibit Tyr phosphorylation of GluN2, suggesting that tyrosine phosphatases have an important role in mediating the effects of alcohol on NMDAR function (Ferrani-Kile et al., 2003).

Recently, it was suggested that STEP mediates aspects of the effects of alcohol on neurons. Hicklin et al. (2011) demonstrated that ethanol's inhibition of NMDAR function (as measured by electrophysiology) was lost in STEP KO mice. The functional consequence of ethanol in inhibiting fear-conditioned responses was also attenuated in STEP KO mice. Moreover, restoring STEP in neuronal cultures and slices derived from STEP KO mice restored ethanol-induced biochemical and electrophysiological deficits. These results suggest that STEP may be required for the amnesiac effects of ethanol by dephosphorylating GluN2B subunits.

These experiments were conducted using hippocampal neurons, but acute ethanol administration in the dorsomedial striatum similarly impairs NMDAR-dependent LTP and dose-dependently promotes LTD (Yin et al., 2007). Wang et al. (2007, 2010) demonstrated that chronic administration of ethanol increases GluN2B phosphorylation and Fyn activation in the dorsomedial striatum. These results are unsurprising, however, considering the differential effects of acute and chronic ethanol treatments on NMDAR function. For example, in cortical neurons, chronic ethanol treatment has the opposite effect as acute ethanol treatment, enhancing NMDAR binding (Hu and Ticku, 1995) and potentiating NMDAR-mediated excitotoxicity (Chandler et al., 1993). Future studies should determine whether STEP mediates the effects of ethanol in the striatum as it does in the hippocampus. In addition, chronic ethanol treatments should be utilized to determine the potential role of STEP in the development of NMDAR upregulation and sensitization.

HYPOXIC-ISCHEMIC BRAIN INJURY

Hypoxia is the reduction of blood oxygenation to the brain whereas ischemia is the reduction of blood perfusion to the brain. Hypoxic-ischemic brain injury caused by perinatal complications during childbirth or stroke is a leading cause of mental retardation and cognitive impairment. In addition, hypoxic-ischemic brain injury caused by stroke increases the risk for dementia; it is estimated that up to a third of stroke patients develop dementia within 3 months (Pohjasvaara et al., 1998). Hypoxia-ischemia results in neuronal loss from glutamate excitotoxicity and increased intracellular Ca^{2+} levels (Benveniste, 1991). Excessive stimulation of NMDARs leads to a massive and rapid influx of Ca^{2+} , which

generates nitric oxide and free radicals (Iadecola, 1997). In addition, intracellular changes in NMDAR subunit phosphorylation as well as MAPK activation following ischemia further exacerbate glutamate excitotoxicity and neuronal loss (Nozaki et al., 2001; Matsumoto et al., 2002). Hippocampal regions, such as CA1, are especially vulnerable to glutamate excitotoxicity (Kirino and Sano, 1984; Nitatori et al., 1995). Damage to the hippocampus is commonly observed in both vascular and senile dementia, and it is associated with cognitive impairment (Ball et al., 1985; Kril et al., 2002).

Gurd et al. (1999) were the first to report that cerebral hypoxia–ischemia results in the calpain-mediated proteolysis of STEP₆₁ to STEP₃₃, which is released into the cytoplasm. Soon after, Nguyen et al. (1999) demonstrated that STEP₆₁ cleavage requires NMDAR-mediated Ca²⁺ influx. The cleavage site is in the middle of the substrate-binding KIM domain (Xu et al., 2009), and it was later determined that although STEP₃₃ is catalytically active, it is unable to bind or dephosphorylate its substrates (Braithwaite et al., 2008; Xu et al., 2009). Extrasynaptic NMDAR stimulation results in the cleavage of STEP₆₁ by calpain and the activation of p38 and cell death (Xu et al., 2009). Thus, cleavage of STEP₆₁ to STEP₃₃ contributes to glutamate excitotoxicity by inhibiting NMDAR internalization from synaptosomal surface membranes and promoting cell death pathways.

FUTURE DIRECTIONS

Homeostasis, a cornerstone of biological organisms, regulates physiological states to promote stability despite fluctuating conditions. Neural activity is subject to homeostatic control, optimizing activity-dependent synaptic plasticity to avoid over- or under-excitation (Turrigiano and Nelson, 2004). STEP may contribute to this homeostatic control of synaptic plasticity by providing a tonic brake on the induction of LTP (Pelkey et al., 2002). In other words, NMDAR-mediated STEP activation modulates LTP by removing glutamate receptors from synaptic membranes. Conversely, STEP may be inactivated during periods of synaptic quiescence, resulting in increased surface expression of glutamate receptors. Finally, note that in the absence of STEP, glutamate receptors would rapidly accumulate on synaptic membranes and neurons would no longer be responsive to synaptic or modulatory inputs.

Striatal-enriched protein tyrosine phosphatase is uniquely positioned to modulate LTP by internalizing NMDARs and AMPARs from the plasma membrane and curtailing the enhancement of LTP by the MAPK pathway. Because the removal of NMDARs and AMPARs is believed to be one mechanism involved in LTD, STEP can balance the degree to which synapses strengthen and weaken during learning and memory, maintaining neural homeostasis (Xiao et al., 1994). The involvement of STEP in homeoplastic synaptic regulation requires further investigation.

Elevated STEP levels and excessive internalization of NMDARs or AMPARs in AD, SZ, and FXS would result in a homeostatic imbalance, shifting synaptic plasticity toward LTD and synaptic weakening. As a result, systems critical for learning, memory, and cognition, such as those involving the hippocampus, would settle into a quiescent state, resulting in cognitive impairment. Conversely, the absence of STEP might facilitate learning. Indeed, Venkitaramani et al. (2011) demonstrated that STEP KO mice display enhanced hippocampal-dependent learning and cognitive

performance. This effect parallels increased phosphorylation of STEP substrates (ERK1/2, GluN2B, Pyk2) and increased synaptosomal expression of GluN1/GluN2B-containing NMDARs and GluA1/GluA2-containing AMPARs.

Striatal-enriched protein tyrosine phosphatase levels are decreased in HD, yet HD also results in cognitive impairment. Before the onset of neuropathological abnormalities, mHTT impairs axonal transport and vesicular trafficking (Trushina et al., 2004) and represses nuclear transcription (Kegel et al., 2002). Inadequate neurotransmitter release or protein synthesis in the synapse could result in dysfunctional synaptic plasticity. For example, mHTT results in decreased glutamate neurotransmission (Usdin et al., 1999) and decreased transcription of brain-derived neurotrophic factor (Zuccato et al., 2001), a crucial modulator of synaptic plasticity (Bramham and Messaoudi, 2005). Although STEP is initially neuroprotective in preventing immediate, mHTT-induced excitotoxicity, it is unable to prevent the mHTT-induced synaptic dysfunction and eventual neurite dystrophy caused by aberrant axonal transport and nuclear transcription.

On the other hand, cognitive impairments observed after hypoxic–ischemic brain injury are caused by excitotoxicity and synaptic destruction rather than altered synaptic function. In rodents, neurotoxic lesions of the hippocampus with NMDA result in cognitive deficits, especially in spatial learning and memory (Hicks et al., 1993; Deacon et al., 2002). As previously mentioned, hypoxia–ischemia results in excitotoxicity through NMDAR-mediated cleavage of STEP₆₁ into STEP₃₃, which is no longer able to dephosphorylate and internalize NMDARs on the plasma membrane. Rather than decreasing STEP levels or activity, hypoxic–ischemic brain injury could be attenuated by elevating STEP levels (Braithwaite et al., 2008) or blocking the calpain cleavage site (Xu et al., 2009), thereby enhancing the ability of STEP to dephosphorylate p38 and protect neurons against excitotoxicity.

In conclusion, STEP contributes to cognitive deficits in several disorders by regulating the activity of key signaling proteins and the trafficking NMDARs and AMPARs to synaptic membranes. Although the mechanisms of impaired synaptic plasticity vary, alterations in NMDAR and AMPAR trafficking contribute to the disruption of synaptic function in each disorder. In AD, SZ, and FXS, elevated STEP activity causes excessive glutamate receptor internalization from synaptosomal surface membranes and decreased synaptic plasticity. In HD, STEP downregulation is initially neuroprotective to mHTT-induced glutamate excitotoxicity, but at the cost of disrupting synaptic plasticity and causing cognitive impairment. In hypoxic–ischemic brain injury, STEP cleavage and the loss of substrate-binding facilitates NMDAR-mediated glutamate excitotoxicity, resulting in neuronal loss, especially in the hippocampus. Although specific inhibitors are being developed, such as glutamate antagonists for schizophrenia and calpain inhibitors for ischemia–hypoxia, STEP may prove to be a novel therapeutic target for multiple disorders that impair cognitive function.

ACKNOWLEDGMENTS

We thank laboratory members for helpful discussions and Dr. Marilee Ogren for critical reading of the manuscript. The work was funded by NIH grants MH091037 and MH052711 (Paul J. Lombroso).

REFERENCES

- Alonso, A. C., Grundke-Iqbal, I., and Iqbal, K. (1996). Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat. Med.* 2, 783–787.
- Alvestad, R. M., Grosshans, D. R., Coultrap, S. J., Nakazawa, T., Yamamoto, T., and Browning, M. D. (2003). Tyrosine dephosphorylation and ethanol inhibition of N-methyl-D-aspartate receptor function. *J. Biol. Chem.* 278, 11020–11025.
- Anderson, C. A., and Arciniegas, D. B. (2010). Cognitive sequelae of hypoxic-ischemic brain injury: a review. *NeuroRehabilitation* 26, 47–63.
- Antar, L. N., Afroz, R., Dichtenberg, J. B., Carroll, R. C., and Bassell, G. J. (2004). Metabotropic glutamate receptor activation regulates fragile x mental retardation protein and FMR1 mRNA localization differentially in dendrites and at synapses. *J. Neurosci.* 24, 2648–2655.
- Apostol, B. L., Illes, K., Pallos, J., Bodai, L., Wu, J., Strand, A., Schweitzer, E. S., Olson, J. M., Kazantsev, A., Marsh, J. L., and Thompson, L. M. (2006). Mutant huntingtin alters MAPK signaling pathways in PC12 and striatal cells: ERK1/2 protects against mutant huntingtin-associated toxicity. *Hum. Mol. Genet.* 15, 273–285.
- Bachoud-Levi, A. C., Maison, P., Bartolomeo, P., Boisse, M. F., Dalla Barba, G., Ergis, A. M., Baudic, S., Degos, J. D., Cesaro, P., and Peschanski, M. (2001). Retest effects and cognitive decline in longitudinal follow-up of patients with early HD. *Neurology* 56, 1052–1058.
- Ball, M. J., Fisman, M., Hachinski, V., Blume, W., Fox, A., Kral, V. A., Kirshen, A. J., Fox, H., and Merskey, H. (1985). A new definition of Alzheimer's disease: a hippocampal dementia. *Lancet* 1, 14–16.
- Bao, J., Sharp, A. H., Wagster, M. V., Becher, M., Schilling, G., Ross, C. A., Dawson, V. L., and Dawson, T. M. (1996). Expansion of polyglutamine repeat in huntingtin leads to abnormal protein interactions involving calmodulin. *Proc. Natl. Acad. Sci. U.S.A.* 93, 5037–5042.
- Bates, M. E., Bowden, S. C., and Barry, D. (2002). Neurocognitive impairment associated with alcohol use disorders: implications for treatment. *Exp. Clin. Psychopharmacol.* 10, 193–212.
- Bear, M. F., Huber, K. M., and Warren, S. T. (2004). The mGluR theory of fragile X mental retardation. *Trends Neurosci.* 27, 370–377.
- Benveniste, H. (1991). The excitotoxin hypothesis in relation to cerebral ischemia. *Cerebrovasc. Brain Metab. Rev.* 3, 213–245.
- Bibb, J. A., Yan, Z., Svenningsson, P., Snyder, G. L., Pieribone, V. A., Horiuchi, A., Nairn, A. C., Messer, A., and Greengard, P. (2000). Severe deficiencies in dopamine signaling in presymptomatic Huntington's disease mice. *Proc. Natl. Acad. Sci. U.S.A.* 97, 6809–6814.
- Bliss, T. V., and Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361, 31–39.
- Boulanger, L. M., Lombroso, P. J., Raghunathan, A., During, M. J., Wahle, P., and Naegele, J. R. (1995). Cellular and molecular characterization of a brain-enriched protein tyrosine phosphatase. *J. Neurosci.* 15, 1532–1544.
- Bourne, C., Clayton, C., Murch, A., and Grant, J. (2006). Cognitive impairment and behavioural difficulties in patients with Huntington's disease. *Nurs. Stand.* 20, 41–44.
- Braithwaite, S. P., Adkisson, M., Leung, J., Nava, A., Masterson, B., Urfer, R., Oksenberg, D., and Nikolich, K. (2006). Regulation of NMDA receptor trafficking and function by striatal-enriched tyrosine phosphatase (STEP). *Eur. J. Neurosci.* 23, 2847–2856.
- Braithwaite, S. P., Xu, J., Leung, J., Urfer, R., Nikolich, K., Oksenberg, D., Lombroso, P. J., and Shamloo, M. (2008). Expression and function of striatal enriched protein tyrosine phosphatase is profoundly altered in cerebral ischemia. *Eur. J. Neurosci.* 27, 2444–2452.
- Bramham, C. R., and Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Prog. Neurobiol.* 76, 99–125.
- Breier, A., Su, T. P., Saunders, R., Carson, R. E., Kolachana, B. S., de Bartolomeis, A., Weinberger, D. R., Weisenfeld, N., Malhotra, A. K., Eckelman, W. C., and Pickar, D. (1997). Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proc. Natl. Acad. Sci. U.S.A.* 94, 2569–2574.
- Bult, A., Zhao, F., Dirks, R. Jr., Raghunathan, A., Solimena, M., and Lombroso, P. J. (1997). STEP: a family of brain-enriched PTPs. Alternative splicing produces transmembrane, cytosolic and truncated isoforms. *Eur. J. Cell Biol.* 72, 337–344.
- Bult, A., Zhao, F., Dirks, R. Jr., Sharma, E., Lukacs, E., Solimena, M., Naegele, J. R., and Lombroso, P. J. (1996). STEP61: a member of a family of brain-enriched PTPs is localized to the endoplasmic reticulum. *J. Neurosci.* 16, 7821–7831.
- Cai, X. D., Golde, T. E., and Younkin, S. G. (1993). Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. *Science* 259, 514–516.
- Calabresi, P., Picconi, B., Tozzi, A., and Di Filippo, M. (2007). Dopamine-mediated regulation of corticostriatal synaptic plasticity. *Trends Neurosci.* 30, 211–219.
- Carty, N., Kurup, P., Xu, J., Austin, D. R., Chen, G., and Lombroso, P. J. (2010). *Striatal Enriched Protein Tyrosine Phosphatase (STEP) Mediates the Beneficial Effects of Antipsychotic Drugs*. Program No. 452.21. 2010 Neuroscience Meeting Planner. Society for Neuroscience. San Diego, CA. [Online].
- Centonze, D., Picconi, B., Gubellini, P., Bernardi, G., and Calabresi, P. (2001). Dopaminergic control of synaptic plasticity in the dorsal striatum. *Eur. J. Neurosci.* 13, 1071–1077.
- Cha, J. H., Kosinski, C. M., Kerner, J. A., Alsdorf, S. A., Mangiarini, L., Davies, S. W., Penney, J. B., Bates, G. P., and Young, A. B. (1998). Altered brain neurotransmitter receptors in transgenic mice expressing a portion of an abnormal human huntington disease gene. *Proc. Natl. Acad. Sci. U.S.A.* 95, 6480–6485.
- Chandler, L. J., Newsom, H., Sumners, C., and Crews, F. (1993). Chronic ethanol exposure potentiates NMDA excitotoxicity in cerebral cortical neurons. *J. Neurochem.* 60, 1578–1581.
- Chen, J., Rusnak, M., Lombroso, P. J., and Sidhu, A. (2009). Dopamine promotes striatal neuronal apoptotic death via ERK signaling cascades. *Eur. J. Neurosci.* 29, 287–306.
- Chin, J., Palop, J. J., Puolivali, J., Massaro, C., Bien-Ly, N., Gerstein, H., Scarce-Levie, K., Masliah, E., and Mucke, L. (2005). Fyn kinase induces synaptic and cognitive impairments in a transgenic mouse model of Alzheimer's disease. *J. Neurosci.* 25, 9694–9703.
- Comery, T. A., Harris, J. B., Willems, P. J., Oostra, B. A., Irwin, S. A., Weiler, I. J., and Greenough, W. T. (1997). Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proc. Natl. Acad. Sci. U.S.A.* 94, 5401–5404.
- Craufurd, D., Thompson, J. C., and Snowden, J. S. (2001). Behavioral changes in Huntington disease. *Neuropsychiatry Neuropsychol. Behav. Neurol.* 14, 219–226.
- Creese, I., Burt, D. R., and Snyder, S. H. (1976). Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192, 481–483.
- Cruz-Martin, A., Crespo, M., and Portera-Cailliau, C. (2010). Delayed stabilization of dendritic spines in fragile X mice. *J. Neurosci.* 30, 7793–7803.
- Cullen, W. K., Wu, J., Anwyl, R., and Rowan, M. J. (1996). Beta-amyloid produces a delayed NMDA receptor-dependent reduction in synaptic transmission in rat hippocampus. *Neuroreport* 8, 87–92.
- Dajas-Bailador, F., and Wonnacott, S. (2004). Nicotinic acetylcholine receptors and the regulation of neuronal signalling. *Trends Pharmacol. Sci.* 25, 317–324.
- Deacon, R. M., Bannerman, D. M., Kirby, B. P., Croucher, A., and Rawlins, J. N. (2002). Effects of cytotoxic hippocampal lesions in mice on a cognitive test battery. *Behav. Brain Res.* 133, 57–68.
- DiFiglia, M. (1990). Excitotoxic injury of the neostriatum: a model for Huntington's disease. *Trends Neurosci.* 13, 286–289.
- Dolan, R. J., Fletcher, P., Frith, C. D., Friston, K. J., Frackowiak, R. S., and Grasby, P. M. (1995). Dopaminergic modulation of impaired cognitive activation in the anterior cingulate cortex in schizophrenia. *Nature* 378, 180–182.
- Dougherty, J. J., Wu, J., and Nichols, R. A. (2003). Beta-amyloid regulation of presynaptic nicotinic receptors in rat hippocampus and neocortex. *J. Neurosci.* 23, 6740–6747.
- Eadie, B. D., Cushman, J., Kannangara, T. S., Fanselow, M. S., and Christie, B. R. (2010). NMDA receptor hypofunction in the dentate gyrus and impaired context discrimination in adult Fmr1 knockout mice. *Hippocampus*. doi: 10.1002/hipo.20890. [Epub ahead of print].
- Fan, M. M., and Raymond, L. A. (2007). N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Prog. Neurobiol.* 81, 272–293.
- Ferrari-Kile, K., Randall, P. K., and Leslie, S. W. (2003). Acute ethanol affects phosphorylation state of the NMDA receptor complex: implication of tyrosine phosphatases and protein kinase A. *Brain Res. Mol. Brain Res.* 115, 78–86.

- Folstein, M. F., and Whitehouse, P. J. (1983). Cognitive impairment of Alzheimer disease. *Neurobehav. Toxicol. Teratol.* 5, 631–634.
- Freund, L. S., and Reiss, A. L. (1991). Cognitive profiles associated with the fra(X) syndrome in males and females. *Am. J. Med. Genet.* 38, 542–547.
- Garber, K., Smith, K. T., Reines, D., and Warren, S. T. (2006). Transcription, translation and fragile X syndrome. *Curr. Opin. Genet. Dev.* 16, 270–275.
- Gines, S., Seong, I. S., Fossale, E., Ivanova, E., Trettel, F., Gusella, J. F., Wheeler, V. C., Persichetti, F., and MacDonald, M. E. (2003). Specific progressive cAMP reduction implicates energy deficit in presymptomatic Huntington's disease knock-in mice. *Hum. Mol. Genet.* 12, 497–508.
- Goebel-Goody, S. M., Davies, K. D., Alvestad Linger, R. M., Freund, R. K., and Browning, M. D. (2009). Phospho-regulation of synaptic and extrasynaptic N-methyl-D-aspartate receptors in adult hippocampal slices. *Neuroscience* 158, 1446–1459.
- Goebel-Goody, S. M., Wallis, E. D., Li, L., Hall, D. V., Royston, S., Baum, M., Naegele, J. R., and Lombroso, P. J. (2010). *Loss of Striatal-Enriched Protein Tyrosine Phosphatase (STEP) Reverses Deficits in a Fragile X Syndrome Mouse Model*. Program No. 452.4. 2010 Neuroscience Meeting Planner. Society for Neuroscience. San Diego, CA. [Online].
- Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D., and Crowther, R. A. (1989). Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron* 3, 519–526.
- Gold, J. M., and Harvey, P. D. (1993). Cognitive deficits in schizophrenia. *Psychiatr. Clin. North Am.* 16, 295–312.
- Gurd, J. W., Bissoon, N., Nguyen, T. H., Lombroso, P. J., Rider, C. C., Beesley, P. W., and Vannucci, S. J. (1999). Hypoxia-ischemia in perinatal rat brain induces the formation of a low molecular weight isoform of striatal enriched tyrosine phosphatase (STEP). *J. Neurochem.* 73, 1990–1994.
- Hansson, O., Guatteo, E., Mercuri, N. B., Bernardi, G., Li, X. J., Castilho, R. F., and Brundin, P. (2001). Resistance to NMDA toxicity correlates with appearance of nuclear inclusions, behavioural deficits and changes in calcium homeostasis in mice transgenic for exon 1 of the huntington gene. *Eur. J. Neurosci.* 14, 1492–1504.
- Hansson, O., Petersen, A., Leist, M., Nicotera, P., Castilho, R. F., and Brundin, P. (1999). Transgenic mice expressing a Huntington's disease mutation are resistant to quinolinic acid-induced striatal excitotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* 96, 8727–8732.
- Hardy, J. A., and Higgins, G. A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184–185.
- Heresco-Levy, U., Javitt, D. C., Ermilov, M., Mordel, C., Silipo, G., and Lichtenstein, M. (1999). Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. *Arch. Gen. Psychiatry* 56, 29–36.
- Heyes, C., and Huber, L. (2000). *The Evolution Of Cognition (Vienna series in theoretical biology)*. Cambridge: MIT Press.
- Hicklin, T. R., Wu, P. H., Radcliffe, R. A., Freund, R. K., Goebel-Goody, S. M., Correa, P. R., Proctor, W. R., Lombroso, P. J., and Browning, M. D. (2011). Alcohol inhibition of the NMDA receptor function, long-term potentiation, and fear learning requires striatal-enriched protein tyrosine phosphatase. *Proc. Natl. Acad. Sci. U.S.A.* 108, 6650–6655.
- Hicks, R. R., Smith, D. H., Lowenstein, D. H., Saint Marie, R., and McIntosh, T. K. (1993). Mild experimental brain injury in the rat induces cognitive deficits associated with regional neuronal loss in the hippocampus. *J. Neurotrauma* 10, 405–414.
- Hu, X. J., and Ticku, M. K. (1995). Chronic ethanol treatment upregulates the NMDA receptor function and binding in mammalian cortical neurons. *Brain Res. Mol. Brain Res.* 30, 347–356.
- Huber, K. M., Gallagher, S. M., Warren, S. T., and Bear, M. F. (2002). Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc. Natl. Acad. Sci. U.S.A.* 99, 7746–7750.
- Huber, K. M., Kayser, M. S., and Bear, M. F. (2000). Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* 288, 1254–1257.
- Huber, K. M., Roder, J. C., and Bear, M. F. (2001). Chemical induction of mGluR5- and protein synthesis-dependent long-term depression in hippocampal area CA1. *J. Neurophysiol.* 86, 321–325.
- Huff, F. J., Becker, J. T., Belle, S. H., Nebes, R. D., Holland, A. L., and Boller, F. (1987). Cognitive deficits and clinical diagnosis of Alzheimer's disease. *Neurology* 37, 1119–1124.
- Humphries, C., Mortimer, A., Hirsch, S., and de Bellerche, J. (1996). NMDA receptor mRNA correlation with antemortem cognitive impairment in schizophrenia. *Neuroreport* 7, 2051–2055.
- Iadecola, C. (1997). Bright and dark sides of nitric oxide in ischemic brain injury. *Trends Neurosci.* 20, 132–139.
- Irwin, S. A., Galvez, R., and Greenough, W. T. (2000). Dendritic spine structural anomalies in fragile-X mental retardation syndrome. *Cereb. Cortex* 10, 1038–1044.
- Jentsch, J. D., and Roth, R. H. (1999). The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* 20, 201–225.
- Jin, R., Clark, S., Weeks, A. M., Dudman, J. T., Gouaux, E., and Partin, K. M. (2005). Mechanism of positive allosteric modulators acting on AMPA receptors. *J. Neurosci.* 25, 9027–9036.
- Kantrowitz, J. T., Malhotra, A. K., Cornblatt, B., Silipo, G., Balla, A., Suckow, R. F., D'Souza, C., Saksa, J., Woods, S. W., and Javitt, D. C. (2010). High dose D-serine in the treatment of schizophrenia. *Schizophr. Res.* 121, 125–130.
- Kegel, K. B., Meloni, A. R., Yi, Y., Kim, Y. J., Doyle, E., Cuiffo, B. G., Sapp, E., Wang, Y., Qin, Z. H., Chen, J. D., Nevins, J. R., Aronin, N., and DiFiglia, M. (2002). Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. *J. Biol. Chem.* 277, 7466–7476.
- Kirino, T., and Sano, K. (1984). Selective vulnerability in the gerbil hippocampus following transient ischemia. *Acta Neuropathol.* 62, 201–208.
- Koukoui, S. D., and Chaudhuri, A. (2007). Neuroanatomical, molecular genetic, and behavioral correlates of fragile X syndrome. *Brain Res. Rev.* 53, 27–38.
- Kril, J. J., Patel, S., Harding, A. J., and Halliday, G. M. (2002). Patients with vascular dementia due to microvascular pathology have significant hippocampal neuronal loss. *J. Neurol. Neurosurg. Psychiatr.* 72, 747–751.
- Kurup, P., Zhang, Y., Xu, J., Venkataramani, D. V., Haroutunian, V., Greengard, P., Nairn, A. C., and Lombroso, P. J. (2010). Abeta-mediated NMDA receptor endocytosis in Alzheimer's disease involves ubiquitination of the tyrosine phosphatase STEP61. *J. Neurosci.* 30, 5948–5957.
- Lacor, P. N., Buniel, M. C., Furlow, P. W., Clemente, A. S., Velasco, P. T., Wood, M., Viola, K. L., and Klein, W. L. (2007). Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J. Neurosci.* 27, 796–807.
- Laggerbauer, B., Ostareck, D., Keidel, E. M., Ostareck-Lederer, A., and Fischer, U. (2001). Evidence that fragile X mental retardation protein is a negative regulator of translation. *Hum. Mol. Genet.* 10, 329–338.
- Li, J., Pelletier, M. R., Perez Velazquez, J. L., and Carlen, P. L. (2002). Reduced cortical synaptic plasticity and GluR1 expression associated with fragile X mental retardation protein deficiency. *Mol. Cell. Neurosci.* 19, 138–151.
- Li, Z., Zhang, Y., Ku, L., Wilkinson, K. D., Warren, S. T., and Feng, Y. (2001). The fragile X mental retardation protein inhibits translation via interacting with mRNA. *Nucleic Acids Res.* 29, 2276–2283.
- Lombroso, P. J., Murdoch, G., and Lerner, M. (1991). Molecular characterization of a protein-tyrosine-phosphatase enriched in striatum. *Proc. Natl. Acad. Sci. U.S.A.* 88, 7242–7246.
- Lombroso, P. J., Naegele, J. R., Sharma, E., and Lerner, M. (1993). A protein tyrosine phosphatase expressed within dopaminergic neurons of the basal ganglia and related structures. *J. Neurosci.* 13, 3064–3074.
- Lue, L. F., Kuo, Y. M., Roher, A. E., Brachova, L., Shen, Y., Sue, L., Beach, T., Kurth, J. H., Rydel, R. E., and Rogers, J. (1999). Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am. J. Pathol.* 155, 853–862.
- Malenka, R. C., and Bear, M. F. (2004). LTP and LTD: an embarrassment of riches. *Neuron* 44, 5–21.
- Mandelkow, E. M., Stamer, K., Vogel, R., Thies, E., and Mandelkow, E. (2003). Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. *Neurobiol. Aging* 24, 1079–1085.
- Matsumoto, S., Shamloo, M., Isshiki, A., and Wieloch, T. (2002). Persistent phosphorylation of synaptic proteins following middle cerebral artery occlusion. *J. Cereb. Blood Flow Metab.* 22, 1107–1113.

- Mega, M. S., Cummings, J. L., Fiorello, T., and Gornbein, J. (1996). The spectrum of behavioral changes in Alzheimer's disease. *Neurology* 46, 130–135.
- Mohn, A. R., Gainetdinov, R. R., Caron, M. G., and Koller, B. H. (1999). Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell* 98, 427–436.
- Mony, L., Kew, J. N., Gunthrope, M. J., and Paoletti, P. (2009). Allosteric modulators of NR2B-containing NMDA receptors: molecular mechanisms and therapeutic potential. *Br. J. Pharmacol.* 157, 1301–1317.
- Munoz, J. J., Tarrega, C., Blanco-Aparicio, C., and Pulido, R. (2003). Differential interaction of the tyrosine phosphatases PTP-SL, STEP and HePTP with the mitogen-activated protein kinases ERK1/2 and p38alpha is determined by a kinase specificity sequence and influenced by reducing agents. *Biochem. J.* 372, 193–201.
- Murphy, K. P., Carter, R. J., Lione, L. A., Mangiarini, L., Mahal, A., Bates, G. P., Dunnett, S. B., and Morton, A. J. (2000). Abnormal synaptic plasticity and impaired spatial cognition in mice transgenic for exon 1 of the human Huntington's disease mutation. *J. Neurosci.* 20, 5115–5123.
- Nakamoto, M., Nalavadi, V., Epstein, M. P., Narayanan, U., Bassell, G. J., and Warren, S. T. (2007). Fragile X mental retardation protein deficiency leads to excessive mGluR5-dependent internalization of AMPA receptors. *Proc. Natl. Acad. Sci. U.S.A.* 104, 15537–15542.
- Nguyen, T. H., Liu, J., and Lombroso, P. J. (2002). Striatal enriched phosphatase 61 dephosphorylates Fyn at phosphotyrosine 420. *J. Biol. Chem.* 277, 24274–24279.
- Nguyen, T. H., Paul, S., Xu, Y., Gurd, J. W., and Lombroso, P. J. (1999). Calcium-dependent cleavage of striatal enriched tyrosine phosphatase (STEP). *J. Neurochem.* 73, 1995–2001.
- Nitatori, T., Sato, N., Waguri, S., Karasawa, Y., Araki, H., Shibana, K., Kominami, E., and Uchiyama, Y. (1995). Delayed neuronal death in the CA1 pyramidal cell layer of the gerbil hippocampus following transient ischemia is apoptosis. *J. Neurosci.* 15, 1001–1011.
- Nosyreva, E. D., and Huber, K. M. (2006). Metabotropic receptor-dependent long-term depression persists in the absence of protein synthesis in the mouse model of fragile X syndrome. *J. Neurophysiol.* 95, 3291–3295.
- Nozaki, K., Nishimura, M., and Hashimoto, N. (2001). Mitogen-activated protein kinases and cerebral ischemia. *Mol. Neurobiol.* 23, 1–19.
- Oberle, I., Rousseau, F., Heitz, D., Kretz, C., Devys, D., Hanauer, A., Boue, J., Bertheas, M., and Mandel, J. (1991). Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science* 252, 1097–1102.
- Pascoli, V., Besnard, A., Hervé, D., Pagès, C., Heck, N., Girault, J. A., Caboche, J., and Vanhoutte, P. (2011). Cyclic adenosine monophosphate-independent tyrosine phosphorylation of N2RB mediates cocaine-induced extracellular signal-related kinase activation. *Biol. Psychiatry* 69, 218–227.
- Paul, S., Nairn, A. C., Wang, P., and Lombroso, P. J. (2003). NMDA-mediated activation of the tyrosine phosphatase STEP regulates the duration of ERK signaling. *Nat. Neurosci.* 6, 34–42.
- Paul, S., Snyder, G. L., Yokakura, H., Picciotto, M. R., Nairn, A. C., and Lombroso, P. J. (2000). The dopamine/D1 receptor mediates the phosphorylation and inactivation of the protein tyrosine phosphatase STEP via a PKA-dependent pathway. *J. Neurosci.* 20, 5630–5638.
- Pelkey, K. A., Askalan, R., Paul, S., Kalia, L. V., Nguyen, T. H., Pitcher, G. M., Salter, M. W., and Lombroso, P. J. (2002). Tyrosine phosphatase STEP is a tonic brake on induction of long-term potentiation. *Neuron* 34, 127–138.
- Pfeiffer, B. E., and Huber, K. M. (2007). Fragile X mental retardation protein induces synapse loss through acute postsynaptic translational regulation. *J. Neurosci.* 27, 3120–3130.
- Pilowsky, L. S., Bressan, R. A., Stone, J. M., Erlandsson, K., Mulligan, R. S., Krystal, J. H., and Ell, P. J. (2006). First in vivo evidence of an NMDA receptor deficit in medication-free schizophrenic patients. *Mol. Psychiatry* 11, 118–119.
- Pohjasvaara, T., Erkinjuntti, T., Ylikoski, R., Hietanen, M., Vataja, R., and Kaste, M. (1998). Clinical determinants of poststroke dementia. *Stroke* 29, 75–81.
- Qin, M., Kang, J., Burlin, T. V., Jiang, C., and Smith, C. B. (2005). Postadolescent changes in regional cerebral protein synthesis: an in vivo study in the FMR1 null mouse. *J. Neurosci.* 25, 5087–5095.
- Reiner, A., Albin, R. L., Anderson, K. D., D'Amato, C. J., Penney, J. B., and Young, A. B. (1988). Differential loss of striatal projection neurons in Huntington disease. *Proc. Natl. Acad. Sci. U.S.A.* 85, 5733–5737.
- Ron, D. (2004). Signaling cascades regulating NMDA receptor sensitivity to ethanol. *Neuroscientist* 10, 325–336.
- Ronesi, J. A., and Huber, K. M. (2008). Metabotropic glutamate receptors and fragile x mental retardation protein: partners in translational regulation at the synapse. *Sci. Signal.* 1, pe6.
- Saavedra, A., Giral, A., Rué, L., Xifró, X., Xu, J., Ortega, Z., Lucas, J. J., Lombroso, P. J., Alberch, J., and Pérez-Navarro, E. (2011). Striatal-enriched protein tyrosine phosphatase expression and activity in Huntington's disease: a STEP in the resistance to excitotoxicity. *J. Neurosci.* 31, 8150–8162.
- Seeman, P., Chau-Wong, M., Tedesco, J., and Wong, K. (1975). Brain receptors for antipsychotic drugs and dopamine: direct binding assays. *Proc. Natl. Acad. Sci. U.S.A.* 72, 4376–4380.
- Shankar, G. M., Bloodgood, B. L., Townsend, M., Walsh, D. M., Selkoe, D. J., and Sabatini, B. L. (2007). Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *J. Neurosci.* 27, 2866–2875.
- Shankar, G. M., Li, S., Mehta, T. H., Garcia-Munoz, A., Shepardson, N. E., Smith, I., Brett, F. M., Farrell, M. A., Rowan, M. J., Lemere, C. A., Regan, C. M., Walsh, D. M., Sabatini, B. L., and Selkoe, D. J. (2008). Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat. Med.* 14, 837–842.
- Sharma, E., Zhao, F., Bult, A., and Lombroso, P. J. (1995). Identification of two alternatively spliced transcripts of STEP: a subfamily of brain-enriched protein tyrosine phosphatases. *Brain Res. Mol. Brain Res.* 32, 87–93.
- Silva, A. J. (2003). Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. *J. Neurobiol.* 54, 224–237.
- Smith, S. E. (1993). Cognitive deficits associated with fragile X syndrome. *Ment. Retard.* 31, 279–283.
- Snyder, E. M., Nong, Y., Almeida, C. G., Paul, S., Moran, T., Choi, E. Y., Nairn, A. C., Salter, M. W., Lombroso, P. J., Gouras, G. K., and Greengard, P. (2005). Regulation of NMDA receptor trafficking by amyloid-beta. *Nat. Neurosci.* 8, 1051–1058.
- Snyder, E. M., Philpot, B. D., Huber, K. M., Dong, X., Fallon, J. R., and Bear, M. F. (2001). Internalization of ionotropic glutamate receptors in response to mGluR activation. *Nat. Neurosci.* 4, 1079–1085.
- Stoof, J. C., and Kebabian, J. W. (1981). Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature* 294, 366–368.
- Suvrathan, A., Hoeffler, C. A., Wong, H., Klann, E., and Chattarji, S. (2010). Characterization and reversal of synaptic defects in the amygdala in a mouse model of fragile X syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11591–11596.
- Suzuki, N., Cheung, T. T., Cai, X. D., Odaka, A., Otvos, L. Jr., Eckman, C., Golde, T. E., and Younkin, S. G. (1994). An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. *Science* 264, 1336–1340.
- Tanzi, R. E., Gusella, J. F., Watkins, P. C., Bruns, G. A., St George-Hyslop, P., Van Keuren, M. L., Patterson, D., Pan, S., Kurnit, D. M., and Neve, R. L. (1987). Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* 235, 880–884.
- Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A., and Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann. Neurol.* 30, 572–580.
- The Huntington's Disease Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72, 971–983.
- Todd, P. K., Mack, K. J., and Malter, J. S. (2003). The fragile X mental retardation protein is required for type-I metabotropic glutamate receptor-dependent translation of PSD-95. *Proc. Natl. Acad. Sci. U.S.A.* 100, 14374–14378.
- Trushina, E., Dyer, R. B., Badger, J. D. II, Ure, D., Eide, L., Tran, D. D., Vrieze, B. T., Legendre-Guillemin, V., McPherson, P. S., Mandavilli, B. S., Van Houten, B., Zeitlin, S., McNiven, M., Aebersold, R., Hayden, M., Parisi, J. E., Seeberg, E., Dragatsis, I., Doyle, K., Bender, A., Chacko, C., and McMurray, C. T. (2004). Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. *Mol. Cell. Biol.* 24, 8195–8209.

- Turalba, A. V., Leite-Morris, K. A., and Kaplan, G. B. (2004). Antipsychotics regulate cyclic AMP-dependent protein kinase and phosphorylated cyclic AMP response element-binding protein in striatal and cortical brain regions. *Neurosci. Lett.* 357, 53–57.
- Turrigiano, G. G., and Nelson, S. B. (2004). Homeostatic plasticity in the developing nervous system. *Nat. Rev. Neurosci.* 5, 97–107.
- Ule, J., and Darnell, R. B. (2006). RNA binding proteins and the regulation of neuronal synaptic plasticity. *Curr. Opin. Neurobiol.* 16, 102–110.
- Usdin, M. T., Shelbourne, P. F., Myers, R. M., and Madison, D. V. (1999). Impaired synaptic plasticity in mice carrying the Huntington's disease mutation. *Hum. Mol. Genet.* 8, 839–846.
- Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., Stipanovich, A., Caboche, J., Lombroso, P. J., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 102, 491–496.
- Van Raamsdonk, J. M., Pearson, J., Slow, E. J., Hossain, S. M., Leavitt, B. R., and Hayden, M. R. (2005). Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. *J. Neurosci.* 25, 4169–4180.
- Vanhoose, A. M., and Winder, D. G. (2003). NMDA and beta1-adrenergic receptors differentially signal phosphorylation of glutamate receptor type 1 in area CA1 of hippocampus. *J. Neurosci.* 23, 5827–5834.
- Venkitaramani, D. V., Moura, P. J., Picciotto, M. R., and Lombroso, P. J. (2011). Striatal-enriched protein tyrosine phosphatase (STEP) knockout mice have enhanced hippocampal memory. *Eur. J. Neurosci.* 33, 2288–2298.
- Visoitsak, J., Warren, S. T., Anido, A., and Graham, J. M. Jr. (2005). Fragile X syndrome: an update and review for the primary pediatrician. *Clin. Pediatr. (Phila.)* 44, 371–381.
- Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J., and Selkoe, D. J. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416, 535–539.
- Wang, J., Carnicella, S., Phamluong, K., Jeanblanc, J., Ronesi, J. A., Chaudhri, N., Janak, P. H., Lovinger, D. M., and Ron, D. (2007). Ethanol induces long-term facilitation of NR2B-NMDA receptor activity in the dorsal striatum: implications for alcohol drinking behavior. *J. Neurosci.* 27, 3593–3602.
- Wang, J., Lanfranco, M. F., Gibb, S. L., Yowell, Q. V., Carnicella, S., and Ron, D. (2010). Long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum play a crucial role in alcohol consumption and relapse. *J. Neurosci.* 30, 10187–10198.
- Weiler, I. J., and Greenough, W. T. (1999). Synaptic synthesis of the fragile X protein: possible involvement in synapse maturation and elimination. *Am. J. Med. Genet.* 83, 248–252.
- White, A. M., Matthews, D. B., and Best, P. J. (2000). Ethanol, memory, and hippocampal function: a review of recent findings. *Hippocampus* 10, 88–93.
- Xiao, M. Y., Wigstrom, H., and Gustafsson, B. (1994). Long-term depression in the hippocampal CA1 region is associated with equal changes in AMPA and NMDA receptor-mediated synaptic potentials. *Eur. J. Neurosci.* 6, 1055–1057.
- Xifro, X., Giral, A., Saavedra, A., Garcia-Martinez, J. M., Diaz-Hernandez, M., Lucas, J. J., Alberch, J., and Perez-Navarro, E. (2009). Reduced calcineurin protein levels and activity in exon-1 mouse models of Huntington's disease: role in excitotoxicity. *Neurobiol. Dis.* 36, 461–469.
- Xu, J., Kurup, P., Bartos, J. A., Hell, J. W., and Lombroso, P. J. (2010). Inhibition of *pyk2* Signaling by Striatal-Enriched Tyrosine Phosphatase (STEP). Program No. 452.13. 2010 Neuroscience Meeting Planner. Society for Neuroscience. San Diego, CA. [Online].
- Xu, J., Kurup, P., Zhang, Y., Goebel-Goody, S. M., Wu, P. H., Hawasli, A. H., Baum, M. L., A. Bibb, J., and Lombroso, P. J. (2009). Extrasynaptic NMDA receptors couple preferentially to excitotoxicity via calpain-mediated cleavage of STEP. *J. Neurosci.* 29, 9330–9343.
- Yin, H. H., Park, B. S., Adermark, L., and Lovinger, D. M. (2007). Ethanol reverses the direction of long-term synaptic plasticity in the dorsomedial striatum. *Eur. J. Neurosci.* 25, 3226–3232.
- Zhang, Y., Kurup, P., Xu, J., Carty, N., Fernandez, S. M., Nygaard, H. B., Pittenger, C., Greengard, P., Strittmatter, S. M., Nairn, A. C., and Lombroso, P. J. (2010). Genetic reduction of striatal-enriched tyrosine phosphatase (STEP) reverses cognitive and cellular deficits in an Alzheimer's disease mouse model. *Proc. Natl. Acad. Sci. U.S.A.* 107, 19014–19019.
- Zhang, Y., Venkitaramani, D. V., Gladding, C. M., Kurup, P., Molnar, E., Collingridge, G. L., and Lombroso, P. J. (2008). The tyrosine phosphatase STEP mediates AMPA receptor endocytosis after metabotropic glutamate receptor stimulation. *J. Neurosci.* 28, 10561–10566.
- Zuccato, C., Ciammola, A., Rigamonti, D., Leavitt, B. R., Goffredo, D., Conti, L., MacDonald, M. E., Friedlander, R. M., Silani, V., Hayden, M. R., Timmusk, T., Sipione, S., and Cattaneo, E. (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 293, 493–498.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 April 2011; accepted: 13 July 2011; published online: 29 July 2011.
Citation: Fitzpatrick CJ and Lombroso PJ (2011) The role of striatal-enriched protein tyrosine phosphatase (STEP) in cognition. *Front. Neuroanat.* 5:47. doi: 10.3389/fnana.2011.00047
Copyright © 2011 Fitzpatrick and Lombroso. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Striatal signal transduction and drug addiction

Scott D. Philibin^{1*}, Adan Hernandez¹, David W. Self¹ and James A. Bibb^{1,2}

¹ Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX, USA

² Department of Neurology and Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, TX, USA

Edited by:

Emmanuel Valjent, Université
Montpellier 1 & 2, France

Reviewed by:

Marina Picciotto, Yale University, USA
Bruce Thomas Hope, National
Institute on Drug Abuse, USA

*Correspondence:

Scott D. Philibin, Laboratory of
Dr. James A. Bibb, Department of
Psychiatry, University of Texas
Southwestern Medical Center, 5323
Harry Hines Boulevard, Dallas, TX
75390-9070, USA.
e-mail: scott.philibin@
utsouthwestern.edu

Drug addiction is a severe neuropsychiatric disorder characterized by loss of control over motivated behavior. The need for effective treatments mandates a greater understanding of the causes and identification of new therapeutic targets for drug development. Drugs of abuse subjugate normal reward-related behavior to uncontrollable drug-seeking and -taking. Contributions of brain reward circuitry are being mapped with increasing precision. The role of synaptic plasticity in addiction and underlying molecular mechanisms contributing to the formation of the addicted state are being delineated. Thus we may now consider the role of striatal signal transduction in addiction from a more integrative neurobiological perspective. Drugs of abuse alter dopaminergic and glutamatergic neurotransmission in medium spiny neurons of the striatum. Dopamine receptors important for reward serve as principle targets of drugs abuse, which interact with glutamate receptor signaling critical for reward learning. Complex networks of intracellular signal transduction mechanisms underlying these receptors are strongly stimulated by addictive drugs. Through these mechanisms, repeated drug exposure alters functional and structural neuroplasticity, resulting in transition to the addicted biological state and behavioral outcomes that typify addiction. Ca^{2+} and cAMP represent key second messengers that initiate signaling cascades, which regulate synaptic strength and neuronal excitability. Protein phosphorylation and dephosphorylation are fundamental mechanisms underlying synaptic plasticity that are dysregulated by drugs of abuse. Increased understanding of the regulatory mechanisms by which protein kinases and phosphatases exert their effects during normal reward learning and the addiction process may lead to novel targets and pharmacotherapeutics with increased efficacy in promoting abstinence and decreased side effects, such as interference with natural reward, for drug addiction.

Keywords: addiction, signaling, plasticity, spines, dopamine, glutamate, phosphorylation

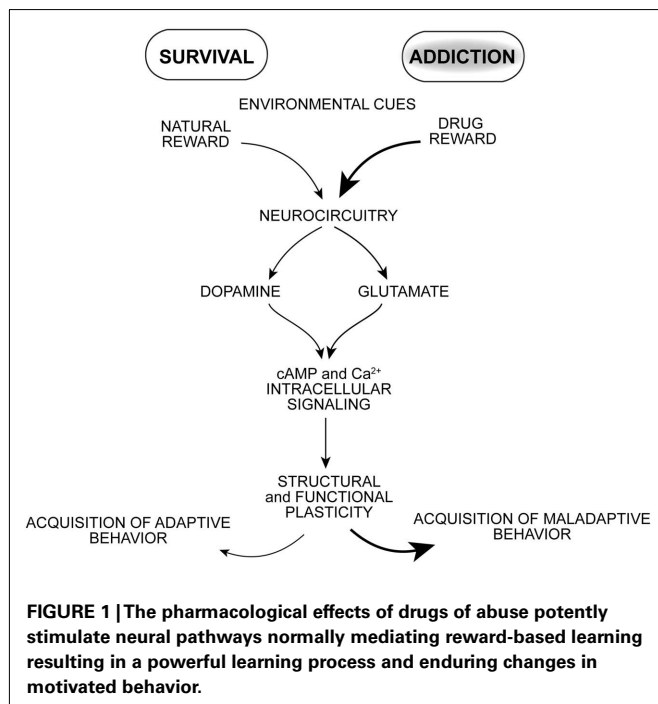
INTRODUCTION

Drug addiction is a behavioral disorder influenced by a complex interaction of genetic, environmental, and developmental factors (Crabbe, 2002; Volkow, 2005). Addictive substances include illicit drugs like the psychostimulant cocaine and the opiate heroin, as well as legally available tobacco/nicotine and alcohol (ethanol; EtOH). Prescription drugs, such as opioids, stimulants, or depressants are also increasingly being used for non-medical reasons (Compton and Volkow, 2006). Drug addiction affects not only the individual, but also society as a whole evidenced by severed interpersonal relations, failure in school or job performance, outbreaks of domestic violence and child abuse, and even the degradation of entire communities. There are few pharmacotherapeutics that exist to treat drug addiction and risk of relapse remains substantial despite sincere efforts to remain abstinent. Therefore, it is important to understand the neurobiological basis of behaviors characteristic of addiction in order to identify novel mechanisms that lead to better treatments.

Chronic drug addiction is associated with the development of pharmacological tolerance, sensitization, and dependence, and the impact of these processes is manifested in drug intake, craving, and relapse. The transition from impulsive to compulsive drug

use corresponds to enhanced euphoric drug experiences, and an overshadowing of adverse consequences (Robbins et al., 2008; Torregrossa et al., 2011). These adaptations are seen clinically as reduced responding to natural rewards and a loss of control over drug intake in response to drug-associated cues (Kalivas and Volkow, 2005). Motivation to alleviate drug withdrawal-induced negative affect (i.e., stress, anxiety, depression, dysphoria) may also contribute to relapse via their negative reinforcing properties (Koob and Le Moal, 2001; Koob and Volkow, 2010).

Drugs of abuse target neural processes that normally mediate adaptive reward-based learning, but with overriding and repeated stimulation that appears to seize these neural processes (Hyman et al., 2006; Kalivas and O'Brien, 2008; Milton and Everitt, 2010; Torregrossa et al., 2011). Once brain reward circuitry is activated by convergent neuronal inputs, synapses undergo functional, and structural plasticity important for learning associated with the conditioned stimulus effects of drugs of abuse (**Figure 1**). What drives a drug addict to uncontrollable self-destructive behavior? Pioneering behavioral psychologists emphasized the formation of stimulus-response associations as important mediators of learned behaviors. Drugs of abuse can serve as powerful rewarding, discriminative, and reinforcing stimuli. Motoric actions leading to



obtaining drug are processed in tandem with environmental cues that also come to drive drug-seeking and -taking behavior. Triggers of relapse during abstinence include re-exposure to the drug itself, environmental drug-associated cues, stress, or some combination of these factors (Schmidt et al., 2005; Milton and Everitt, 2010; Weiss, 2010).

The striatum is a subcortical brain structure that is major target for drugs of abuse. It is comprised of ventral and dorsal subregions. The ventral striatum consists of the nucleus accumbens (NAc), olfactory tubercle, and islands of Calleja. The NAc includes shell and core subregions and plays an integral role in three dissociable aspects of psychological reward associated with addiction: “liking” (hedonic impact), “wanting” (incentive salience), and learning (predictive associations and cognition; Berridge et al., 2009). While both natural and drug rewards increase extracellular dopamine in the NAc, dopamine activity in the NAc shell does not adapt or habituate to repeated drug exposure by decreasing dopamine activity as it does with natural rewards (Wise et al., 1995a,b; Hemby et al., 1997) and repeated drug exposure induces sensitization of dopamine transmission in the NAc core (Cadoni and Di Chiara, 1999, 2000; Cadoni et al., 2000). Dopamine in the NAc shell is activated in response to primary, unconditioned, and unanticipated stimuli, for example, to an appetitive unfamiliar taste (sweet chocolate) infused intra-orally (Bassareo et al., 2002). Interestingly, food-conditioned stimuli increase dopamine in the NAc shell whereas drug-conditioned stimuli increase dopamine in the NAc core (Bassareo et al., 2011). Repeated and sustained levels of drug-induced dopamine stimulation in the shell could pathologically strengthen the acquisition (learning) of incentive stimuli predictive of drug availability through Pavlovian conditioning, while sensitization of dopamine transmission in the core might be more directly related to the initiation of behavioral activity to

obtain drug reward (instrumental action). Thus, incentive learning of drug-associated cues through Pavlovian conditioning is critical for the reinforcing effects of drugs that increase the probability of future occurrences of drug-seeking and -taking behaviors (Bower and Grusec, 1964) and has been denoted as Pavlovian instrumental transfer (PIT; Ettenberg, 1990).

While reward processed in the NAc is critical for acquisition of goal-directed behaviors, these behaviors repeated over time develop into more automatic repertoires characteristic of stimulus–response learning, largely understood to involve dorsal striatal circuits (Mitchell et al., 1985; Mitchell and Hall, 1988). The dorsal striatum includes the caudate and putamen in primates, analogous to the dorsomedial and dorsolateral striatum in rodents, respectively. The transition to the addicted state appears to correspond to a recruitment of neurocircuitry from ventral to dorsal striatum (Gerdeman et al., 2003; Porrino et al., 2007; Pierce and Vanderschuren, 2010). The dorsal striatum is critical for formation of rigid or inflexible behaviors characterizing drug-seeking and -taking habits (Gerdeman et al., 2003; Pierce and Vanderschuren, 2010). This ventral to dorsal progression does not, however, negate involvement of the NAc over time. In fact, a functionally linked progression of activity from ventral to dorsal striatum in response to chronic drug exposure may invoke widespread neuroplasticity underlying cognitive and behavioral perturbations associated with drug addiction (Koob and Volkow, 2010).

Dorsal and ventral striatum are composed of medium spiny neurons (MSNs) that are predominantly γ -aminobutyric acid (GABA) projection neurons (Tepper et al., 2007). These MSNs receive dopaminergic input from afferent projections of the substantia nigra and ventral tegmental areas of the midbrain. MSNs also receive glutamatergic input from afferent projections of the prefrontal cortex, predominantly in layer 5 of the cortex.

EFFECTS OF DRUGS OF ABUSE ON SIGNAL TRANSDUCTION

Addictive substances can be generally classified into drugs that target monoamine transporters, G protein-coupled receptors (GPCRs), or ion channels and ionotropic receptors (Luscher and Ungless, 2006). Drugs of abuse mimic and surpass endogenous dopamine activation subsequent to phasic dopamine cell firing coding for salience and reward (Schultz et al., 2000). Human brain imaging studies show that drug-induced increases in striatal dopamine are associated with subjective reward (Volkow et al., 1996; Drevets et al., 2001).

All dopamine receptors are GPCRs (Neve et al., 2004). Dopamine receptors fall into two major classes: D₁-like receptors (D₁ and D₅) and D₂-like receptors (D₂, D₃, and D₄). They are primarily distinguished via their opposing actions on adenylate cyclase (AC). D₁-like receptors stimulate AC through coupling to stimulatory (G α_s) G proteins that increase cyclic adenosine 3',5'-monophosphate (cAMP) levels, whereas D₂-like receptors inhibit AC through coupling to inhibitory (G α_i) G proteins that decrease cAMP levels. Both D₁ and D₂ receptors contribute to aspects addiction, despite the fact that their opposing G protein-coupled functions might suggest that the two classes would interact antagonistically. One level at which this paradox resolves is the neuroanatomical circuitry. Specifically, D₁ mediated effects on midbrain GABA release are direct, while D₂ mediated effects are

indirect. Beyond this discrepancy, interactions between D₁ and D₂ receptors and their circuitry during reward learning, motivated behavior, and addiction are quite complex.

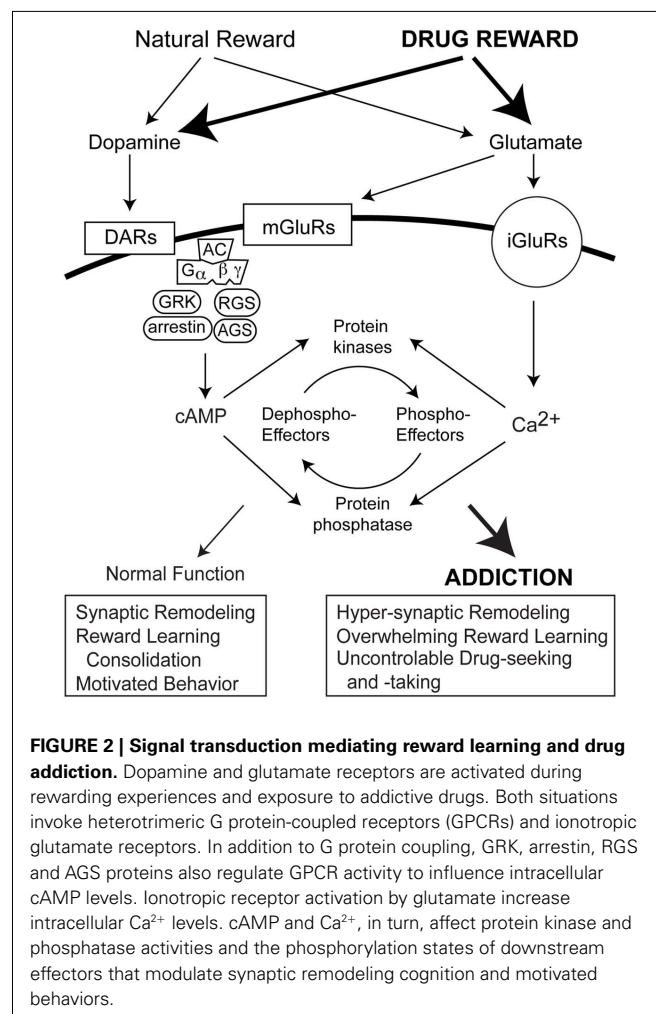
While the mechanisms for the synergism between D₁ and D₂ receptors in mediating behavioral effects are not yet completely understood, the roles of D₁- and D₂-like receptors in reward can be dissociated. Either selective D₁- or D₂-like receptor agonists maintain intravenous drug self-administration in rats (Yokel and Wise, 1978; Self and Stein, 1992), monkeys (Woolverton et al., 1984; Weed et al., 1993), and mice (Caine et al., 2007). However, self-administration of D₂-like receptor agonists requires an extensive history of self-administration with non-selective dopamine agonists like cocaine. Converse to systemic studies, intracranial self-administration studies show a requirement for concomitant D₁- and D₂-like receptor activation in the NAc shell but not the core subregion (Ikemoto et al., 1997). Interestingly, infusion of cocaine into the more ventral olfactory tubercle regions is even more efficacious in maintaining self-administration than in the NAc shell, and this effect is blocked by co-administration of D₁- or D₂-like antagonists (Ikemoto, 2003).

In contrast to voluntary drug intake modeled in self-administration procedures, compulsive drug-seeking modeled in extinction/reinstatement procedures show a predominant role for D₂- but not D₁-like receptors. Extinction/reinstatement procedures typically measure the level of effort an animal with prior history of drug self-administration will exert to obtain drug, as an index of craving that would precipitate relapse in humans (Wise and Rompre, 1989; Berridge and Robinson, 1998). D₂-like receptor activation triggers cocaine-seeking during or after extinction of behavioral responses (Koeltzow and Vezina, 2005; Edwards et al., 2007). Conversely, D₁-like receptor activation essentially has no effect, even at doses that stimulate locomotor activity comparable to D₂-like receptor activation (Dias et al., 2004; Koeltzow and Vezina, 2005). However, pretreatment with a D₁-like agonist blocks the ability of cocaine priming or cocaine-associated cues to induce reinstatement (Alleweireldt et al., 2003; Edwards et al., 2007), whereas, D₂-like agonist pretreatment facilitates cocaine-primed reinstatement (Self et al., 1996). Thus, D₂-like receptors are critical for relapse to cocaine-seeking induced by environmental cues such as cocaine-related stimuli or stress that activates the mesolimbic dopamine system (Phillips et al., 2003) and D₁-like receptor tone may provide an inhibitory mechanism over cocaine-seeking via saturation of primary reward processes. However, differences between systemic and intracranial studies of D₁- versus D₂-like effects on drug-seeking also exist. In contrast to systemic pretreatment of a D₁-like agonist, direct infusion of a D₁-like agonist intra-NAc triggers cocaine-seeking (Bachtell et al., 2005; Schmidt et al., 2006). Similarly, intra-NAc infusions of D₂-like agonists reinstate cocaine-seeking (Bachtell et al., 2005; Schmidt et al., 2006). Thus, dopamine effects critical for reward-related behavior can vary according to receptor subtype and systemic versus direct infusion into the NAc and either D₁- or D₂-like receptors have the ability to modulate the effects of the other.

MODULATION OF GPCR SIGNALING

The effects of GPCRs is modulated via transmembrane signaling pathways consisting of metabotropic cell surface receptors, G

proteins, and effectors (Engelhardt and Rochais, 2007; **Figure 2**). In addition to G protein coupling, GPCR activation triggers a negative feedback mechanism known as desensitization (Hausdorff et al., 1990). Desensitization may be mediated via agonist-induced receptor phosphorylation by a GPCR kinase (GRK; Krupnick and Benovic, 1998). GRK-dependent GPCR phosphorylation increases receptor affinity for cytosolic arrestin protein family members. For example, the resulting phosphorylated receptor/arrestin complex prevents further coupling of the D₁ receptor to its G protein, progressively reducing second messenger synthesis of cAMP. Resensitization is triggered by internalization of an uncoupled receptor to endosomal compartments, allowing protein phosphatase-dependent dephosphorylation of the receptor and recycling back to the cell surface, or degradation. Modulation of GRKs occurs via mechanisms involving Ca²⁺-binding proteins, phosphorylation, targeting proteins, or mechanisms governing their localizations and expressions (Penela et al., 2003). In addition to GRKs that regulate GPCR activity, several families of accessory proteins have also been discovered that control G protein signaling (Blumer and Lanier, 2003). The regulators of G protein signaling (RGS) and activators of G protein signaling (AGS) G protein modulators both are implicated in addition.



RGS proteins are a large family that function as GTPase-accelerating proteins (GAP). GAP action at GTP-bound $G\alpha_{i/o}$ - or $G\alpha_q$ -coupled receptors increases inactivation of the $G\alpha$ -GDP state and expedites recombination of the $G\alpha$ and $\beta\gamma$ signaling molecules. This allows RGS proteins to restrict G protein signal strength at steady state and negatively modulate G protein signaling, controlling potency, and efficacy of agonist effects (Garzon et al., 2008; Hooks et al., 2008; Traynor, 2010). Some RGS family members are highly enriched in striatal subregions. RGS2 is expressed in caudate putamen (Grafstein-Dunn et al., 2001; Taymans et al., 2002). RGS4 is expressed in the caudate putamen and NAc (Gold et al., 1997; Nomoto et al., 1997). The long form of RGS9 (RGS9-2) is expressed predominantly in caudate putamen, NAc, islands of Calleja, and olfactory tubercle (Thomas et al., 1998; Rahman et al., 1999). Based on striatal regional distributions, RGS2, RGS4, and especially RGS9-2 have potential in negatively regulating the effects of drugs of abuse. Correspondingly, administration of various drugs of abuse induces an up-regulation of RGS family members in striatum. RGS2, 4, and 9 have all been implicated in addiction (Burchett et al., 1998; Zachariou et al., 2003; Psifogeorgou et al., 2007; Hooks et al., 2008; Traynor, 2010). By acting at a relatively early stage of the signaling pathway, RGS promoters can affect multiple branches of D_1 - as well as D_2 -like receptor signaling to AC and its effectors and RGS proteins can function to dampen dopamine signaling in the striatum in response to various drugs of abuse.

AGS proteins constitute another family of negative G protein modulators. The AGS family contains three functionally distinct members (Blumer et al., 2005). AGS3 is the only one currently associated with addictive drug-induced neuroadaptations (Kelley and Schiltz, 2004; Bowers, 2010). AGS3 binds to and stabilizes the inactive GDP-bound $G\alpha_i$ conformation, preventing GDP release and, inhibiting $G\alpha_i$ -mediated signaling (Natochin et al., 2000). AGS3 expression increases in NAc core during protracted withdrawal (three or more weeks) from either repeated experimenter-administered injections of cocaine or 3 weeks of extinction training in rats that had self-administered cocaine (Bowers et al., 2004). Furthermore, cocaine-induced reinstatement of drug-seeking behavior was attenuated by AGS3 knock-down, but then restored by antisense washout and return of AGS3 protein. These studies suggest that AGS3 up-regulation requires protracted withdrawal from chronic drug exposure and that decreased $G\alpha_i$ signaling may support addiction pathogenesis.

In addition to dopamine receptors (Surmeier et al., 2007), group II metabotropic glutamate (mGlu) receptors (mGlu_{2/3}) also modulate striatal MSNs function (Richards et al., 2005), and through these receptors cortical glutamatergic input into striatum may counter balance and potentially reverse drug-induced neuroadaptations. These receptors are G_i -coupled and are also subject to negative regulation via AGS3. G_i coupling to mGlu_{2/3} receptors can modulate neurotransmitter release via activation of presynaptic K^+ channels, inhibition of presynaptic Ca^{2+} channels, or direct interference with vesicular release. Dopamine release in NAc is regulated by mGlu_{2/3} receptors (Karasawa et al., 2006; Xi et al., 2010). NAc mGlu_{2/3} receptor-G protein coupling is reduced after

chronic cocaine (Xi et al., 2002; Bowers et al., 2004; Ghasemzadeh et al., 2009) or EtOH (Bowers et al., 2008). Furthermore it has been suggested that mGlu_{2/3} auto receptors may mediate enhanced glutamate release in NAc during protracted drug withdrawal in response to the drug itself or associated cues (Madayag et al., 2007; Lalumiere and Kalivas, 2008). Therefore, mGlu_{2/3} receptors are emerging as a therapeutic target to possibly reduce drug abuse relapse (Moussawi and Kalivas, 2010).

IONOTROPIC MECHANISMS OF SYNAPTIC PLASTICITY

G protein-coupled receptors signaling via dopamine receptors and metabotropic glutamate receptors mediates important aspects of reward and reinstatement through regulation of excitability, which is dependent upon glutamatergic activation of ionotropic receptors. The two classes of ionotropic glutamate receptors are *N*-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-propionate (AMPA) receptors and they contribute in important ways to addiction. For example, cocaine administration increases the NR1 NMDA and GluA1 AMPA (Figure 3) receptor subunit in the NAc (Lu et al., 2003; Hemby et al., 2005b; Schumann and Yaka, 2009) and NMDA and AMPA receptors are involved in the ability of addictive drugs to produce long-term plasticity in learning and memory circuits associated with cue-reward associations and habit formation.

Drugs of abuse evoke an NMDA receptor-dependent long-term potentiation (LTP) of AMPA receptor current in dopamine neurons, possibly reflecting an early memory trace in the acquisition of drug addiction (Ungless et al., 2001; Saal et al., 2003; Borgland et al., 2004; Liu et al., 2005). LTP is induced when a

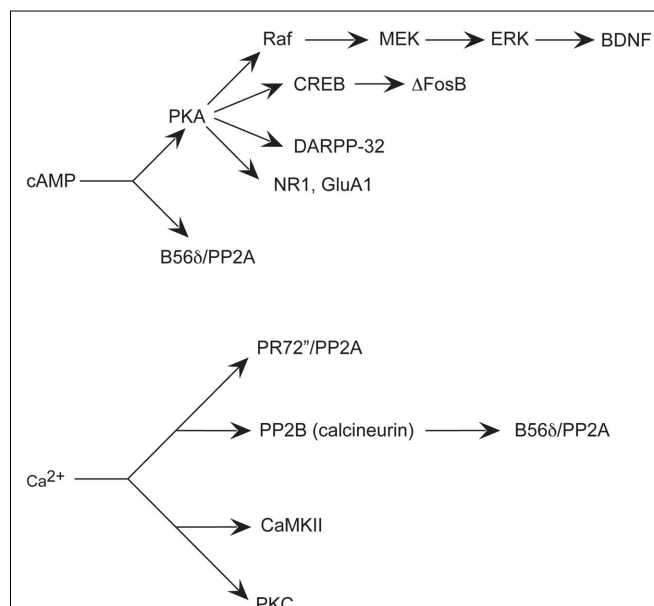


FIGURE 3 | Simplified schematic of signaling pathways involving major effectors of cAMP and Ca^{2+} implicated in aspects of addiction.

Increased cAMP levels activate PKA, which influences multiple effectors, such as other kinases, phosphatases, transcription factors, phosphatase inhibitors, and neurotransmitter receptor subunits. Increased Ca^{2+} levels also affect kinases, and phosphatases.

brief high frequency train of stimuli (a tetanus) is applied to a synaptic pathway that increases the amplitude of the excitatory postsynaptic potentials in target neurons. Long-term depression (LTD) is induced when tetanus application decreases the amplitude of the excitatory postsynaptic potentials in target neurons. Induction of LTP is associated with the formation of spines *de novo* and enlargement of existing spines, whereas induction of LTD is associated with contraction and retraction of spines (Nagerl et al., 2004; Okamoto et al., 2004).

Subsequent to induction of LTP, synaptic strengthening can manifest through insertion of GluA2-lacking AMPA receptors (Kauer and Malenka, 2007). The synaptic insertion of AMPA receptors may create a temporal opportunity when the acquisition of cocaine-related cues corresponds to increased synaptic plasticity (Wolf, 2010). Restricting AMPA receptor activation could reverse LTP associated with continued cocaine-seeking. AMPA receptor antagonists attenuate reinstatement of drug-seeking behavior induced by drug, cue-, or stress (Mcfarland et al., 2004; Backstrom and Hyttia, 2007; Ping et al., 2008). However, it has also been shown that degrading basal AMPA receptor function in NAc neurons is sufficient to facilitate relapse and elevating basal AMPA receptor function attenuates this behavioral effect (Bachtell et al., 2008). The role of glutamatergic mechanisms in the modulation of drug self-administration and risk of relapse is complex. Nonetheless, several non-specific glutamatergic agents have displayed potential as pharmacotherapeutics for addiction (Bowers et al., 2010).

Converse to LTP, LTD corresponds to removal of AMPA receptors from synapses (Malinow and Malenka, 2002). Persistent impairment in LTD has been associated with rigid drug-seeking behaviors resistant to modulation by environmental contingencies (Kasanez et al., 2010). Moreover, operant cocaine self-administration attenuates LTD in both the NAc core and shell; however, LTD was abolished only in the NAc core after protracted withdrawal, suggesting long-term plasticity in the core could underlie drug-seeking behavior and relapse (Martin et al., 2006). Also, animals sensitized to repeated cocaine administration, displayed a ratio of AMPA to NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) that was reduced in MSNs of the NAc shell that corresponded to decreased amplitude of miniature EPSCs and magnitude of LTD (Thomas et al., 2001). Thus aspects of both LTP and LTD in NAc subregions appear to be involved in drug-taking and -seeking behaviors.

Drug-seeking and -taking induced by exposure to cues associated with drug use imply the involvement of long-term memories, such as those induced via strong glutamatergic stimulation. At the same time, protein kinase A (PKA; see below) activation in the NAc has been shown to be necessary for reward learning in which properties of drugs become associated with environmental cues (Sutton et al., 2000; Beninger et al., 2003). These two converging observations, that glutamatergic input is elevated and necessary for drug-seeking and reward learning, and that PKA signaling invoked via activation of D₁ dopamine receptors is also critical for reward associated learning, suggest that any comprehensive explanation for the biochemical mechanisms of addiction would require obligatory integration of both NMDA/AMPA receptor-dependent Ca²⁺ and dopamine receptor-dependent cAMP signaling cascades.

PROTEIN KINASES, PHOSPHATASES, AND DOWNSTREAM EFFECTORS

Drugs of abuse induce dopaminergic D₁ receptor activation that increases intracellular cAMP. Glutamatergic input that activates NMDA and AMPA receptors increases intracellular Ca²⁺ concentration. Intracellular signaling cascades involving specific kinases and phosphatases affected by cAMP and Ca²⁺, in turn, influence phospho- and dephospho-effectors that modulate neuroplasticity to affect behavioral outcome (Figure 2).

CYCLIC AMP-DEPENDENT KINASE (PROTEIN KINASE A)

Under basal conditions, PKA exists as a heterotetramer composed of two regulatory and two catalytic subunits. Activation of AC through G_{αs}-coupled receptors causes rapid increase in intracellular cAMP. PKA is subsequently activated as cAMP binds the regulatory subunits triggering dissociation of the holoenzyme, releasing the active catalytic subunits from inhibition by the regulatory subunits (Pearce et al., 2010). There are four regulatory subunit isoforms classified as type I (RI α , RI β) and type II (RII α , RII β) and three catalytic isoforms (C α , C β , C γ). Any change in cAMP directly affects function of PKA, the most important effector for cAMP.

For example, cAMP-induced activation of PKA activates cAMP response element-binding protein (CREB; Figure 3). Induction of cAMP liberates the PKA C α subunit that then passively diffuses into the nucleus and induces cellular gene expression by phosphorylation of CREB on Ser133 (Gonzalez and Montminy, 1989). CREB then signals transcription of genes containing a cAMP response element (CRE) in their promoter regions to regulate gene transcription (Mayr and Montminy, 2001). Both opiates and stimulants activate CREB-mediated transcription within the NAc (Cole et al., 1995; Turgeon et al., 1997; Shaw-Lutchman et al., 2002). Increased CREB in the NAc attenuates rewarding effects of cocaine (Carlezon et al., 1998) and morphine (Barrot et al., 2002) and these effects are also seen in transgenic mice with increased (McClung and Nestler, 2003) or decreased (Walters and Blendy, 2001) CREB. Although the CREB-regulated target genes contributing to these effects are unknown, one candidate gene in the NAc encodes preprodynorphin (Carlezon et al., 1998), the precursor gene product of the endogenous opioid peptide dynorphin, which activates the κ opioid receptor. Dynorphin is upregulated in the striatum subsequent to cocaine exposure (Cole et al., 1995) and is largely understood to contribute to the inhibition of dopamine transmission that leads to the downregulation of reward mechanisms (Shippenberg and Rea, 1997). For example, microinjection of a κ opioid receptor antagonist into the NAc of CREB overexpressing mice attenuates the inhibition of the rewarding effects of cocaine (Carlezon et al., 1998). Thus, CREB activation in the NAc may function as negative feedback to reduce sensitivity to the behavioral effects of repeated drug administration (Carlezon et al., 2005).

Another transcription factor induced in striatum by chronic drugs of abuse is Δ FosB (Figure 3). In contrast to CREB, Δ FosB appears to mediate the development of drug sensitization. Δ FosB shares homology with other Fos family transcription factors that include c-Fos, FosB, Fra1, and Fra2 (Morgan and Curran, 1995). Heterodimerization of Fos family proteins with Jun family proteins

(c-Jun, JunB, or JunD) form activator protein-1 (AP-1) transcription factors. AP-1 transcription factors bind to AP-1 sites in the promoters of specific genes to regulate their transcription. Fos family proteins are highly unstable and induced rapidly and transiently in the striatum after acute drug administration. Conversely, chronic drug administration induces persistent increases in Δ FosB (M_r 35–37 kD) in striatal subregions unlike other Fos family members (Chen et al., 1995, 1997; Hiroi et al., 1997). Similar to CREB, some drugs of abuse appear to induce Δ FosB selectively in dynorphin-containing MSNs of the striatum (Nye et al., 1995; Moratalla et al., 1996; Muller and Unterwald, 2005; Lee et al., 2006). Δ FosB-overexpressing mice have augmented locomotor response to acute and chronic cocaine (Kelz et al., 1999) and exhibit enhanced conditioned place preference (CPP), a Pavlovian, or classical conditioned behavior used as an indirect measure of the rewarding (or aversive) effects of drugs (Prus et al., 2009) with either cocaine (Kelz et al., 1999) or morphine (Zachariou et al., 2006). Further, Δ FosB-overexpressing mice self-administer lower doses of cocaine than do their wild-type littermates, indicating increased sensitivity to drug reinforcing effects (Colby et al., 2003). Interestingly, persistent increases in Δ FosB vary according to different striatal subregions, such as the NAc core, shell and dorsal striatum and do not differ between contingent and non-contingent drug administration procedures (Perrotti et al., 2008).

It is important to note here that volition is a critical factor in understanding the signaling mechanisms of addiction. Different outcomes between contingent versus non-contingent drug administration studies exist (Hemby et al., 2005a; Jacobs et al., 2005). While experimenter-administered drug paradigms, such as CPP and locomotor sensitization, have greatly increased understanding of neuroplasticity induced by repeated drug exposure, it has been proposed that molecular mechanisms of drug relapse may be probed with greater clinical relevance using operant drug self-administration procedures (Shaham et al., 2003; Epstein et al., 2006).

Another major substrate of the dopamine D_1 /cAMP/PKA pathway implicated in the mechanisms of various drugs of abuse is Dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa (DARPP-32; Svenningsson et al., 2005; **Figure 3**). DARPP-32 is highly enriched in striatal MSNs (Ouimet et al., 1998). DARPP-32 can serve a dual function as a protein kinase inhibitor or a protein phosphatase inhibitor, depending on the site of DARPP-32 phosphorylation. Changes in the phosphorylation state of DARPP-32, through activation of its phosphatase- or kinase-inhibitory activity, indirectly influence the phosphorylation state of other proteins and thereby mediate some of the diverse physiological effects of first messengers on cellular function. For example, activation of DARPP-32 by dopamine and cAMP can act as a positive feedback signal by inhibiting dephosphorylation of other substrates for PKA. DARPP-32 can also attenuate dephosphorylation of other substrates for other protein kinases and thereby mediate effects of first- and second-messenger systems on one another. Furthermore, DARPP-32, via phosphorylation by PKA and dephosphorylation by the Ca^{2+} -calmodulin-dependent phosphatase, protein phosphatase 2B (PP2B; calcineurin) can integrate physiological effects of first messengers that influence cAMP and Ca^{2+}

systems (**Figure 3**). For example, dopamine activation of D_1 receptors that activate the cAMP pathway phosphorylates DARPP-32, whereas NMDA or AMPA signals that activate the Ca^{2+} pathway dephosphorylates DARPP-32.

PKA phosphorylates DARPP-32 at Thr34, rendering DARPP-32 a potent inhibitor of the multifunctional protein phosphatase 1 (PP1; Hemmings et al., 1984). Altered activity of PP1 leads to altered dephosphorylation of one of its prominent substrates: Na^+ , K^+ -ATPase. Changes in the phosphorylation state of Na^+ , K^+ -ATPase results in altered Na^+ transport across the cell membrane and altered membrane potential in excitable cells (Li et al., 1995). Analogous mechanisms appear to underlie modulation of Ca^{2+} channel activity (Surmeier et al., 1995).

CYCLIN-DEPENDENT KINASE 5

Cyclin-dependent kinase 5 (Cdk5) is another important kinase implicated in drug addiction. Cdk5 inhibition, whether by pharmacological or genetic means, potentiates both natural and cocaine reward-related behaviors. Repeated intra-NAc infusions of the Cdk5 pharmacological inhibitor roscovitine prior to cocaine injections alter both the development and expression of cocaine-induced locomotor sensitization (Taylor et al., 2007). Repeated intra-NAc infusion of roscovitine also enhances the incentive-motivational effects of cocaine as measured by responding for drug-associated cues and this effect persisted for at least 2 weeks after the last exposure of roscovitine (Taylor et al., 2007). NAc infusions of another Cdk5 inhibitor, olomoucine, produces acute, and persistent increases in “breakpoints” on a progressive ratio schedule for cocaine reinforcement (Taylor et al., 2007), an index of the degree of effort an animal will exert to obtain drug.

Cre/loxP conditional knockout systems permit temporal and spatial control of Cdk5 expression in the adult brain (Benavides et al., 2007). Mice lacking Cdk5 in adult forebrain exhibit increased locomotor-activating effects of cocaine, increased breakpoints on a progressive ratio schedule of food reinforcement and enhanced cocaine CPP corresponding to increased excitability of MSNs in the NAc, a form of functional plasticity (Benavides et al., 2007). These combined results suggest that Cdk5 acts as a homeostatic mechanism of neuronal excitability in the NAc and that Cdk5 may govern behaviors driven to obtain drug, drug-associated cues, and natural reward.

The AP-1 site in the Cdk5 gene promoter is a downstream target of Δ FosB. Chronic cocaine administration as well as overexpression of Δ FosB raises striatal Cdk5 mRNA, protein, and activity (Bibb et al., 2001). Unlike protein kinases that are activated by second messengers, Cdk5 is constitutively activated through its interactions with its cofactor p35. In this constitutively active basal state, Cdk5 appears to provide a negative tonus toward D_1 dopamine receptor/cAMP/PKA signaling, through its phosphorylation of DARPP-32, for example. Cdk5 phosphorylates DARPP-32 at Thr75, rendering DARPP-32 a potent inhibitor of PKA that effectively antagonizes effects of chronic cocaine on striatal dopamine levels (Bibb et al., 1999). These data suggest that chronic cocaine induces up-regulation of Cdk5 mediated by Δ FosB that alters dopamine D_1 receptor signaling. While up-regulation of Cdk5 may functionally repress hyper stimulated cAMP/PKA signaling pathways, it may

also alter neuroplasticity in other ways that may contribute to addiction.

PP2A is a predominant mechanism for dephosphorylation of DARPP-32 at Thr75. Dephosphorylation of DARPP-32 at Thr34 involves primarily the Ca^{2+} -dependent protein phosphatase calcineurin. Dephosphorylation of Thr75 primarily involves PP2A. Dephosphorylation of Thr75 is subject to control by both cAMP- and Ca^{2+} -dependent regulatory mechanisms. The PR72 (or B'' or PPP2R3A; **Figure 3**) regulatory subunit of PP2A is enriched in striatum and contains two Ca^{2+} binding sites formed by E and F helices (EF-hands 1 and 2), of which EF-hands 1 is required for the ability of PP2A activity to be regulated by Ca^{2+} *in vitro* and *in vivo* (Ahn et al., 2007b). These data demonstrate that the PR72-containing form of PP2A is required for glutamate acting at AMPA and NMDA receptors to regulate Thr75 dephosphorylation. In addition, PP2A may be activated through cAMP-dependent signaling through the B56 δ regulatory subunit (**Figure 3**), rendering it responsive to D₁ receptor activation and subject to dysregulation by drugs of abuse. It has been suggested that the B56 δ /AC/PP2A complex also regulates the dephosphorylation of DARPP-32 at Thr75 (Ahn et al., 2007a). As either Ca^{2+} or cAMP through these two regulatory subunits may activate it, PP2A represents a particularly interesting signaling molecule that may contribute to reward learning and addiction.

Ongoing studies in our laboratory suggest that Cdk5 may regulate PKA signaling through additional mechanisms. While the activation of Cdk5 is dependent upon associating with p35, this may also serve as an important regulatory target. Specifically, NMDAR-dependent Ca^{2+} influx causes calpain to convert p35 to p25 (Wei et al., 2005; Meyer et al., 2008; **Figure 4**). The Cdk5/p25 holoenzyme is more soluble than Cdk5/p35 and its specificity may shift away from substrates by which it mediates attenuation of cAMP/PKA signaling. Thus, while Cdk5/p25 has been broadly implicated in neuronal injury and disease (Barnett and Bibb, 2010; Hisanaga and Endo, 2010), it may play an important role in integrating Ca^{2+} and cAMP signaling in localized domains within synapses invoked during reward learning. While there is growing evidence for this concept, it remains to be proven.

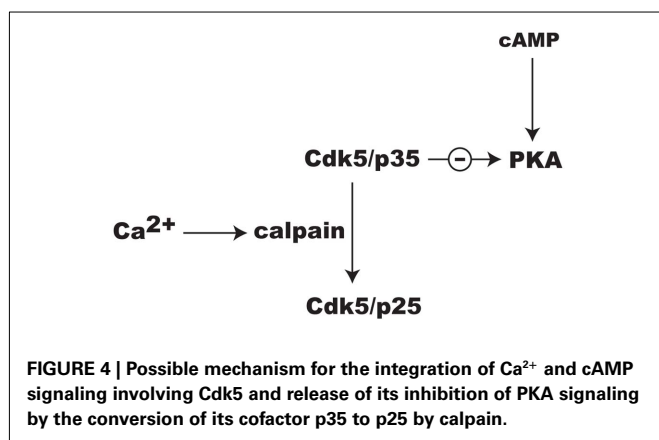
Cyclin-dependent kinase 5 also modulates cocaine-induced plasticity in dendritic spine morphology, a form of structural

plasticity. Rats received intra-NAc infusions of roscovitine or saline during chronic cocaine administration and Cdk5 inhibition attenuated cocaine-induced dendritic spine outgrowth in NAc core and shell 24–48 h after cocaine exposure (Norrholm et al., 2003). Cdk5 phosphorylates and inhibits myocyte enhancing factor 2 (MEF2) that increases dendritic spines in MSNs of the NAc (Pulipparacharuvil et al., 2008). Inhibition of MEF2 activity in response to cocaine can permit transcription of the cytoskeleton-associated genes, Wiskott–Aldrich syndrome protein (WASP), N-WASP, and WASP-family verprolin homologs (WAVEs) that have putative MEF binding sites in their proximal promoter regions. WAVE1 also appears to modulate spine morphogenesis in a Cdk5-dependent manner (Kim et al., 2006; Sung et al., 2008). Thus, increased activation of Cdk5 by repeated cocaine exposure via Δ FosB potentially results in modulation of WAVE activity, whereas MEF2 can modulate its expression level to mediate persistent alterations that could underlie aspects of cocaine-induced plasticity and addiction. Thus, Cdk5 could modulate functional and structural plasticity underlying cognitive inflexibility and maladaptive behaviors characteristic of addiction. The role of Cdk5 in cytoskeletal dynamics, synaptic function, and cell survival (Lopes and Agostinho, 2011) and cognition and plasticity (Angelo et al., 2006; Barnett and Bibb, 2010; Bibb et al., 2010) suggest targeting Cdk5 signaling may be a viable approach to attenuating drug-induced changes in synaptic plasticity that influence addiction behaviors.

EXTRACELLULAR SIGNAL-REGULATED KINASES

Extracellular signal-regulated kinases (ERK) is also involved in the D₁/cAMP/PKA signaling cascade (**Figure 3**; Greengard et al., 1999; Svenningsson et al., 2004). The MAPK kinases responsible for phosphorylating ERK are the ERK kinases II (MEKs). The MAPK kinase responsible for MEK activation include kinases termed Raf. Raf is activated by one of three forms of Ras that is, in turn, activated in response to many types of growth factors to their receptors, such as brain-derived neurotrophic factor (BDNF). ERKs are implicated in long-term neuroplasticity induced by various drugs of abuse (Lu et al., 2006; Girault et al., 2007; Thomas et al., 2008).

Acute cocaine injections rapidly increase ERK phosphorylation in NAc MSNs and dorsal striatum that are blocked by MEK pharmacological inhibition (Valjent et al., 2000) and cocaine-induced ERK phosphorylation is attenuated in the NAc and dorsal striatum in DARPP-32 mutant mice (Valjent et al., 2005). Increased ERK phosphorylation in the NAc has been shown with Δ 9-tetrahydrocannabinol (THC; Valjent et al., 2001, 2004), D-amphetamine (Choe et al., 2002), 3,4-methylene-dioxy-methamphetamine (MDMA; Salzman et al., 2003), morphine (Valjent et al., 2004), and nicotine (Valjent et al., 2004). Morphine-dependent mice undergoing naloxone-precipitated withdrawal show increased ERK activity in NAc but not dorsal striatum compared to controls (Li et al., 2010). Pharmacological inhibition of ERK, abolishes, or attenuates LTP induced by high frequency stimulation of population spikes in the dorsomedial striatum in rat and this effect is, in part, mediated by the ERK pathway coupling to NMDA receptors (Xie et al., 2009). Blockade of ERK phosphorylation in the NAc by direct infusion of a MEK inhibitor



attenuates cocaine-induced activation of CREB (Mattson et al., 2005). Furthermore, the blockade of ERK activity corresponds to the blockade of CREB and activity of the intermediary kinase MSK1 (that directly activates CREB) in the NAc and dorsal striatum (Brami-Cherrier et al., 2005). MEK pharmacological inhibition also blocked cocaine-induced activation of the transcription factor Elk-1 and expression of Fos in the NAc and dorsal striatum (Valjent et al., 2000; Zhang et al., 2004).

Cocaine-induced CPP corresponds to increased ERK phosphorylation in the NAc and dorsal striatum (Valjent et al., 2006) and exposure to the cocaine-paired context increases ERK phosphorylation in the NAc core but not shell (Miller and Marshall, 2005). CPP corresponding to increased ERK activity in striatum has also been obtained with methamphetamine (Mizoguchi et al., 2004). Effects of MEK inhibitors in ERK1 knockout mice suggest it is the ERK2 isoform that is critical for morphine CPP (Mazzucchelli et al., 2002), cocaine CPP, and locomotor sensitization (Ferguson et al., 2006). ERK2 can inhibit ERK1 signaling, possibly by competition for phosphorylation by MEK, suggesting ERK2 is more potent than ERK1 (Valjent et al., 2005). Indeed, ERK1 and ERK2 appear to have opposing roles in drug-induced synaptic plasticity and behavior (Girault et al., 2007), thus ERK2 but not ERK1 appears to be the isoform most critical for modulating behavioral effects of drugs of abuse.

It has also been suggested that processes of memory disruption may underlie the effects of ERK blockade during CPP training on subsequent drug CPP expression (Gerdjikov et al., 2004). The modulation of ERK by drugs of abuse via dopamine and glutamate receptor interactions integrates many second messengers suggesting ERK may function in learned associations between reward and contextual cues (Girault et al., 2007). In addition to a role in classical conditioning, ERK activation is implicated in learning and performance of instrumental responding via regulation of transcription factors affecting intrinsic cellular excitability (Shiflett and Balleine, 2011a,b). ERK signaling cascades and BDNF growth factors influence glutamate transmission modulating LTP and LTD in MSNs of the striatum (McGinty et al., 2010). Chronic EtOH intake attenuates ERK phosphorylation and LTD induction, whereas withdrawal for 1 day potentiates ERK phosphorylation and LTD induction (Cui et al., 2011). Direct infusions of BDNF into the prefrontal cortex decreases responding during extinction training and prevents cocaine-induced decreases in ERK phosphorylation in the NAc but not the dorsal striatum 1 day after drug exposure (McGinty et al., 2010) and prevents cocaine-induced aberrations in extracellular glutamate in the NAc (Berglind et al., 2007, 2009). Thus, the ERK signaling cascade appears to mediate drug-induced synaptic plasticity linked to reward.

Ca²⁺/CALMODULIN-DEPENDENT KINASE II

The most prominent example of a Ca²⁺/calmodulin-dependent kinases (CaMK) with neuronal involvement in addiction and plasticity is CaMKII (Wayman et al., 2008). CaMKII can mediate many of the second messenger actions of Ca²⁺ in neurons (Figure 3). The CaMKII family consists of four subunits termed α , β , γ , and δ isoforms that are encoded by four different genes. CaMKII

contains a catalytic, regulatory, and self-association domain. The regulatory domain contains (1) the autoinhibitory site that binds to and inhibits the catalytic domain while in the resting state, (2) the Ca²⁺/calmodulin-binding site and (3) several regulatory phosphorylation sites. Disinhibition of the autoinhibitory site occurs upon binding of Ca²⁺/calmodulin to the regulatory domain. CaMKII α at Thr286 and CaMKII β at Thr287 undergo autophosphorylation independent of Ca²⁺ that switches the kinase into an autonomous activity mode (Lisman et al., 2002; Wayman et al., 2008). Due to the stimulation-sensor capability of this autophosphorylation switch, CaMKII has been proposed to act as a memory storage mechanism (Lisman et al., 2002).

The effects of psychostimulants on CaMKII activity can vary according to striatal region, drug, and dosing regimen. Acute cocaine increases CaMKII activity in the NAc (Mattson et al., 2005). Acute amphetamine increases CaMKII activity in dorsal striatum and alters the CaMKII substrates, ERK1/2, CREB, and Elk-1 (Choe and Wang, 2002) that is dependent on group I mGlu receptors (Choe and Wang, 2001). Acute methamphetamine actually reduces CaMKII in the NAc and dorsal striatum (Akiyama and Suemaru, 2000). Chronic amphetamine administration increases CaMKII phosphorylation in the striatum but not protein levels (Iwata et al., 1997). Chronic intermittent amphetamine increases striatal levels of CaMKII mRNA (Greenstein et al., 2007). Pharmacological inhibition of CaMKII activity blunts acute amphetamine-induced dopamine efflux via the dopamine transporter (DAT) in mouse midbrain dopamine cultured neurons, striatal brain slices, and *in vivo* (Fog et al., 2006). Acute administration of a D₁-like agonist (Das et al., 1997; Dudman et al., 2003) or NMDA agonist (Das et al., 1997) increases phosphorylation of CREB that is attenuated by CaMKII pharmacological inhibitors in rat striatal neurons.

There is also cross talk between CaMKII and PKA signaling pathways. PKA may promote LTP by stabilizing and/or recycling pools of GluA1 AMPA receptors that are subsequently recruited to the synaptic membrane by Ca²⁺ stimulation of CaMKII (or protein kinase C PKC; see below)-driven trafficking events and GluA1 phosphorylation at Ser818 and Ser831 (Boehm et al., 2006; Guire et al., 2008). Single channel AMPA receptor activity may also be increased during LTP via PKA phosphorylation of Ser845 to increase probability of open channels and CaMKII/PKC phosphorylation of Ser831 to increase single channel conductance (Derkach et al., 2007).

Intriguingly, CaMKII has been implicated as an important negative regulator of the dopamine D₃ receptor subtype that relieves the dopamine receptor-mediated inhibition on sensitized behavior (Liu et al., 2009). It was shown that D₃ receptor activation inhibits AC in acute NAc slices and that NMDA receptor-mediated Ca²⁺ influx restores this inhibition through stimulating CaMKII-binding to the D₃ receptor. Importantly, a peptide derived from the CaMKII-binding site on the D₃ receptor prevented this disinhibition induced by Ca²⁺ influx.

PROTEIN KINASE C

PKC comprises more than 14 isoforms subdivided into categories according to their structure, Ca²⁺ dependence, and lipid

activators (Pearce et al., 2010). All PKC isoforms contain a catalytic domain harboring an ATP binding site and a substrate-binding site. Ca^{2+} in conjunction with the lipid signaling intermediate diacylglycerol (DAG) activate the classical PKCs (α , β I, β II, and γ ; **Figure 3**). DAG but not Ca^{2+} activate novel PKCs (δ , ϵ , η , and θ). Lipids such as phosphatidic and arachidonic acid activate atypical PKCs (ζ , and ι/λ) that are considered to be constitutively active as they require neither DAG nor Ca^{2+} . Ca^{2+} and/or DAG or lipids bind to the R domain to disinhibit the C domain. PKC, similar to CaMKII, mediates multiple second messenger functions of Ca^{2+} to influence substrates such as receptors and ion channels.

There are numerous downstream substrates of PKCs involved in mechanisms of drugs of abuse that include neurotransmitter receptor subtypes such as α 4 subunit of the nicotinic acetylcholine receptor (nAChR) phosphorylated at Ser550 (Pollock et al., 2007), dopamine D_2 receptors phosphorylated at Ser229, Ser228, Thr352, Thr354, and Ser355 (Namkung and Sibley, 2004), the γ_2 subunit of the GABA_A receptor phosphorylated at Ser327 of the γ_2 subunit of the GABA_A receptor (Qi et al., 2007), and cannabinoid CB1 receptors phosphorylated at Ser317 (Garcia et al., 1998). Other PKC substrate proteins are involved in exocytosis (e.g., Ser187 of SNAP-25; Nagy et al., 2002) and synaptic plasticity (e.g., Ser36 of neurogranin and Ser41 of neuromodulin; Huang et al., 1993; Oehrlein et al., 1996) are also targeted by PKC.

PKC also phosphorylates Ser818 of the AMPA glutamate receptor subunit GluA1 that facilitates AMPA receptor externalization and LTP (Boehm et al., 2006). PKC and MAPK signaling pathways appear to be involved in AMPA receptor trafficking underlying an *in vitro* model of classical conditioning in pond turtles, *Pseudemys scripta elegans* (Zheng and Keifer, 2008). PKC activation facilitates autophosphorylated CaMKII and increased association with NMDA receptors in conjunction with NMDA postsynaptic receptor insertion that was, along with PKC-induced LTP of the AMPA receptor-mediated response, abolished by a CaMKII antagonist or by disruption of CaMKII interaction with NR2A or NR2B (Yan et al., 2011).

Due to the lack of selectivity of pharmacological inhibitors for specific PKC isoforms, targeted gene disruption in mouse models is increasingly being used to explore the precise role of PKC isoforms in behaviors related to addiction (Olive and Newton, 2010). PKC β knockout mice have a reduced locomotor-activating effect of acute amphetamine and this isozyme is critical for effects on DAT (Chen et al., 2009). PKC γ knockout mice fail to exhibit morphine CPP (Narita et al., 2001) and show increased EtOH consumption in a two-bottle choice procedure (Bowers and Wehner, 2001). PKC ϵ knockout mice show enhanced locomotor stimulant effects of lower doses of EtOH (Hodge et al., 1999, 2002) and potentiated rewarding and reinforcing effects of morphine (Newton et al., 2007). However, PKC ϵ knockout mice consumed approximately 75% less EtOH and exhibit weaker EtOH preference in a two-bottle choice task compared to wildtype controls (Hodge et al., 1999). Another study showed that PKC ϵ knockout mice exhibit reduced EtOH self-administration and attenuated alcohol deprivation effect (Olive et al., 2000). PKC ϵ knockout mice

are more sensitive to the aversive and less sensitive to the rewarding effects of EtOH in CPP (Newton and Messing, 2007). PKC ϵ knockout mice acquire morphine intravenous self-administration at doses that do not maintain self-administration in wild-type controls (Newton et al., 2007).

These pharmacological and genetic techniques suggest a meaningful role for PKC in neuroadaptations and behavioral alterations pertinent to addiction. Indeed, the wealth of data especially on PKC ϵ obtained in drug self-administration has led to its inclusion on a panel of markers for haplotype analysis of addiction-related genes (Hodgkinson et al., 2008). Thus, the operation of PKC isozymes and their signaling pathways provide another route to the development of pharmacotherapeutics for addiction.

FUTURE DIRECTIONS

Here we have made attempt to integrate different aspects of addiction including behavioral considerations and animal models, considerations of circuitry and its mediation of both reward and addiction learning, and have cataloged a small portion of the underlying signaling mechanism that mediate normal and addiction-related motivated behaviors. Focus on the cellular and molecular neurobiology of signal transduction has allowed the elucidation of mechanisms beyond the synaptic level that modulate addictive drug-induced functional and structural plasticity. Behavioral assays related to drug addiction that dissociates measures of relapse from reinforcement and dependence provides powerful tools to evaluate the effects of targeting specific intracellular signaling pathways. However, understanding of the molecular mechanism involved and relating them complex pathophysiological and behavioral basis of addiction is incomplete and remains a challenge. Current and future research on addiction driven toward reversing drug-induced pathology that impairs synaptic plasticity will undoubtedly include the systematic manipulation of mechanisms of protein phosphorylation.

The transition to the addicted state may initiate with a cascade of neuroadaptations from the ventral striatum to dorsal striatum. However, the delineation of drug-induced plasticity in neurocircuitry corresponding to stages of binge intoxication, withdrawal-induced negative affect and preoccupation/anticipation involves other critical brain regions that include but are not limited to the ventral tegmental area, extended amygdala, prefrontal cortex, cingulate gyrus, and hippocampus. A better understanding of the neurocircuitry altered by drugs of abuse will help form a heuristic basis in the search for genetic, molecular, and pharmacological neuroadaptations conferring risk for or protection against addiction and aid in the identification of novel targets for pharmacotherapeutics with increased therapeutic efficacy and decreased side effect liability.

ACKNOWLEDGMENTS

This work was supported by a NARSAD Young Investigator Award (to Scott D. Philibin); National Institute of Drug Abuse grants DA026482, DA010460, DA008227 (to David W. Self), DA16672 (to James A. Bibb); and National Institute of Mental Health grants MH079710 and MH083711 (to James A. Bibb).

REFERENCES

- Ahn, J. H., Mcavoy, T., Rakhilin, S. V., Nishi, A., Greengard, P., and Nairn, A. C. (2007a). Protein kinase A activates protein phosphatase 2A by phosphorylation of the B56delta subunit. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2979–2984.
- Ahn, J. H., Sung, J. Y., Mcavoy, T., Nishi, A., Janssens, V., Goris, J., Greengard, P., and Nairn, A. C. (2007b). The B α /PR72 subunit mediates Ca $^{2+}$ -dependent dephosphorylation of DARPP-32 by protein phosphatase 2A. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9876–9881.
- Akiyama, K., and Suemaru, J. (2000). Effect of acute and chronic administration of methamphetamine on calcium-calmodulin dependent protein kinase II activity in the rat brain. *Ann. N. Y. Acad. Sci.* 914, 263–274.
- Alleweireldt, A. T., Kirschner, K. F., Blake, C. B., and Neisewander, J. L. (2003). D1-receptor drugs and cocaine-seeking behavior: investigation of receptor mediation and behavioral disruption in rats. *Psychopharmacology (Berl.)* 168, 109–117.
- Angelo, M., Plattner, E., and Giese, K. P. (2006). Cyclin-dependent kinase 5 in synaptic plasticity, learning and memory. *J. Neurochem.* 99, 353–370.
- Bachtell, R. K., Choi, K. H., Simmons, D. L., Falcon, E., Monteggia, L. M., Neve, R. L., and Self, D. W. (2008). Role of GluR1 expression in nucleus accumbens neurons in cocaine sensitization and cocaine-seeking behavior. *Eur. J. Neurosci.* 27, 2229–2240.
- Bachtell, R. K., Whisler, K., Karanian, D., and Self, D. W. (2005). Effects of intra-nucleus accumbens shell administration of dopamine agonists and antagonists on cocaine-taking and cocaine-seeking behaviors in the rat. *Psychopharmacology (Berl.)* 183, 41–53.
- Backstrom, P., and Hyttia, P. (2007). Involvement of AMPA/kainate, NMDA, and mGlu5 receptors in the nucleus accumbens core in cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl.)* 192, 571–580.
- Barnett, D. G., and Bibb, J. A. (2010). The role of Cdk5 in cognition and neuropsychiatric and neurological pathology. *Brain Res. Bull.* 85, 9–13.
- Barrot, M., Olivier, J. D., Perrotti, L. I., Dileone, R. J., Berton, O., Eisch, A. J., Impey, S., Storm, D. R., Neve, R. L., Yin, J. C., Zachariou, V., and Nestler, E. J. (2002). CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11435–11440.
- Bassareo, V., De Luca, M. A., and Di Chiara, G. (2002). Differential expression of motivational stimulus properties by dopamine in nucleus accumbens shell versus core and prefrontal cortex. *J. Neurosci.* 22, 4709–4719.
- Bassareo, V., Musio, P., and Di Chiara, G. (2011). Reciprocal responsiveness of nucleus accumbens shell and core dopamine to food- and drug-conditioned stimuli. *Psychopharmacology (Berl.)* 214, 687–697.
- Benavides, D. R., Quinn, J. J., Zhong, P., Hawasli, A. H., Dileone, R. J., Kansy, J. W., Olausson, P., Yan, Z., Taylor, J. R., and Bibb, J. A. (2007). Cdk5 modulates cocaine reward, motivation, and striatal neuron excitability. *J. Neurosci.* 27, 12967–12976.
- Beninger, R. J., Nakonechny, P. L., and Savina, I. (2003). cAMP-dependent protein kinase and reward-related learning: intra-accumbens Rp-cAMPS blocks amphetamine-produced place conditioning in rats. *Psychopharmacology (Berl.)* 170, 23–32.
- Berglind, W. J., See, R. E., Fuchs, R. A., Ghee, S. M., Whitfield, T. W. Jr., Miller, S. W., and McGinty, J. F. (2007). A BDNF infusion into the medial prefrontal cortex suppresses cocaine seeking in rats. *Eur. J. Neurosci.* 26, 757–766.
- Berglind, W. J., Whitfield, T. W. Jr., Lalumiere, R. T., Kalivas, P. W., and McGinty, J. F. (2009). A single intra-PFC infusion of BDNF prevents cocaine-induced alterations in extracellular glutamate within the nucleus accumbens. *J. Neurosci.* 29, 3715–3719.
- Berridge, K. C., and Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Brain Res. Rev.* 28, 309–369.
- Berridge, K. C., Robinson, T. E., and Aldridge, J. W. (2009). Dissecting components of reward: “liking,” “wanting,” and learning. *Curr. Opin. Pharmacol.* 9, 65–73.
- Bibb, J. A., Chen, J., Taylor, J. R., Svenningsson, P., Nishi, A., Snyder, G. L., Yan, Z., Sagawa, Z. K., Ouimet, C. C., Nairn, A. C., Nestler, E. J., and Greengard, P. (2001). Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature* 410, 376–380.
- Bibb, J. A., Mayford, M. R., Tsien, J. Z., and Alberini, C. M. (2010). Cognition enhancement strategies. *J. Neurosci.* 30, 14987–14992.
- Bibb, J. A., Snyder, G. L., Nishi, A., Yan, Z., Meijer, L., Fienberg, A. A., Tsai, L. H., Kwon, Y. T., Girault, J. A., Czernik, A. J., Haganir, R. L., Hemmings, H. C., Jr., Nairn, A. C., and Greengard, P. (1999). Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature* 402, 669–671.
- Blumer, J. B., Cismowski, M. J., Sato, M., and Lanier, S. M. (2005). AGS proteins: receptor-independent activators of G-protein signaling. *Trends Pharmacol. Sci.* 26, 470–476.
- Blumer, J. B., and Lanier, S. M. (2003). Accessory proteins for G protein-signaling systems: activators of G protein signaling and other nonreceptor proteins influencing the activation state of G proteins. *Recept. Channels* 9, 195–204.
- Boehm, J., Kang, M. G., Johnson, R. C., Esteban, J., Haganir, R. L., and Malinow, R. (2006). Synaptic incorporation of AMPA receptors during LTP is controlled by a PKC phosphorylation site on GluR1. *Neuron* 51, 213–225.
- Borgland, S. L., Malenka, R. C., and Bonci, A. (2004). Acute and chronic cocaine-induced potentiation of synaptic strength in the ventral tegmental area: electrophysiological and behavioral correlates in individual rats. *J. Neurosci.* 24, 7482–7490.
- Bower, G., and Grusec, T. (1964). Effect of prior pavlovian discrimination training upon learning an operant discrimination. *J. Exp. Anal. Behav.* 7, 401–404.
- Bowers, B. J., and Wehner, J. M. (2001). Ethanol consumption and behavioral impulsivity are increased in protein kinase Cgamma null mutant mice. *J. Neurosci.* 21, RC180.
- Bowers, M. S. (2010). Activators of G-protein signaling 3: a drug addiction molecular gateway. *Behav. Pharmacol.* 21, 500–513.
- Bowers, M. S., Chen, B. T., and Bonci, A. (2010). AMPA receptor synaptic plasticity induced by psychostimulants: the past, present, and therapeutic future. *Neuron* 67, 11–24.
- Bowers, M. S., Hopf, F. W., Chou, J. K., Guillery, A. M., Chang, S. J., Janak, P. H., Bonci, A., and Diamond, I. (2008). Nucleus accumbens AGS3 expression drives ethanol seeking through G betagamma. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12533–12538.
- Bowers, M. S., McFarland, K., Lake, R. W., Peterson, Y. K., Lapish, C. C., Gregory, M. L., Lanier, S. M., and Kalivas, P. W. (2004). Activator of G protein signaling 3: a gatekeeper of cocaine sensitization and drug seeking. *Neuron* 42, 269–281.
- Brami-Cherrier, K., Valjent, E., Herve, D., Darrah, J., Corvol, J. C., Pages, C., Arruth, S. J., Girault, J. A., and Caboche, J. (2005). Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. *J. Neurosci.* 25, 11444–11454.
- Burchett, S. A., Volk, M. L., Bannan, M. J., and Granneman, J. G. (1998). Regulators of G protein signaling: rapid changes in mRNA abundance in response to amphetamine. *J. Neurochem.* 70, 2216–2219.
- Cadoni, C., and Di Chiara, G. (1999). Reciprocal changes in dopamine responsiveness in the nucleus accumbens shell and core and in the dorsal caudate-putamen in rats sensitized to morphine. *Neuroscience* 90, 447–455.
- Cadoni, C., and Di Chiara, G. (2000). Differential changes in accumbens shell and core dopamine in behavioral sensitization to nicotine. *Eur. J. Pharmacol.* 387, R23–R25.
- Cadoni, C., Solinas, M., and Di Chiara, G. (2000). Psychostimulant sensitization: differential changes in accumbal shell and core dopamine. *Eur. J. Pharmacol.* 388, 69–76.
- Caine, S. B., Thomsen, M., Gabriel, K. I., Berkowitz, J. S., Gold, L. H., Koob, G. F., Tonegawa, S., Zhang, J., and Xu, M. (2007). Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. *J. Neurosci.* 27, 13140–13150.
- Carlezon, W. A. Jr., Duman, R. S., and Nestler, E. J. (2005). The many faces of CREB. *Trends Neurosci.* 28, 436–445.
- Carlezon, W. A. Jr., Thome, J., Olson, V. G., Lane-Ladd, S. B., Brodtkin, E. S., Hiroi, N., Duman, R. S., Neve, R. L., and Nestler, E. J. (1998). Regulation of cocaine reward by CREB. *Science* 282, 2272–2275.
- Chen, J., Kelz, M. B., Hope, B. T., Nakabeppu, Y., and Nestler, E. J. (1997). Chronic Fos-related antigens: stable variants of deltaFosB induced in brain by chronic treatments. *J. Neurosci.* 17, 4933–4941.
- Chen, J., Nye, H. E., Kelz, M. B., Hiroi, N., Nakabeppu, Y., Hope, B. T., and Nestler, E. J. (1995). Regulation of delta FosB and FosB-like proteins by electroconvulsive seizure and cocaine treatments. *Mol. Pharmacol.* 48, 880–889.
- Chen, R., Furman, C. A., Zhang, M., Kim, M. N., Gereau, R. W. T., Leitges, M., and Gnegy, M. E. (2009). Protein kinase Cbeta is a critical regulator of dopamine transporter trafficking and regulates the behavioral response to amphetamine in mice. *J. Pharmacol. Exp. Ther.* 328, 912–920.
- Choe, E. S., Chung, K. T., Mao, L., and Wang, J. Q. (2002). Amphetamine increases phosphorylation of extracellular signal-regulated kinase and transcription factors in the rat striatum via group I metabotropic glutamate receptors.

- Neuropsychopharmacology* 27, 565–575.
- Choe, E. S., and Wang, J. Q. (2001). Group I metabotropic glutamate receptors control phosphorylation of CREB, Elk-1 and ERK via a CaMKII-dependent pathway in rat striatum. *Neurosci. Lett.* 313, 129–132.
- Choe, E. S., and Wang, J. Q. (2002). CaMKII regulates amphetamine-induced ERK1/2 phosphorylation in striatal neurons. *Neuroreport* 13, 1013–1016.
- Colby, C. R., Whisler, K., Steffen, C., Nestler, E. J., and Self, D. W. (2003). Striatal cell type-specific overexpression of deltaFosB enhances incentive for cocaine. *J. Neurosci.* 23, 2488–2493.
- Cole, R. L., Konradi, C., Douglass, J., and Hyman, S. E. (1995). Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. *Neuron* 14, 813–823.
- Compton, W. M., and Volkow, N. D. (2006). Abuse of prescription drugs and the risk of addiction. *Drug Alcohol Depend.* 83(Suppl. 1), S4–S7.
- Crabbe, J. C. (2002). Genetic contributions to addiction. *Annu. Rev. Psychol.* 53, 435–462.
- Cui, S. Z., Wang, S. J., Li, J., Xie, G. Q., Zhou, R., Chen, L., and Yuan, X. R. (2011). Alteration of synaptic plasticity in rat dorsal striatum induced by chronic ethanol intake and withdrawal via ERK pathway. *Acta Pharmacol. Sin.* 32, 175–181.
- Das, S., Grunert, M., Williams, L., and Vincent, S. R. (1997). NMDA and D1 receptors regulate the phosphorylation of CREB and the induction of c-fos in striatal neurons in primary culture. *Synapse* 25, 227–233.
- Derkach, V. A., Oh, M. C., Guire, E. S., and Soderling, T. R. (2007). Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat. Rev. Neurosci.* 8, 101–113.
- Dias, C., Lachize, S., Boilet, V., Huitel, E., and Cador, M. (2004). Differential effects of dopaminergic agents on locomotor sensitization and on the reinstatement of cocaine-seeking and food-seeking behaviour. *Psychopharmacology (Berl.)* 175, 414–427.
- Drevets, W. C., Gautier, C., Price, J. C., Kupfer, D. J., Kinahan, P. E., Grace, A. A., Price, J. L., and Mathis, C. A. (2001). Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. *Biol. Psychiatry* 49, 81–96.
- Dudman, J. T., Eaton, M. E., Rajadhyaksha, A., Macias, W., Taher, M., Barczak, A., Kameyama, K., Haganir, R., and Konradi, C. (2003). Dopamine D1 receptors mediate CREB phosphorylation via phosphorylation of the NMDA receptor at Ser897-NR1. *J. Neurochem.* 87, 922–934.
- Edwards, S., Whisler, K. N., Fuller, D. C., Orsulak, P. J., and Self, D. W. (2007). Addiction-related alterations in D1 and D2 dopamine receptor behavioral responses following chronic cocaine self-administration. *Neuropsychopharmacology* 32, 354–366.
- Engelhardt, S., and Rochais, F. (2007). G proteins: more than transducers of receptor-generated signals? *Circ. Res.* 100, 1109–1111.
- Epstein, D. H., Preston, K. L., Stewart, J., and Shaham, Y. (2006). Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology (Berl.)* 189, 1–16.
- Ettenberg, A. (1990). Haloperidol prevents the reinstatement of amphetamine-rewarded runway responding in rats. *Pharmacol. Biochem. Behav.* 36, 635–638.
- Ferguson, S. M., Fasano, S., Yang, P., Brambilla, R., and Robinson, T. E. (2006). Knockout of ERK1 enhances cocaine-evoked immediate early gene expression and behavioral plasticity. *Neuropsychopharmacology* 31, 2660–2668.
- Fog, J. U., Khoshbouei, H., Holy, M., Owens, W. A., Vaegter, C. B., Sen, N., Nikandrova, Y., Bowton, E., McMahon, D. G., Colbran, R. J., Daws, L. C., Sitte, H. H., Javitch, J. A., Galli, A., and Gether, U. (2006). Calmodulin kinase II interacts with the dopamine transporter C terminus to regulate amphetamine-induced reverse transport. *Neuron* 51, 417–429.
- Garcia, D. E., Brown, S., Hille, B., and Mackie, K. (1998). Protein kinase C disrupts cannabinoid actions by phosphorylation of the CB1 cannabinoid receptor. *J. Neurosci.* 18, 2834–2841.
- Garzon, J., Rodriguez-Munoz, M., and Sanchez-Blazquez, P. (2008). Do pharmacological approaches that prevent opioid tolerance target different elements in the same regulatory machinery? *Curr. Drug Abuse Rev.* 1, 222–238.
- Gerdeman, G. L., Partridge, J. G., Lupica, C. R., and Lovinger, D. M. (2003). It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends Neurosci.* 26, 184–192.
- Gerdjikov, T. V., Ross, G. M., and Beninger, R. J. (2004). Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of ERK or p38 MAP kinases in rats. *Behav. Neurosci.* 118, 740–750.
- Ghasemzadeh, M. B., Mueller, C., and Vasudevan, P. (2009). Behavioral sensitization to cocaine is associated with increased glutamate receptor trafficking to the postsynaptic density after extended withdrawal period. *Neuroscience* 159, 414–426.
- Girault, J. A., Valjent, E., Caboche, J., and Herve, D. (2007). ERK2: a logical and gate critical for drug-induced plasticity? *Curr. Opin. Pharmacol.* 7, 77–85.
- Gold, S. J., Ni, Y. G., Dohman, H. G., and Nestler, E. J. (1997). Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. *J. Neurosci.* 17, 8024–8037.
- Gonzalez, G. A., and Montminy, M. R. (1989). Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell* 59, 675–680.
- Grafstein-Dunn, E., Young, K. H., Cockett, M. I., and Khawaja, X. Z. (2001). Regional distribution of regulators of G-protein signaling (RGS) 1, 2, 13, 14, 16, and GAIIP messenger ribonucleic acids by in situ hybridization in rat brain. *Brain Res. Mol. Brain Res.* 88, 113–123.
- Greengard, P., Allen, P. B., and Nairn, A. C. (1999). Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* 23, 435–447.
- Greenstein, R., Novak, G., and Seeman, P. (2007). Amphetamine sensitization elevates CaMKIIbeta mRNA. *Synapse* 61, 827–834.
- Guire, E. S., Oh, M. C., Soderling, T. R., and Derkach, V. A. (2008). Recruitment of calcium-permeable AMPA receptors during synaptic potentiation is regulated by CaM-kinase I. *J. Neurosci.* 28, 6000–6009.
- Hausdorff, W. P., Caron, M. G., and Lefkowitz, R. J. (1990). Turning off the signal: desensitization of beta-adrenergic receptor function. *FASEB J.* 4, 2881–2889.
- Hemby, S. E., Co, C., Koves, T. R., Smith, J. E., and Dworkin, S. I. (1997). Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. *Psychopharmacology (Berl.)* 133, 7–16.
- Hemby, S. E., Horman, B., and Tang, W. (2005a). Differential regulation of ionotropic glutamate receptor subunits following cocaine self-administration. *Brain Res.* 1064, 75–82.
- Hemby, S. E., Tang, W., Muly, E. C., Kuhar, M. J., Howell, L., and Mash, D. C. (2005b). Cocaine-induced alterations in nucleus accumbens ionotropic glutamate receptor subunits in human and non-human primates. *J. Neurochem.* 95, 1785–1793.
- Hemmings, H. C. Jr., Greengard, P., Tung, H. Y., and Cohen, P. (1984). DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. *Nature* 310, 503–505.
- Hiroi, N., Brown, J. R., Haile, C. N., Ye, H., Greenberg, M. E., and Nestler, E. J. (1997). FosB mutant mice: loss of chronic cocaine induction of Fos-related proteins and heightened sensitivity to cocaine's psychomotor and rewarding effects. *Proc. Natl. Acad. Sci. U.S.A.* 94, 10397–10402.
- Hisanaga, S., and Endo, R. (2010). Regulation and role of cyclin-dependent kinase activity in neuronal survival and death. *J. Neurochem.* 115, 1309–1321.
- Hodge, C. W., Mehmert, K. K., Kelley, S. P., McMahon, T., Haywood, A., Olive, M. F., Wang, D., Sanchez-Perez, A. M., and Messing, R. O. (1999). Supersensitivity to allosteric GABA(A) receptor modulators and alcohol in mice lacking PKCepsilon. *Nat. Neurosci.* 2, 997–1002.
- Hodge, C. W., Raber, J., McMahon, T., Walter, H., Sanchez-Perez, A. M., Olive, M. F., Mehmert, K., Morrow, A. L., and Messing, R. O. (2002). Decreased anxiety-like behavior, reduced stress hormones, and neurosteroid supersensitivity in mice lacking protein kinase Cepsilon. *J. Clin. Invest.* 110, 1003–1010.
- Hodgkinson, C. A., Yuan, Q., Xu, K., Shen, P. H., Heinz, E., Lobos, E. A., Binder, E. B., Cubells, J., Ehlers, C. L., Gelernter, J., Mann, J., Riley, B., Roy, A., Tabakoff, B., Todd, R. D., Zhou, Z., and Goldman, D. (2008). Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. *Alcohol Alcohol.* 43, 505–515.
- Hooks, S. B., Martemyanov, K., and Zachariou, V. (2008). A role of RGS proteins in drug addiction. *Biochem. Pharmacol.* 75, 76–84.
- Huang, K. P., Huang, F. L., and Chen, H. C. (1993). Characterization of a 7.5-kDa protein kinase C substrate (RC3 protein, neurogranin) from rat brain. *Arch. Biochem. Biophys.* 305, 570–580.
- Hyman, S. E., Malenka, R. C., and Nestler, E. J. (2006). Neural mechanisms of addiction: the role of reward-related learning and

- memory. *Annu. Rev. Neurosci.* 29, 565–598.
- Ikemoto, S. (2003). Involvement of the olfactory tubercle in cocaine reward: intracranial self-administration studies. *J. Neurosci.* 23, 9305–9311.
- Ikemoto, S., Glazier, B. S., Murphy, J. M., and McBride, W. J. (1997). Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. *J. Neurosci.* 17, 8580–8587.
- Iwata, S. I., Hewlett, G. H., Ferrell, S. T., Kantor, L., and Gnegy, M. E. (1997). Enhanced dopamine release and phosphorylation of synapsin I and neuromodulin in striatal synaptosomes after repeated amphetamine. *J. Pharmacol. Exp. Ther.* 283, 1445–1452.
- Jacobs, E. H., Smit, A. B., De Vries, T. J., and Schoffelmeier, A. N. (2005). Long-term gene expression in the nucleus accumbens following heroin administration is subregion-specific and depends on the nature of drug administration. *Addict. Biol.* 10, 91–100.
- Kalivas, P. W., and O'Brien, C. (2008). Drug addiction as a pathology of staged neuroplasticity. *Neuropsychopharmacology* 33, 166–180.
- Kalivas, P. W., and Volkow, N. D. (2005). The neural basis of addiction: a pathology of motivation and choice. *Am. J. Psychiatry* 162, 1403–1413.
- Karasawa, J., Yoshimizu, T., and Chaki, S. (2006). A metabotropic glutamate 2/3 receptor antagonist, MGS0039, increases extracellular dopamine levels in the nucleus accumbens shell. *Neurosci. Lett.* 393, 127–130.
- Kasanetz, F., Deroche-Gamonet, V., Berson, N., Balado, E., Lafourcade, M., Manzoni, O., and Piazza, P. V. (2010). Transition to addiction is associated with a persistent impairment in synaptic plasticity. *Science* 328, 1709–1712.
- Kauer, J. A., and Malenka, R. C. (2007). Synaptic plasticity and addiction. *Nat. Rev. Neurosci.* 8, 844–858.
- Kelley, A. E., and Schiltz, C. A. (2004). Accessories to addiction: G protein regulators play a key role in cocaine seeking and neuroplasticity. *Neuron* 42, 181–183.
- Kelz, M. B., Chen, J., Carlezon, W. A. Jr., Whisler, K., Gilden, L., Beckmann, A. M., Steffen, C., Zhang, Y. J., Marotti, L., Self, D. W., Tkatch, T., Baranaukas, G., Surmeier, D. J., Neve, R. L., Duman, R. S., Picciotto, M. R., and Nestler, E. J. (1999). Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature* 401, 272–276.
- Kim, Y., Sung, J. Y., Ceglia, I., Lee, K. W., Ahn, J. H., Halford, J. M., Kim, A. M., Kwak, S. P., Park, J. B., Ho Ryu, S., Schenck, A., Bardoni, B., Scott, J. D., Nairn, A. C., and Greengard, P. (2006). Phosphorylation of WAVE1 regulates actin polymerization and dendritic spine morphology. *Nature* 442, 814–817.
- Koeltzow, T. E., and Vezina, P. (2005). Locomotor activity and cocaine-seeking behavior during acquisition and reinstatement of operant self-administration behavior in rats. *Behav. Brain Res.* 160, 250–259.
- Koob, G. F., and Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* 24, 97–129.
- Koob, G. F., and Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology* 35, 217–238.
- Krupnick, J. G., and Benovic, J. L. (1998). The role of receptor kinases and arrestins in G protein-coupled receptor regulation. *Annu. Rev. Pharmacol. Toxicol.* 38, 289–319.
- Lalumiere, R. T., and Kalivas, P. W. (2008). Glutamate release in the nucleus accumbens core is necessary for heroin seeking. *J. Neurosci.* 28, 3170–3177.
- Lee, K. W., Kim, Y., Kim, A. M., Helmin, K., Nairn, A. C., and Greengard, P. (2006). Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proc. Natl. Acad. Sci. U.S.A.* 103, 3399–3404.
- Li, D., Aperia, A., Celsi, G., Da Cruz E Silva, E. F., Greengard, P., and Meister, B. (1995). Protein phosphatase-1 in the kidney: evidence for a role in the regulation of medullary Na(+)-K(+)-ATPase. *Am. J. Physiol.* 269, F673–F680.
- Li, T., Hou, Y., Cao, W., Yan, C. X., Chen, T., and Li, S. B. (2010). Naloxone-precipitated withdrawal enhances ERK phosphorylation in prefrontal association cortex and accumbens nucleus of morphine-dependent mice. *Neurosci. Lett.* 468, 348–352.
- Lisman, J., Schulman, H., and Cline, H. (2002). The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat. Rev. Neurosci.* 3, 175–190.
- Liu, Q. S., Pu, L., and Poo, M. M. (2005). Repeated cocaine exposure in vivo facilitates LTP induction in mid-brain dopamine neurons. *Nature* 437, 1027–1031.
- Liu, X. Y., Mao, L. M., Zhang, G. C., Papasian, C. J., Fibuch, E. E., Lan, H. X., Zhou, H. F., Xu, M., and Wang, J. Q. (2009). Activity-dependent modulation of limbic dopamine D3 receptors by CaMKII. *Neuron* 61, 425–438.
- Lopes, J. P., and Agostinho, P. (2011). Cdk5: multitasking between physiological and pathological conditions. *Prog. Neurobiol.* 94, 49–63.
- Lu, L., Grimm, J. W., Shaham, Y., and Hope, B. T. (2003). Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats. *J. Neurochem.* 85, 1604–1613.
- Lu, L., Koya, E., Zhai, H., Hope, B. T., and Shaham, Y. (2006). Role of ERK in cocaine addiction. *Trends Neurosci.* 29, 695–703.
- Luscher, C., and Ungless, M. A. (2006). The mechanistic classification of addictive drugs. *PLoS Med.* 3, e437. doi: 10.1371/journal.pmed.0030437
- Madayag, A., Lobner, D., Kau, K. S., Mantsch, J. R., Abdulhameed, O., Hearing, M., Grier, M. D., and Baker, D. A. (2007). Repeated N-acetylcysteine administration alters plasticity-dependent effects of cocaine. *J. Neurosci.* 27, 13968–13976.
- Malinow, R., and Malenka, R. C. (2002). AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* 25, 103–126.
- Martin, M., Chen, B. T., Hopf, F. W., Bowers, M. S., and Bonci, A. (2006). Cocaine self-administration selectively abolishes LTD in the core of the nucleus accumbens. *Nat. Neurosci.* 9, 868–869.
- Mattson, B. J., Bossert, J. M., Simmons, D. E., Nozaki, N., Nagarkar, D., Kreuter, J. D., and Hope, B. T. (2005). Cocaine-induced CREB phosphorylation in nucleus accumbens of cocaine-sensitized rats is enabled by enhanced activation of extracellular signal-related kinase, but not protein kinase A. *J. Neurochem.* 95, 1481–1494.
- Mayr, B., and Montminy, M. (2001). Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat. Rev. Mol. Cell Biol.* 2, 599–609.
- Mazzucchi, C., Vantaggiato, C., Ciamei, A., Fasano, S., Pakhotin, P., Krezel, W., Welzl, H., Wolfer, D. P., Pages, G., Valverde, O., Marowsky, A., Porrazzo, A., Orban, P. C., Maldonado, R., Ehrenguber, M. U., Cestari, V., Lipp, H. P., Chapman, P. F., Pouyssegur, J., and Brambilla, R. (2002). Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. *Neuron* 34, 807–820.
- McClung, C. A., and Nestler, E. J. (2003). Regulation of gene expression and cocaine reward by CREB and deltaFosB. *Nat. Neurosci.* 6, 1208–1215.
- McFarland, K., Davidge, S. B., Lapish, C. C., and Kalivas, P. W. (2004). Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. *J. Neurosci.* 24, 1551–1560.
- McGinty, J. F., Whitfield, T. W. Jr., and Berglund, W. J. (2010). Brain-derived neurotrophic factor and cocaine addiction. *Brain Res.* 1314, 183–193.
- Meyer, D. A., Richer, E., Benkovic, S. A., Hayashi, K., Kansy, J. W., Hale, C. F., Moy, L. Y., Kim, Y., O'callaghan, J. P., Tsai, L. H., Greengard, P., Nairn, A. C., Cowan, C. W., Miller, D. B., Antich, P., and Bibb, J. A. (2008). Striatal dysregulation of Cdk5 alters locomotor responses to cocaine, motor learning, and dendritic morphology. *Proc. Natl. Acad. Sci. U.S.A.* 105, 18561–18566.
- Miller, C. A., and Marshall, J. F. (2005). Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. *Neuron* 47, 873–884.
- Milton, A. L., and Everitt, B. J. (2010). The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. *Eur. J. Neurosci.* 31, 2308–2319.
- Mitchell, J. A., Channell, S., and Hall, G. (1985). Response-reinforcer associations after caudate-putamen lesions in the rat: spatial discrimination and overshadowing-potential effects in instrumental learning. *Behav. Neurosci.* 99, 1074–1088.
- Mitchell, J. A., and Hall, G. (1988). Learning in rats with caudate-putamen lesions: unimpaired classical conditioning and beneficial effects of redundant stimulus cues on instrumental and spatial learning deficits. *Behav. Neurosci.* 102, 504–514.
- Mizoguchi, H., Yamada, K., Mizuno, M., Mizuno, T., Nitta, A., Noda, Y., and Nabeshima, T. (2004). Regulations of methamphetamine reward by extracellular signal-regulated kinase 1/2/ets-like gene-1 signaling pathway via the activation of dopamine receptors. *Mol. Pharmacol.* 65, 1293–1301.
- Moratalla, R., Elibol, B., Vallejo, M., and Graybiel, A. M. (1996). Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. *Neuron* 17, 147–156.
- Morgan, J. I., and Curran, T. (1995). Immediate-early genes: ten years on. *Trends Neurosci.* 18, 66–67.

- Moussawi, K., and Kalivas, P. W. (2010). Group II metabotropic glutamate receptors (mGlu2/3) in drug addiction. *Eur. J. Pharmacol.* 639, 115–122.
- Muller, D. L., and Unterwald, E. M. (2005). D1 dopamine receptors modulate deltaFosB induction in rat striatum after intermittent morphine administration. *J. Pharmacol. Exp. Ther.* 314, 148–154.
- Nagerl, U. V., Eberhorn, N., Cambridge, S. B., and Bonhoeffer, T. (2004). Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron* 44, 759–767.
- Nagy, G., Matti, U., Nehring, R. B., Binz, T., Rettig, J., Neher, E., and Sorensen, J. B. (2002). Protein kinase C-dependent phosphorylation of synaptosome-associated protein of 25 kDa at Ser187 potentiates vesicle recruitment. *J. Neurosci.* 22, 9278–9286.
- Namkung, Y., and Sibley, D. R. (2004). Protein kinase C mediates phosphorylation, desensitization, and trafficking of the D2 dopamine receptor. *J. Biol. Chem.* 279, 49533–49541.
- Narita, M., Aoki, T., Ozaki, S., Yajima, Y., and Suzuki, T. (2001). Involvement of protein kinase Cgamma isoform in morphine-induced reinforcing effects. *Neuroscience* 103, 309–314.
- Natocin, M., Lester, B., Peterson, Y. K., Bernard, M. L., Lanier, S. M., and Artemyev, N. O. (2000). AGS3 inhibits GDP dissociation from galpha subunits of the Gi family and rhodopsin-dependent activation of transducin. *J. Biol. Chem.* 275, 40981–40985.
- Neve, K. A., Seamans, J. K., and Trantham-Davidson, H. (2004). Dopamine receptor signaling. *J. Recept. Signal Transduct. Res.* 24, 165–205.
- Newton, P. M., Kim, J. A., Mcgeehan, A. J., Paredes, J. P., Chu, K., Wallace, M. J., Roberts, A. J., Hodge, C. W., and Messing, R. O. (2007). Increased response to morphine in mice lacking protein kinase C epsilon. *Genes Brain Behav.* 6, 329–338.
- Newton, P. M., and Messing, R. O. (2007). Increased sensitivity to the aversive effects of ethanol in PKCepsilon null mice revealed by place conditioning. *Behav. Neurosci.* 121, 439–442.
- Nomoto, S., Adachi, K., Yang, L. X., Hirata, Y., Muraguchi, S., and Kiuchi, K. (1997). Distribution of RGS4 mRNA in mouse brain shown by in situ hybridization. *Biochem. Biophys. Res. Commun.* 241, 281–287.
- Norrholm, S. D., Bibb, J. A., Nestler, E. J., Ouimet, C. C., Taylor, J. R., and Greengard, P. (2003). Cocaine-induced proliferation of dendritic spines in nucleus accumbens is dependent on the activity of cyclin-dependent kinase-5. *Neuroscience* 116, 19–22.
- Nye, H. E., Hope, B. T., Kelz, M. B., Iadarola, M., and Nestler, E. J. (1995). Pharmacological studies of the regulation of chronic FOS-related antigen induction by cocaine in the striatum and nucleus accumbens. *J. Pharmacol. Exp. Ther.* 275, 1671–1680.
- Oehrlein, S. A., Parker, P. J., and Herget, T. (1996). Phosphorylation of GAP-43 (growth-associated protein of 43 kDa) by conventional, novel and atypical isoforms of the protein kinase C gene family: differences between oligopeptide and polypeptide phosphorylation. *Biochem. J.* 317(Pt 1), 219–224.
- Okamoto, K., Nagai, T., Miyawaki, A., and Hayashi, Y. (2004). Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. *Nat. Neurosci.* 7, 1104–1112.
- Olive, M. F., Mehmert, K. K., Messing, R. O., and Hodge, C. W. (2000). Reduced operant ethanol self-administration and in vivo mesolimbic dopamine responses to ethanol in PKCepsilon-deficient mice. *Eur. J. Neurosci.* 12, 4131–4140.
- Olive, M. F., and Newton, P. M. (2010). Protein kinase C isozymes as regulators of sensitivity to and self-administration of drugs of abuse—studies with genetically modified mice. *Behav. Pharmacol.* 21, 493–499.
- Ouimet, C. C., Langley-Gullion, K. C., and Greengard, P. (1998). Quantitative immunocytochemistry of DARPP-32-expressing neurons in the rat caudateputamen. *Brain Res.* 808, 8–12.
- Pearce, L. R., Komander, D., and Alessi, D. R. (2010). The nuts and bolts of AGC protein kinases. *Nat. Rev. Mol. Cell Biol.* 11, 9–22.
- Penela, P., Ribas, C., and Mayor, F. Jr. (2003). Mechanisms of regulation of the expression and function of G protein-coupled receptor kinases. *Cell. Signal.* 15, 973–981.
- Perrotti, L. I., Weaver, R. R., Robinson, B., Renthall, W., Maze, I., Yazdani, S., Elmore, R. G., Knapp, D. J., Selley, D. E., Martin, B. R., Sim-Selley, L., Bachtell, R. K., Self, D. W., and Nestler, E. J. (2008). Distinct patterns of deltaFosB induction in brain by drugs of abuse. *Synapse* 62, 358–369.
- Phillips, P. E., Stuber, G. D., Heien, M. L., Wightman, R. M., and Carelli, R. M. (2003). Subsecond dopamine release promotes cocaine seeking. *Nature* 422, 614–618.
- Pierce, R. C., and Vanderschuren, L. J. (2010). Kicking the habit: the neural basis of ingrained behaviors in cocaine addiction. *Neurosci. Biobehav. Rev.* 35, 212–219.
- Ping, A., Xi, J., Prasad, B. M., Wang, M. H., and Kruzich, P. J. (2008). Contributions of nucleus accumbens core and shell GluR1 containing AMPA receptors in AMPA- and cocaine-primed reinstatement of cocaine-seeking behavior. *Brain Res.* 1215, 173–182.
- Pollock, V. V., Pastoor, T. E., and Wecker, L. (2007). Cyclic AMP-dependent protein kinase (PKA) phosphorylates Ser362 and 467 and protein kinase C phosphorylates Ser550 within the M3/M4 cytoplasmic domain of human nicotinic receptor alpha4 subunits. *J. Neurochem.* 103, 456–466.
- Porrino, L. J., Smith, H. R., Nader, M. A., and Beveridge, T. J. (2007). The effects of cocaine: a shifting target over the course of addiction. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31, 1593–1600.
- Prus, A. J., James, J. R., and Rosecrans, J. A. (2009). “Conditioned place preference,” in *Methods of Behavior Analysis in Neuroscience*, 2nd Edn., Chap. 4, ed. J. J. Buccafusco (Boca Raton, FL: CRC Press).
- Psifogeorgou, K., Papakosta, P., Russo, S. J., Neve, R. L., Kardassis, D., Gold, S. J., and Zachariou, V. (2007). RGS9-2 is a negative modulator of mu-opioid receptor function. *J. Neurochem.* 103, 617–625.
- Pulipparacharuvil, S., Renthall, W., Hale, C. F., Taniguchi, M., Xiao, G., Kumar, A., Russo, S. J., Sikder, D., Dewey, C. M., Davis, M. M., Greengard, P., Nairn, A. C., Nestler, E. J., and Cowan, C. W. (2008). Cocaine regulates MEF2 to control synaptic and behavioral plasticity. *Neuron* 59, 621–633.
- Qi, Z. H., Song, M., Wallace, M. J., Wang, D., Newton, P. M., McMahon, T., Chou, W. H., Zhang, C., Shokat, K. M., and Messing, R. O. (2007). Protein kinase C epsilon regulates gamma-aminobutyrate type A receptor sensitivity to ethanol and benzodiazepines through phosphorylation of gamma2 subunits. *J. Biol. Chem.* 282, 33052–33063.
- Rahman, Z., Gold, S. J., Potenza, M. N., Cowan, C. W., Ni, Y. G., He, W., Wensel, T. G., and Nestler, E. J. (1999). Cloning and characterization of RGS9-2: a striatal-enriched alternatively spliced product of the RGS9 gene. *J. Neurosci.* 19, 2016–2026.
- Richards, G., Messer, J., Malherbe, P., Pink, R., Brockhaus, M., Stadler, H., Wichmann, J., Schaffhauser, H., and Mutel, V. (2005). Distribution and abundance of metabotropic glutamate receptor subtype 2 in rat brain revealed by [3H]LY354740 binding in vitro and quantitative radioautography: correlation with the sites of synthesis, expression, and agonist stimulation of [35S]GTPgamma binding. *J. Comp. Neurol.* 487, 15–27.
- Robbins, T. W., Ersche, K. D., and Everitt, B. J. (2008). Drug addiction and the memory systems of the brain. *Ann. N. Y. Acad. Sci.* 1141, 1–21.
- Saal, D., Dong, Y., Bonci, A., and Malenka, R. C. (2003). Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron* 37, 577–582.
- Salzmann, J., Marie-Claire, C., Le Guen, S., Roques, B. P., and Noble, F. (2003). Importance of ERK activation in behavioral and biochemical effects induced by MDMA in mice. *Br. J. Pharmacol.* 140, 831–838.
- Schmidt, H. D., Anderson, S. M., Famous, K. R., Kumaresan, V., and Pierce, R. C. (2005). Anatomy and pharmacology of cocaine priming-induced reinstatement of drug seeking. *Eur. J. Pharmacol.* 526, 65–76.
- Schmidt, H. D., Anderson, S. M., and Pierce, R. C. (2006). Stimulation of D1-like or D2 dopamine receptors in the shell, but not the core, of the nucleus accumbens reinstates cocaine-seeking behavior in the rat. *Eur. J. Neurosci.* 23, 219–228.
- Schultz, W., Tremblay, L., and Hollerman, J. R. (2000). Reward processing in primate orbitofrontal cortex and basal ganglia. *Cereb. Cortex* 10, 272–284.
- Schumann, J., and Yaka, R. (2009). Prolonged withdrawal from repeated noncontingent cocaine exposure increases NMDA receptor expression and ERK activity in the nucleus accumbens. *J. Neurosci.* 29, 6955–6963.
- Self, D. W., Barnhart, W. J., Lehman, D. A., and Nestler, E. J. (1996). Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. *Science* 271, 1586–1589.

- Self, D. W., and Stein, L. (1992). The D1 agonists SKF 82958 and SKF 77434 are self-administered by rats. *Brain Res.* 582, 349–352.
- Shaham, Y., Shalev, U., Lu, L., De Wit, H., and Stewart, J. (2003). The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology (Berl.)* 168, 3–20.
- Shaw-Lutchman, T. Z., Barrot, M., Wallace, T., Gilden, L., Zachariou, V., Impey, S., Duman, R. S., Storm, D., and Nestler, E. J. (2002). Regional and cellular mapping of cAMP response element-mediated transcription during naltrexone-precipitated morphine withdrawal. *J. Neurosci.* 22, 3663–3672.
- Shiflett, M. W., and Balleine, B. W. (2011a). Contributions of ERK signaling in the striatum to instrumental learning and performance. *Behav. Brain Res.* 218, 240–247.
- Shiflett, M. W., and Balleine, B. W. (2011b). Molecular substrates of action control in cortico-striatal circuits. *Prog. Neurobiol.* 95, 1–13.
- Shippenberg, T. S., and Rea, W. (1997). Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. *Pharmacol. Biochem. Behav.* 57, 449–455.
- Sung, J. Y., Engmann, O., Teylan, M. A., Nairn, A. C., Greengard, P., and Kim, Y. (2008). WAVE1 controls neuronal activity-induced mitochondrial distribution in dendritic spines. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3112–3116.
- Surmeier, D. J., Bargas, J., Hemmings, H. C. Jr., Nairn, A. C., and Greengard, P. (1995). Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* 14, 385–397.
- Surmeier, D. J., Ding, J., Day, M., Wang, Z., and Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci.* 30, 228–235.
- Sutton, M. A., McGibney, K., and Beninger, R. J. (2000). Conditioned locomotion in rats following amphetamine infusion into the nucleus accumbens: blockade by coincident inhibition of protein kinase A. *Behav. Pharmacol.* 11, 365–376.
- Svenningsson, P., Nairn, A. C., and Greengard, P. (2005). DARPP-32 mediates the actions of multiple drugs of abuse. *AAPS J.* 7, E353–E360.
- Svenningsson, P., Nishi, A., Fissue, G., Girault, J. A., Nairn, A. C., and Greengard, P. (2004). DARPP-32: an integrator of neurotransmission. *Annu. Rev. Pharmacol. Toxicol.* 44, 269–296.
- Taylor, J. R., Lynch, W. J., Sanchez, H., Olausson, P., Nestler, E. J., and Bibb, J. A. (2007). Inhibition of Cdk5 in the nucleus accumbens enhances the locomotor-activating and incentive-motivational effects of cocaine. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4147–4152.
- Taymans, J. M., Wintmolders, C., Te Riele, P., Jurzak, M., Groenewegen, H. J., Leysen, J. E., and Langlois, X. (2002). Detailed localization of regulator of G protein signaling 2 messenger ribonucleic acid and protein in the rat brain. *Neuroscience* 114, 39–53.
- Tepper, J. M., Abercrombie, E. D., and Bolam, J. P. (2007). Basal ganglia macrocircuits. *Prog. Brain Res.* 160, 3–7.
- Thomas, E. A., Danielson, P. E., and Sutcliffe, J. G. (1998). RGS9: a regulator of G-protein signalling with specific expression in rat and mouse striatum. *J. Neurosci. Res.* 52, 118–124.
- Thomas, M. J., Beurrier, C., Bonci, A., and Malenka, R. C. (2001). Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat. Neurosci.* 4, 1217–1223.
- Thomas, M. J., Kalivas, P. W., and Shaham, Y. (2008). Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. *Br. J. Pharmacol.* 154, 327–342.
- Torregrassa, M. M., Corlett, P. R., and Taylor, J. R. (2011). Aberrant learning and memory in addiction. *Neurobiol. Learn. Mem.* PMID: 21376820. [Epub ahead of print].
- Traynor, J. (2010). Regulator of G protein-signaling proteins and addictive drugs. *Ann. N. Y. Acad. Sci.* 1187, 341–352.
- Turgeon, S. M., Pollack, A. E., and Fink, J. S. (1997). Enhanced CREB phosphorylation and changes in c-Fos and FRA expression in striatum accompany amphetamine sensitization. *Brain Res.* 749, 120–126.
- Ungless, M. A., Whistler, J. L., Malenka, R. C., and Bonci, A. (2001). Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature* 411, 583–587.
- Valjent, E., Corbille, A. G., Bertran-Gonzalez, J., Herve, D., and Girault, J. A. (2006). Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2932–2937.
- Valjent, E., Corvol, J. C., Pages, C., Besson, M. J., Maldonado, R., and Caboche, J. (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J. Neurosci.* 20, 8701–8709.
- Valjent, E., Pages, C., Herve, D., Girault, J. A., and Caboche, J. (2004). Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. *Eur. J. Neurosci.* 19, 1826–1836.
- Valjent, E., Pages, C., Rogard, M., Besson, M. J., Maldonado, R., and Caboche, J. (2001). Delta 9-tetrahydrocannabinol-induced MAPK/ERK and Elk-1 activation in vivo depends on dopaminergic transmission. *Eur. J. Neurosci.* 14, 342–352.
- Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., Stipanovich, A., Caboche, J., Lombroso, P. J., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 102, 491–496.
- Volkow, N. D. (2005). What do we know about drug addiction? *Am. J. Psychiatry* 162, 1401–1402.
- Volkow, N. D., Wang, G. J., Fowler, J. S., Gatley, S. J., Ding, Y. S., Logan, J., Dewey, S. L., Hitzemann, R., and Lieberman, J. (1996). Relationship between psychostimulant-induced “high” and dopamine transporter occupancy. *Proc. Natl. Acad. Sci. U.S.A.* 93, 10388–10392.
- Walters, C. L., and Blendy, J. A. (2001). Different requirements for cAMP response element binding protein in positive and negative reinforcing properties of drugs of abuse. *J. Neurosci.* 21, 9438–9444.
- Wayman, G. A., Lee, Y. S., Tokumitsu, H., Silva, A. J., and Soderling, T. R. (2008). Calmodulin-kinases: modulators of neuronal development and plasticity. *Neuron* 59, 914–931.
- Weed, M. R., Vanover, K. E., and Woolverton, W. L. (1993). Reinforcing effect of the D1 dopamine agonist SKF 81297 in rhesus monkeys. *Psychopharmacology (Berl.)* 113, 51–52.
- Wei, F. Y., Tomizawa, K., Ohshima, T., Asada, A., Saito, T., Nguyen, C., Bibb, J. A., Ishiguro, K., Kulkarni, A. B., Pant, H. C., Mikoshiba, K., Matsui, H., and Hisanaga, S. (2005). Control of cyclin-dependent kinase 5 (Cdk5) activity by glutamatergic regulation of p35 stability. *J. Neurochem.* 93, 502–512.
- Weiss, F. (2010). “Advances in animal models of relapse for addiction research,” in *Advances in the Neuroscience of Addiction*, 2nd Edn., Chap. 1, eds C. M. Kuhn and G. F. Koob (Boca Raton, FL: CRC Press).
- Wise, R. A., Leone, P., Rivest, R., and Leeb, K. (1995a). Elevations of nucleus accumbens dopamine and DOPAC levels during intravenous heroin self-administration. *Synapse* 21, 140–148.
- Wise, R. A., Newton, P., Leeb, K., Burnette, B., Pocock, D., and Justice, J. B. Jr. (1995b). Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. *Psychopharmacology (Berl.)* 120, 10–20.
- Wise, R. A., and Rompre, P. P. (1989). Brain dopamine and reward. *Annu. Rev. Psychol.* 40, 191–225.
- Wolf, M. E. (2010). Regulation of AMPA receptor trafficking in the nucleus accumbens by dopamine and cocaine. *Neurotox. Res.* 18, 393–409.
- Woolverton, W. L., Goldberg, L. I., and Ginos, J. Z. (1984). Intravenous self-administration of dopamine receptor agonists by rhesus monkeys. *J. Pharmacol. Exp. Ther.* 230, 678–683.
- Xi, Z. X., Kiyatkin, M., Li, X., Peng, X. Q., Wiggins, A., Spiller, K., Li, J., and Gardner, E. L. (2010). N-acetylaspartylglutamate (NAAG) inhibits intravenous cocaine self-administration and cocaine-enhanced brain-stimulation reward in rats. *Neuropharmacology* 58, 304–313.
- Xi, Z. X., Ramamoorthy, S., Baker, D. A., Shen, H., Samuvel, D. J., and Kalivas, P. W. (2002). Modulation of group II metabotropic glutamate receptor signaling by chronic cocaine. *J. Pharmacol. Exp. Ther.* 303, 608–615.
- Xie, G. Q., Wang, S. J., Li, J., Cui, S. Z., Zhou, R., Chen, L., and Yuan, X. R. (2009). Ethanol attenuates the HFS-induced, ERK-mediated LTP in a dose-dependent manner in rat striatum. *Alcohol. Clin. Exp. Res.* 33, 121–128.
- Yan, J. Z., Xu, Z., Ren, S. Q., Hu, B., Yao, W., Wang, S. H., Liu, S. Y., and Lu, W. (2011). Protein kinase C promotes N-methyl-D-aspartate (NMDA)

- receptor trafficking by indirectly triggering calcium/calmodulin-dependent protein kinase II (CaMKII) autophosphorylation. *J. Biol. Chem.* 286, 25187–25200.
- Yokel, R. A., and Wise, R. A. (1978). Amphetamine-type reinforcement by dopaminergic agonists in the rat. *Psychopharmacology (Berl.)* 58, 289–296.
- Zachariou, V., Bolanos, C. A., Selley, D. E., Theobald, D., Cassidy, M. P., Kelz, M. B., Shaw-Lutchman, T., Berton, O., Sim-Selley, L. J., Dileone, R. J., Kumar, A., and Nestler, E. J. (2006). An essential role for deltaFosB in the nucleus accumbens in morphine action. *Nat. Neurosci.* 9, 205–211.
- Zachariou, V., Georgescu, D., Sanchez, N., Rahman, Z., Dileone, R., Berton, O., Neve, R. L., Sim-Selley, L. J., Selley, D. E., Gold, S. J., and Nestler, E. J. (2003). Essential role for RGS9 in opiate action. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13656–13661.
- Zhang, L., Lou, D., Jiao, H., Zhang, D., Wang, X., Xia, Y., Zhang, J., and Xu, M. (2004). Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. *J. Neurosci.* 24, 3344–3354.
- Zheng, Z., and Keifer, J. (2008). Protein kinase C-dependent and independent signaling pathways regulate synaptic GluR1 and GluR4 AMPAR subunits during in vitro classical conditioning. *Neuroscience* 156, 872–884.
- Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
- Received: 10 May 2011; accepted: 01 September 2011; published online: 20 September 2011.
- Citation: Philibin SD, Hernandez A, Self DW and Bibb JA (2011) Striatal signal transduction and drug addiction. *Front. Neuroanat.* 5:60. doi: 10.3389/fnana.2011.00060
- Copyright © 2011 Philibin, Hernandez, Self and Bibb. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Post-translational modification biology of glutamate receptors and drug addiction

Li-Min Mao¹, Ming-Lei Guo¹, Dao-Zhong Jin¹, Eugene E. Fibuch², Eun Sang Choe^{1,3} and John Q. Wang^{1,2*}

¹ Department of Basic Medical Science, School of Medicine, University of Missouri-Kansas City, Kansas City, MO, USA

² Department of Anesthesiology, School of Medicine, University of Missouri-Kansas City, Kansas City, MO, USA

³ Department of Biological Sciences, Pusan National University, Pusan, Korea

Edited by:

Emmanuel Valjent, Université Montpellier 1 and 2, France

Reviewed by:

Peter Kalivas, University of South Carolina, USA

Karen K. Szumlanski, University of California Santa Barbara, USA

*Correspondence:

John Q. Wang, Department of Basic Medical Science, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108, USA.
e-mail: wangjq@umkc.edu

Post-translational covalent modifications of glutamate receptors remain a hot topic. Early studies have established that this family of receptors, including almost all ionotropic and metabotropic glutamate receptor subtypes, undergoes active phosphorylation at serine, threonine, or tyrosine residues in their intracellular domains. Recent evidence identifies several glutamate receptor subtypes to be direct substrates for palmitoylation at cysteine residues. Other modifications such as ubiquitination and sumoylation at lysine residues also occur to certain glutamate receptors. These modifications are dynamic and reversible in nature and are regulatable by changing synaptic inputs. The regulated modifications significantly impact the receptor in many ways, including interrelated changes in biochemistry (synthesis, subunit assembling, and protein-protein interactions), subcellular redistribution (trafficking, endocytosis, synaptic delivery, and clustering), and physiology, usually associated with changes in synaptic plasticity. Glutamate receptors are enriched in the striatum and cooperate closely with dopamine to regulate striatal signaling. Emerging evidence shows that modification processes of striatal glutamate receptors are sensitive to addictive drugs, such as psychostimulants (cocaine and amphetamine). Altered modifications are believed to be directly linked to enduring receptor/synaptic plasticity and drug-seeking. This review summarizes several major types of modifications of glutamate receptors and analyzes the role of these modifications in striatal signaling and in the pathogenesis of psychostimulant addiction.

Keywords: AMPA, NMDA, mGluR, dopamine, phosphorylation, palmitoylation, ubiquitination, sumoylation

INTRODUCTION

The neurotransmitter l-glutamate (glutamate) interacts with specific ionotropic glutamate receptors (iGluR) or metabotropic glutamate receptors (mGluR; Dingledine et al., 1999; Cull-Candy et al., 2001). The former are the ligand-gated ion channel and are classified into N-methyl-D-aspartate receptors (NMDAR), α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPA), and kainate receptors. The latter are the G protein-coupled receptor. Through various G proteins, they connect to multiple second messenger systems. There are three functional groups of mGluRs (group I–III) classified from eight subtypes (mGluR1–8; Conn and Pin, 1997). Group I mGluRs (mGluR1/5 subtypes) are positively coupled to phospholipase C β 1 through G α_q proteins. Activation of mGluR1/5 increases phosphoinositol hydrolysis, resulting in intracellular Ca²⁺ release and protein kinase C (PKC) activation (Conn and Pin, 1997). Both group II (mGluR2/3) and group III (mGluR4/6/7/8) receptors are negatively coupled to adenylyl cyclase through G α_i/o proteins. Their activation reduces cAMP formation and inhibits protein kinase A (PKA).

Diverse post-translational modifications occur at intracellular domains of glutamate receptors and represent regulatory mechanisms for controlling many properties of modified receptors. Early extensive studies have established a phosphorylation modification of both iGluRs and mGluRs (Swope et al., 1999; Wang et al., 2006). By targeting specific amino acids (serine, threonine, and tyrosine),

multiple protein kinases and phosphatases control phosphorylation levels of glutamate receptors. Recent work identifies fatty acylation, such as palmitoylation, as another important type of modification at cysteine residues. Lysine residues may also be modified by the enzymatic cascades known as ubiquitination and sumoylation. All these modifications are noticeably physiological events and sensitive to changing synaptic inputs. They are labile, dynamic and reversible in nature, unlike other modifications, such as prenylation, farnesylation, and geranylation, which are semi-permanent and serve to anchor proteins to membranes. Thus, these reversible modifications are believed to activity-dependently regulate and assure normal expression and function of glutamate receptors. As such, malfunction of these modifications is frequently associated with the pathogenesis of various neuropsychiatric disorders.

The striatum, i.e., the dorsal caudate putamen and ventral nucleus accumbens (NAc), is a central structure in reward circuits implicated in drug addiction. This region is enriched with glutamatergic innervation and glutamate receptors. The accumulated data support the role of striatal glutamatergic transmission in drug addiction (Tzschantke and Schmidt, 2003; Hyman et al., 2006). Recently, emerging evidence links post-translational modifications of glutamate receptors to excitatory synaptic plasticity and drug-seeking behavior. Generally, modification processes of striatal glutamate receptors are sensitive to addictive drugs such as the psychostimulants (cocaine and amphetamine). Altered modifications

contribute to enduring receptor plasticity and the addictive properties of drugs. This review will primarily discuss post-translational modifications of glutamate receptor and their associations with drug action, while roles of individual glutamate receptors in drug addiction have been thoroughly reviewed elsewhere (Bird and Lawrence, 2009; Kalivas, 2009; Bowers et al., 2010; Moussawi and Kalivas, 2010; Schmidt and Pierce, 2010). We will focus on several major types of modifications appreciated recently, including phosphorylation, palmitoylation, ubiquitination, and sumoylation, based on the available data linking them to drug addiction. Of note, in addition to addiction, abnormalities in the modifications of glutamate receptors can be observed in many other disease models, such as schizophrenia (Li et al., 2009), anxiety (Delawary et al., 2010), and Parkinson's disease (Ba et al., 2006).

PHOSPHORYLATION OF GLUTAMATE RECEPTORS AT SERINE/THREONINE

α -AMINO-3-HYDROXY-5-METHYLISOXAZOLE-4-PROPIONIC ACID RECEPTORS

Phosphorylation of AMPARs at serine/threonine has been well established (Wang et al., 2006). Most AMPARs become functional upon a heteromeric assembly of four subunits (GluA1–4 or GluR1–4). A tetrameric structure seems to be prototypic and is assembled by dimer-of-dimers of GluA2 and either GluA1, GluA3, or GluA4. As a membrane-bound receptor, four subunit proteins share the same conformation in the plasma membrane: four transmembrane domains (TMD; M1–4) with an extracellular N-terminus and an intracellular C-terminus. C-termini are variable in length among subunits and are intracellular domains for protein–protein interactions and serine/threonine phosphorylation (Carvalho et al., 2000). To date, the GluA1 C-terminus has four identified phosphorylation sites at serine 818 (S818), S831, threonine 840 (T840), and S845 (Roche et al., 1996; Barria et al., 1997; Mammen et al., 1997; **Table 1**). Other subunits are also similarly phosphorylated at their C-terminal serine and/or threonine residues (Chung et al., 2000; Wang et al., 2006). S818 is phosphorylated by PKC (Boehm et al., 2006) and S831 by both PKC and Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII; Roche et al., 1996; Barria et al., 1997; Mammen et al., 1997). T840 is not a substrate of the common kinases such as PKA, PKC, or CaMKII, but it appears to be a substrate of p70S6 kinase (Delgado et al., 2007). Finally, S845 is specific for PKA-mediated phosphorylation (Roche et al., 1996).

Phosphorylation is one of the post-translational modifications that are labile and reversible. In fact, AMPAR phosphorylation is subject to the vigorous regulation by synaptic signals. Regulated phosphorylation in turn adjusts expression, distribution (synaptic delivery) and function of the receptor usually in a site-specific fashion. Acute phosphorylation of GluA1 S818 by PKC promoted GluA1 synaptic incorporation and an activity-dependent form of synaptic plasticity, long-term potentiation (LTP; Boehm et al., 2006). PKC/CaMKII-sensitive S831 and PKA-sensitive S845 phosphorylation potentiated AMPAR currents and augmented LTP (Roche et al., 1996; Derkach et al., 1999; Banke et al., 2000). Activity-driven PKA phosphorylation of S845 also drove AMPARs to synapses, which was necessary for LTP (Estaban et al., 2003). T840 is dephosphorylated by NMDAR signals, which is implicated in long-term depression (LTD; Delgado et al., 2007).

AMPA receptors are densely expressed in striatal medium spiny output neurons and certain types of interneurons (Martin et al., 1993; Bernard et al., 1997). These postsynaptic receptors are present in the perikarya, dendrites, and spines with a significant amount of extrasynaptic receptors on these subcellular specializations. The site-specific phosphorylation of striatal GluA1 subunits in response to drug exposure has been extensively studied. It is apparent that dopamine is a strong regulator of their phosphorylation. By stimulating D1 dopamine receptors and associated cAMP/PKA pathways, the D1 agonist and psychostimulants (cocaine and amphetamines) increased GluA1 phosphorylation preferentially at S845 in striatal neurons (Price et al., 1999; Snyder et al., 2000; Chao et al., 2002). This facilitated surface/synaptic delivery of AMPARs and potentiated efficacy and strength of excitatory synapses (Price et al., 1999; Mangiavacchi and Wolf, 2004; Swayze et al., 2004). In contrast to D1 signals, the D2 receptor inhibits S845 phosphorylation (Hakansson et al., 2006).

Plastic changes in AMPAR phosphorylation may constitute an important layer of underlying mechanisms for receptor plasticity and drug addiction. Several studies in chronic drug administration models (passive repeated administration or operant self-administration) have established an association (correlation) between AMPAR phosphorylation and enduring behavioral plasticity (behavioral sensitization and more significantly drug-seeking behavior), although a causal link between them remains to be proven experimentally. For instance, cocaine-sensitized or heroin self-administering rodents were associated with increased phosphorylation at S845 in the NAc (Zhang et al., 2007; Edwards et al., 2009; Chen et al., 2010; but Mattson et al., 2005; Chen and Manev, 2010) and at S831 in the CPu (Kim et al., 2009). Increased accumbens shell S831 phosphorylation was seen in animals showing reinstatement of cocaine-seeking (Anderson et al., 2008). In addition to GluA1, elevated GluA2 S880 phosphorylation in the NAc was related to reinstatement of cocaine-seeking (Famous et al., 2008). These results imply a phosphorylation-dependent mechanism for AMPAR plasticity and drug-seeking. S845/S831 phosphorylation is likely to be upregulated to increase surface AMPAR expression and thereby enhance AMPAR transmission related to behavioral plasticity (Boudreau and Wolf, 2005; Conrad et al., 2008). However, self-administration of cocaine induced lesser S845 phosphorylation in the striatum as compared to acute cocaine injection, establishing a tolerance of S845 phosphorylation in response to chronic cocaine (Edwards et al., 2007). This tolerance may reflect a downregulated GluA1 function in accumbens neurons and may contribute to cocaine sensitization and cocaine-seeking behavior (Sutton et al., 2003; Bachtell et al., 2008). These results underscore the complexity of AMPARs' roles in drug action. Distinct roles of the receptor may derive from differences in subsets of synapses, subpopulations of projection and interneurons, subdivisions of the NAc (core versus shell), early or late withdrawal times and addiction stages, different administration/test regimens, and types of behavior surveyed.

N-METHYL-D-ASPARTATE RECEPTORS

Like AMPARs, NMDARs are tetrameric assemblies of two obligatory GluN1 subunits and two modulatory GluN2 subunits (Dingledine et al., 1999; Cull-Candy et al., 2001). The difference

Table 1 | Post-translational modifications of glutamate receptors.

Modification	Receptor	Subtype	Site	Enzyme	Physiological/ pathophysiological impact	References
Phosphorylation	AMPA	GluA1	S818 (CT)	PKC	Promote synaptic incorporation and critical for LTP	Boehm et al. (2006)
			S831 (CT)	PKC/CaMKII	Increase currents and channel conductance	Roche et al. (1996), Barria et al. (1997), Mammen et al. (1997)
			T840 (CT)	p70S6	Dephosphorylation links to LTD	Delgado et al. (2007)
			S845 (CT)	PKA	Increase synaptic delivery/peak currents and critical for LTP	Roche et al. (1996), Estaban et al. (2003)
		GluA2	S880 (CT)	PKC	Disrupt GRIP1/2 binding and promote endocytosis and LTD	Chung et al. (2000)
		GluA2	Y876 (CT)	Src	Disrupt GRIP1/2 binding and promote endocytosis and LTD	Hayashi and Huganir (2004), Ahmadian et al. (2004), Fox et al. (2007)
	NMDAR	GluN1	S890 (CT)	PKC	Disperse surface clustering	Tingley et al. (1997)
			S896 (CT)	PKC	ND	Tingley et al. (1997)
			S897 (CT)	PKA	ND	Tingley et al. (1997)
		GluN2A	Y1325 (CT)	Src	Increase NMDAR activity and contribute to depression	Taniguchi et al. (2009)
		GluN2B	S1303 (CT)	PKC/CaMKII	Enhance NMDAR function	Omkumar et al. (1996), Liao et al. (2001)
			S1323 (CT)	PKC	Enhance NMDAR function	Liao et al. (2001)
			S1480 (CT)	CK2	Increase endocytosis	Sanz-Clemente et al. (2010)
			Y1336 (CT)	Fyn	Increase calpain cleavage	Wu et al. (2007)
			Y1472 (CT)	Fyn	Link to LTP	Nakazawa et al. (2001)
		GluNC	S1244 (CT)	PKA/PKC	Regulate channel kinetics	Chen et al. (2006)
			S1096 (CT)	PKB/Akt	Increase surface expression	Chen and Roche (2009)
	mGluR	mGluR1a	T695 (IL2)	PKC	Desensitization	Medler and Bruch (1999), Francesconi and Duvoisin (2000)
		mGluR5	T840/S839 (CT)	PKC	Generate oscillatory Ca ²⁺ responses	Kawabata et al. (1996), Kim et al. (2005)
		mGluR5	T606/S613 (IL1), T665/T681 (IL2), S881/S890 (CT)	PKC	Desensitization	Gereau and Heinemann (1998)
		mGluR5	T1164/S1167 (CT)	CDK5	Increase Homer binding	Orlando et al. (2009)
		mGluR2	S843 (CT)	PKA	Inhibit function	Schaffhauser et al. (2000)
		mGluR3	S845 (CT)	PKA	Inhibit function	Cai et al. (2001)
		mGluR4a/7a/8a	S859/S862/S855 (CT)	PKA	Inhibit function	Cai et al. (2001)
		mGluR7	S862 (CT)	PKC	Inhibit calmodulin binding and increase surface expression	Airas et al. (2001), Suh et al. (2008)
Palmitoylation	AMPA	GluA1/2/3/4	C585/C610/C615/C611 (TMD2)	GODZ/DHHC3	Golgi retention	Hayashi et al. (2005)
		GluA1/2/3/4	C811/C836/C841/C817 (CT)	ND	Disrupt 4.1N binding and promote endocytosis	Hayashi et al. (2005)

(Continued)

Table 1 | Continued

Modification	Receptor	Subtype	Site	Enzyme	Physiological/ pathophysiological impact	References
Ubiquitination	NMDAR	GluN2A	C848/C853/ C870 (CT, 1st cluster)	GODZ/ DHHC3	Increase tyrosine phosphorylation and surface expression	Hayashi et al. (2009)
		GluN2A	C1214/C1217/ C1236/C1239 (CT, 2nd cluster)	GODZ/ DHHC3	Golgi retention	Hayashi et al. (2009)
		GluN2B	C848/C854/ C871 (CT, 1st cluster)	GODZ/ DHHC3	Increase tyrosine phosphorylation and surface expression	Hayashi et al. (2009)
		GluN2B	C1215/C1218/ C1239/C1242/ C1245 (CT, 2nd cluster)	GODZ/ DHHC3	Golgi retention	Hayashi et al. (2009)
	Kainate	GluK6	C827/C840 (CT)	ND	Regulate PKC phosphorylation of GluK6	Pickering et al. (1995)
	mGluR	mGluR4	ND	ND	ND	Alaluf et al. (1995)
	NMDAR	GluN1	ND	ND	Decrease expression	Ratnam and Teichberg (2005), Kato et al. (2005)
		GluN2B	ND	Mind bomb-2	Decrease NMDAR activity	Jurd et al. (2008)
Sumoylation	Kainate	GluK6	ND (I884 is critical for binding enzyme)	ND	Decrease surface expression	Salinas et al. (2006)
	mGluR	mGluR1a/5	ND	Siah1A	Decrease expression	Moriyoshi et al. (2004)
	Kainate	GluK6	K886 (CT)	PIAS3	Agonist-induced endocytosis	Martin et al. (2007)
	Kainate	GluK7	ND	SUMO-1	ND	Wilkinson et al. (2008)
	mGluR	mGluR8	K882 (CT)	PIAS1	ND	Tang et al. (2005)
	mGluR	mGluR4/6/7	ND	SUMO-1	ND	Wilkinson et al. (2008)

ND, not determined; IL1, intracellular loop 1; IL2, intracellular loop 2; CT, C-terminus. See text for other abbreviations.

in GluN2 subunit compositions (GluN2A–D) largely determines the distinct gating and pharmacology of the channel. As a transmembrane receptor, each NMDAR subunit shares an extracellular N-terminus and an intracellular C-terminus. The C-terminal region is large especially for GluN2A and GluN2B, which provides a spacious area for protein kinase interactions and serine/threonine phosphorylation. Indeed, three NMDAR subunits (GluN1, GluN2B, and GluN2C) have been found to undergo active phosphorylation at distinct serine/threonine sites. Within the GluN1 C-terminal domain, PKC phosphorylates S890 and S896 (Tingley et al., 1997). An S896-neighboring site, S897, is a substrate of PKA (Tingley et al., 1997). GluN2B S1303 is subject to phosphorylation by CaMKII (Omikumar et al., 1996). PKC may be another kinase for this site as well as S1323 (Liao et al., 2001). A more recently identified site on the GluN2B C-terminus, S1480, is within a PDZ-binding domain and is sensitive to casein kinase (CK2; Sanz-Clemente et al., 2010). The GluN2C C-terminus is phosphorylated at S1244 by both PKA and PKC (Chen et al., 2006) and at S1096 by protein kinase B (PKB)/Akt (Chen and Roche, 2009). The phosphorylation level at these sites has a significant impact on some key properties of

the receptor. PKC phosphorylation of S890 dispersed the surface clusters of GluN1 (Tingley et al., 1997). PKC-sensitive phosphorylation of GluN1 and GluN2B (S1303 and S1323) enhanced NMDAR function (Hisatsune et al., 1997; Liao et al., 2001). CK2 phosphorylation of GluN2B S1480 drove GluN2B endocytosis and facilitated developmental switch from GluN2B to GluN2A at synapses (Sanz-Clemente et al., 2010). PKB phosphorylation of GluN2C S1096 enhanced surface expression of GluN2C-containing NMDARs and supported neuronal survival (Chen and Roche, 2009).

GluN2A and GluN2B are the predominant GluN2 subunits in the striatum. Major tetrameric subtypes of NMDARs in this region are therefore thought to be 2GluN1/2GluN2A, 2GluN1/2GluN2B, or 2GluN1/1GluN2A/1GluN2B, in addition to some binary subtypes containing one GluN1 and one GluN2A or GluN2B subunit (Dunah et al., 2000; Dunah and Standaert, 2003). Both striatonigral and striatopallidal projection neurons and cholinergic interneurons express a high level of NMDARs (Landwehrmeyer et al., 1995; Chen et al., 1996). Most GluN1/GluN2A receptors are incorporated into synapses, while GluN1/GluN2B receptors are present at both synaptic and extrasynaptic sites (Tovar and Westbrook, 1999).

Enriched expression of NMDARs in the striatum implies their roles in drug action and provides an opportunity to investigate adaptive changes in the receptor phosphorylation in response to psychostimulants. Acute amphetamine increased GluN1 S896 phosphorylation primarily in D1 receptor-bearing striatonigral neurons (Liu et al., 2004). Direct stimulation of D1 receptors with D1 agonists enhanced PKA-sensitive GluN1 S897 phosphorylation through the D1/cAMP/PKA pathway (Dudman et al., 2003). The enhanced S897 phosphorylation seems to lead to a cytosolic Ca^{2+} rise, which synergizes with the cAMP/PKA signals to activate the transcription factor Ca^{2+} /cAMP response element binding protein (CREB) to facilitate gene expression. These results show the ability of psychostimulants to modify NMDAR phosphorylation. Through modifying the receptor, stimulants regulate gene expression and construct transcription-dependent neuroadaptations essential for long-lasting drug action.

In addition to the D1 receptor, the D2 receptor is involved in the regulation of NMDAR phosphorylation (Liu et al., 2006). A single dose of cocaine induced a heteroreceptor complex formation between D2 receptors and GluN2B in D2 receptor-bearing striatopallidal neurons. The interaction of D2 receptors with GluN2B disrupted the association of CaMKII with GluN2B, thereby reducing phosphorylation at the CaMKII-sensitive site S1303 and inhibiting NMDAR currents. Behaviorally, this phosphorylation-involved D2–GluN2B interaction suppressed the inhibitory indirect pathway to promote a full motor response to cocaine.

Chronic cocaine reduced GluN1 S896 phosphorylation in the rat frontal cortex at 24 h, although not 14 days, of withdrawal (Loftis and Janowsky, 2002). However, cocaine self-administration had a minimal influence over S896 phosphorylation in the monkey striatum (Hemby et al., 2005). Acute, repeated, and self administration of cocaine increased GluN1 S897 phosphorylation in the rat striatum (Edwards et al., 2007). These results demonstrate that in the striatum at least S897 is a sensitive site modified by cocaine. Future studies are needed to elucidate how S897 phosphorylation mediates NMDAR plasticity and drug craving.

METABOTROPIC GLUTAMATE RECEPTORS

The mGluR is an equally sensitive substrate for phosphorylation and PKC's roles in mGluR phosphorylation have been the most extensively studied (Kim et al., 2008; Mao et al., 2008). The long form group I mGluRs (1a, 5a, and 5b) have a characteristically large C-terminal tail. A threonine residue (T840) or an adjacent S839 in the proximal region of mGluR5a C-terminus undergoes PKC-mediated phosphorylation (Kawabata et al., 1996; Kim et al., 2005). This single site phosphorylation determines the pattern of Ca^{2+} responses to mGluR5 stimulation (Kawabata et al., 1996; Uchino et al., 2004). PKC also possibly phosphorylates other serine/threonine sites (T606, S613, T665, T681, S881, and S890 for mGluR5a; T695 for mGluR1a). Phosphorylation at these sites is involved in rapid desensitization of the receptor (Gereau and Heinemann, 1998; Medler and Bruch, 1999; Francesconi and Duvoisin, 2000). In addition to PKC, cyclin-dependent kinase 5 (CDK5) phosphorylates two residues (T1164/S1167) within the domain of mGluR1/5 C-termini that interacts with the scaffold protein Homer (Orlando et al., 2009). This phosphorylation increased the binding of the receptor to Homer.

Group II/III mGluRs are also phosphorylated at serine/threonine. PKA phosphorylates mGluR2 S843, mGluR3 S845, mGluR4a S859, mGluR7a S862, and mGluR8a S855 on the C-terminal tail to inhibit their function as presynaptic receptors (Schaffhauser et al., 2000; Cai et al., 2001). PKC phosphorylates a conserved site (S862) on mGluR7 C-termini to inhibit calmodulin binding *in vitro* (Airas et al., 2001). In addition, mGluR7 S862 phosphorylation, in combination with the binding of the PDZ domain-containing protein PICK1, stabilized surface mGluR7 expression *in vivo* (Suh et al., 2008). At present, studies on the regulation of mGluR phosphorylation by addictive drugs are limited, partially due to the lack of phospho- and site-specific antibodies. One study revealed that relative serine phosphorylation of the mGluR2/3 monomer was elevated in both the NAc and prefrontal cortex after repeated cocaine, which was associated with an enduring reduction of mGluR2/3 function in inhibiting glutamate release (Xi et al., 2002). How phosphorylation modifications of mGluRs precisely contribute to drug-induced mGluR plasticity and drug-seeking behavior remains an interesting topic in future studies.

PHOSPHORYLATION OF GLUTAMATE RECEPTORS AT TYROSINE

In addition to serine and threonine, tyrosine is another phosphorylation site on glutamate receptors. GluA1 and GluA2 intracellular domains are tyrosine-phosphorylated (Hayashi and Haganir, 2004; Wu et al., 2004). The distal region of the GluA2 C-terminus possesses multiple tyrosine residues. Non-receptor Src family tyrosine kinases phosphorylate tyrosine 876 (Y876; Hayashi and Haganir, 2004), the last tyrosine residue near the end of C-terminus that lies within the PDZ ligand motif. Y876 phosphorylation disrupted the association of GluA2 with the PDZ domain-containing proteins, such as glutamate receptor interacting proteins 1 and 2 (GRIP1/2), and thereby promoted endocytosis of GluA2 (Hayashi and Haganir, 2004) and LTD (Ahmadian et al., 2004; Fox et al., 2007). GluN2, although not GluN1, subunits are also tyrosine-phosphorylated in their C-termini (Lau and Haganir, 1995; Menegoz et al., 1995; Dunah et al., 2000). Both Src-family tyrosine kinases (Src and Fyn) and Src-independent tyrosine kinases can carry out the phosphorylation (Suzuki and Okumura-Noji, 1995; Zheng et al., 1998; Xu et al., 2009). Multiple tyrosine residues (7 of 25, including 1252, 1336, and 1472) within the GluN2B C-terminus are responsive to phosphorylation. Y1472 seems to be a major site (Nakazawa et al., 2001) comparable to a major site (Y1325) on GluN2A (Taniguchi et al., 2009). GluN2 phosphorylation usually enhances NMDAR-mediated currents and NMDAR-dependent LTP via mechanisms involving the regulation of trafficking and protein–protein interactions (Wang and Salter, 1994; Lau and Haganir, 1995; Rostas et al., 1996; Dunah et al., 2004). Moreover, the influence of tyrosine phosphorylation can be site-selective. Y1472 and Y1336 phosphorylation was associated with enrichment of synaptic and extrasynaptic NMDARs, respectively (Goebel-Goody et al., 2009). Fyn-mediated Y1336 phosphorylation site-dependently controlled GluN2B cleavage by calpain (Wu et al., 2007). Regarding mGluRs, mGluR5 was abundantly tyrosine-phosphorylated in striatal neurons *in vivo*, based on pharmacological studies with tyrosine kinase or phosphatase

inhibitors and immunoprecipitation experiments with anti-phosphotyrosine antibodies (Orlando et al., 2002), although accurate site(s) among a few tyrosine residues on the mGluR5 C-terminus have not been determined.

D1 receptor signals increased synaptic delivery of tyrosine-phosphorylated GluN2A and GluN2B in striatal neurons (Dunah and Standaert, 2001). This event is tyrosine phosphorylation-dependent because the tyrosine kinase inhibitor genistein blocked it and the phosphatase inhibitor pervanadate mimicked D1 signals to induce the delivery. In cultured neurons, D1 receptor stimulation increased GluN2B phosphorylation at Y1472, which increased surface expression of GluN2B/NMDARs in prefrontal cortical neurons (Guo and Wolf, 2008) and facilitated the clustering of GluN2B along the dendritic spine shaft in striatal neurons (Hallett et al., 2006). In addition to D1 signals, brain-derived neurotrophic factor (BDNF) rapidly increased Fyn-sensitive Y1472 phosphorylation and NMDAR activity (Xu et al., 2006). In studies with addictive drugs, acute cocaine increased Src-dependent tyrosine phosphorylation of GluN2A, leading to elevated NMDAR expression and activity in the ventral tegmental area (Schumann et al., 2009). Repeated administration of alcohol elevated Fyn activity, Y1472 phosphorylation, and GluN2B–NMDAR function in the dorsomedial striatum (Wang et al., 2010). Inhibition of Src family tyrosine kinases decreased self-administration of alcohol (Wang et al., 2010). Thus, plastic changes in Y1472 phosphorylation can be a critical element linking NMDAR plasticity to drug-taking. In general, available data indicate the linkage between tyrosine phosphorylation of NMDAR GluN2 and drug action. As for AMPARs and mGluRs, little is known about how tyrosine phosphorylation of these receptors responds to drugs and the role of tyrosine phosphorylation might play in determining drug-induced receptor and behavioral adaptations.

PALMITOYLATION OF GLUTAMATE RECEPTORS AT CYSTEINE

Like phosphorylation, fatty acylation, such as palmitoylation, is another important type of modification. Palmitoylation is the covalent attachment of a 16 carbon saturated fatty acid, palmitate (palmitic acid), to a cysteine residue most commonly via a thioester bond. This process is an enzymatic event and is catalyzed by a family of palmitoyl acyltransferases (PATs). All PATs contain a signature Asp-His-His-Cys (DHHC) Cys-rich domain conserved from yeast to mammals (Planey and Zacharias, 2009). At least 23 mammalian DHHC-containing PATs (DHHC1–23) have been identified. These PATs have distinct substrate selectivity, although it is unclear yet if there exists a consensus PAT palmitoylation motif (Planey and Zacharias, 2009). As one type of lipidation, palmitoylation increases the lipophilicity or hydrophobicity of modified proteins. As such, it often results in an altered protein affinity for the plasma membrane, leading to protein trafficking from one membrane system to another. Additionally, palmitoylation can alter interactions of modified proteins with their binding partners, thereby regulating their subcellular distribution and function. Like phosphorylation and unlike other forms of lipidation, palmitoylation (the thioester bond) is labile and reversible. This nature situates it well as an activity-dependent regulator of synaptic proteins, including glutamate receptors. All four AMPAR subunits (GluA1–4) are palmitoylated constitutively in transfected

HEK 293T cells, cultured cortical neurons, and striatal neurons *in vivo* (Hayashi et al., 2005; Van Dolah et al., 2011). Two conserved cysteine residues on these subunits undergo reliable palmitoylation: one within C-terminal regions (GluA1–C811, GluA2–C836, GluA3–C841, and GluA4–C817) and another within the TMD 2 (GluA1–C585, GluA2–C610, GluA3–C615, and GluA4–C611; Hayashi et al., 2005). These sites are surrounded by basic and hydrophobic residues. They are adjacent to TMD, consistent with a pattern of palmitoylation for transmembrane proteins, i.e., palmitoylation at intracellular cysteines near TMD (El-Husseini and Brecht, 2002). Regarding potential PAT subtypes catalyzing AMPAR palmitoylation, a Golgi-specific protein with a DHHC zinc finger domain (GODZ, also known as DHHC3) promoted the palmitoylation on the TMD 2 site, while the exact PAT subtype for the C-terminal site remains to be identified (Hayashi et al., 2005). In addition to DHHC3, a dendritically localized DHHC2 activity-dependently translocated to the postsynaptic density (PSD) to induce rapid palmitoylation of PSD-95 in cultured hippocampal neurons (Noritake et al., 2009).

As aforementioned, palmitoylation controls redistribution of modified proteins usually in an activity-sensitive fashion. According to Hayashi et al. (2005), GODZ increases palmitoylation of AMPARs on the TMD 2 site and retains the receptor in the Golgi apparatus. In contrast, depalmitoylation releases the receptor from the Golgi for surface delivery. On the C-terminal site, palmitoylation disrupts the interaction of receptors with 4.1N, a synapse-enriched cytoskeletal protein that stabilizes surface AMPAR expression, and enhances susceptibility to agonist-induced internalization trafficking. Depalmitoylation increases the receptor affinity for 4.1N and stabilizes the receptor on surface membrane. Apparently, the balance of palmitoylation and depalmitoylation is regulated by synaptic activity. Such regulatable balance determines subcellular distribution of the receptor.

N-methyl-D-aspartate receptors GluN2A and GluN2B subunits have two distinct clusters of palmitoylation sites in their C-terminal regions (Hayashi et al., 2009). The first cluster is proximal to membrane (GluN2A: C848, C853, and C870; GluN2B: C849, C854, and C871). Palmitoylation of these sites increased tyrosine phosphorylation, leading to enhanced surface expression of the receptor. The second cluster resides in the middle of C terminus (GluN2A: C1214, C1217, C1236, and C1239; GluN2B: C1215, C1218, C1239, C1242, and C1245). Their palmitoylation caused receptors to accumulate in the Golgi apparatus and reduced receptor surface expression. Recombinant kainate receptor GluK6 subunits expressed in HEK cells are palmitoylated at C-terminal C827 and C840 (Pickering et al., 1995). mGluR palmitoylation has been less extensively studied. Available data show that mGluR4 but not mGluR1 α is palmitoylated in heterologous cells transfected with these subtypes (Alaluf et al., 1995; Pickering et al., 1995). Little is known about the palmitoylation status of endogenous mGluRs in neurons.

Palmitate is the most abundant fatty acid in the brain. Thus, inducible, regulatable, and reversible palmitoylation could be a common mechanism for regulating normal glutamate receptors and excitatory synapses. An attractive speculation is that glutamate receptor palmitoylation could also be plastic in response to drug exposure and is thus involved in enduring synaptic and behavioral plasticity. At present, the study linking palmitoylation to addiction

is limited partially due to the lack of palmito- and site-specific antibodies that are needed to detect site-specific palmitoylation responses to drugs in *in vivo* adult rodent brains. Nevertheless, one recent study shows that palmitoylation of AMPARs is subject to the regulation by a psychostimulant (Van Dolah et al., 2011). Acute cocaine induced a transient and reversible increase in overall GluA1 and GluA3 palmitoylation in the rat NAc, while cocaine did not affect GluA2 and GluA4 palmitoylation. This increase was correlated well with a temporary loss of surface GluA1/3. Pharmacological inhibition of protein palmitoylation reversed the loss of local GluA1/3. Thus, the increased palmitoylation appears to cause reduction of GluA1/3 surface expression, probably via an enhanced internalization rate due to disrupted interactions with the surface-stabilizing protein 4.1N (see above). Noticeably, AMPAR palmitoylation contributes to the regulation of behavioral sensitivity to cocaine. Since blocking AMPAR palmitoylation responses to cocaine augmented motor responses to the drug, the induced palmitoylation may downregulate surface AMPAR expression to prevent motor overstimulation. It will be interesting to explore how palmitoylation of AMPARs responds to chronic drug administration and whether palmitoylation is another modification that needs to be investigated for elucidating mechanisms underlying AMPAR plasticity and drug addiction.

UBIQUITINATION OF GLUTAMATE RECEPTORS AT LYSINE

Ubiquitination is a stepwise enzymatic process carried out by a set of three enzymes. Initially, a small regulatory protein ubiquitin is activated by the ubiquitin-activating enzyme (E1). Active ubiquitin is then transferred to the ubiquitin-conjugating enzyme (E2). Finally, the C-terminal glycine of ubiquitin recognizes and binds the lysine in the modified protein via the ubiquitin-protein ligase (E3). The whole process can be repeated until a short chain of ubiquitin is formed (polyubiquitination) to target the modified protein to degradation by proteasomes. The ubiquitin-proteasome system (UPS) serves as a fundamental mechanism for the regulation of protein expression and function. Increasing evidence supports its role as an important regulator of synaptic plasticity (Yi and Ehlers, 2005). To date, a subset of synaptic scaffolds, including Shank, guanylate kinase-associated protein (GKAP) and A-kinase anchoring protein (AKAP), have been found to be polyubiquitinated in cultured hippocampal or cortical neurons (Ehlers, 2003) or in the rat striatum *in vivo* (Mao et al., 2009). PSD-95 may arguably be ubiquitinated (Colledge et al., 2003; Bingol and Schuman, 2004). Among glutamate receptor subtypes surveyed, there is evidence showing that GluN1 and GluN2B could be the direct targets of ubiquitin in heterologous cells or in cultured neurons, although results are not always consistent (Ehlers, 2003; Kato et al., 2005; Ratnam and Teichberg, 2005; Jurd et al., 2008). GluK6 is ubiquitinated (Salinas et al., 2006). Group I mGluRs (mGluR1 α and mGluR5) can also undergo polyubiquitination in heterologous cells via a specific E3 ligase, seven in absentia homolog 1A (Siah1A; Moriyoshi et al., 2004). Multiple lysine sites at mGluR5 intracellular loops and the C-terminal tail are among the sites ubiquitinated, although the exact sites remain to be identified. Functionally, polyubiquitination determines the degradation rate and expression level of the receptor, while mono-ubiquitination may carry out some non-proteolytic roles, including the regulation of receptor endocytosis.

Given the regulatory role of ubiquitination and degradation in synaptic plasticity, it is intriguing to explore whether ubiquitination is directly involved in the remodeling of excitatory synapses and behavioral plasticity in response to drug exposure. Mao et al. (2009) have recently found that chronic amphetamine administration enhanced ubiquitin conjugation to synaptic proteins in the rat striatum. The specific substrates sensitive to the drug in their ubiquitination include Shank and GKAP. Since these two scaffold proteins are known to anchor PSD-95 and PSD-95-linked GluN2B to the PSD microdomain, the ubiquitination-dependent removal of Shank/GKAP may have a significant impact on the stability of PSD-95/GluN2B, even though PSD-95/GluN2B are not direct ubiquitin targets. In fact, striatal PSD-95 (Yao et al., 2004) and GluN2B proteins were reduced in parallel with reduced levels of Shank and GKAP (Mao et al., 2009). Inhibition of proteasomes reversed the reduction of all four proteins (Shank, GKAP, PSD-95, and GluN2B) in the striatum of amphetamine-treated rats. Destabilization of GluN2B through this mechanism further translates to the inhibitory modulation of synaptic plasticity in the form of LTD at cortico-accumbal glutamatergic synapses and contributes to behavioral sensitization. These data link ubiquitination to glutamate receptor plasticity in relation to drug action and pave the way for further studies to characterize this new layer of mechanisms.

NAC-1 is a transcription factor in brain cells and is localized in the nucleus. Its mRNA levels were increased in the rat NAc 3 weeks after chronic cocaine self-administration (Cha et al., 1997). NAC-1 is deemed to play an important role in modifying long-term behavioral effects of cocaine (Mackler et al., 2000). Interestingly, NAC-1 forms complexes with proteins in the UPS (Shen et al., 2007). NAC-1 and the proteasome were cotranslocated from the nucleus into dendritic spines and the PSD microdomain when synaptic activity was disinhibited by bicuculline (Shen et al., 2007). Thus, NAC-1 has a potential to modify drug-induced synaptic plasticity via recruiting the UPS into synaptic sites.

SUMOYLATION OF GLUTAMATE RECEPTORS AT LYSINE

Analogous to ubiquitination, sumoylation is a sequential enzymatic modification of proteins at lysine residues. Through a defined cascade involving three enzymes (E1–3), small ubiquitin-like modifier or SUMO proteins, a family of small proteins similar to ubiquitin, are covalently attached to and detached from target proteins (Geiss-Friedlander and Melchior, 2007; Heun, 2007). Specifically, at the final step, the C-terminal glycine of SUMO forms an isopeptide bond with an acceptor lysine on the target protein. In contrast to ubiquitin, SUMO is not used to tag proteins for degradation. It instead has diverse functions most frequently linked to protein protection from ubiquitination (stability), protein–protein interactions, cytosol-nuclear transport, and transcriptional regulation (Zhao, 2007). These functions are directly derived from the sumoylation-induced changes in trafficking/subcellular redistribution and binding properties of modified proteins with their interactors. Typically, sumoylation is carried out at a consensus motif Ψ -K-x-D/E where Ψ is a hydrophobic residue, K is the lysine conjugated to SUMO, x is any amino acid, D or E is an acidic residue. Free online engines, such as SUMOplot or SUMOsp (Ren et al., 2009),

can be accessed to predict and score potential sumoylation sites for a given protein of interest. Of note, sumoylation can also be reversibly regulated by desumoylating enzymes.

Early work focused on nuclear proteins. It is now appreciated that proteins in all subcellular compartments are subject to sumoylation, including synaptic proteins such as glutamate receptors (Scheschonka et al., 2007; Sen and Snyder, 2010). GluK6 is a SUMO substrate in rat hippocampal neurons (Martin et al., 2007). GluK6 sumoylation is required for agonist-evoked endocytosis of the receptor. Noticeably, GluK6 is sumoylated at the C-terminal K886 site (Martin et al., 2007), which is adjacent to I884 critical for binding and formation of a complex for GluK6 ubiquitination (Salinas et al., 2006). The proximity of two types of modifications implies possible crosstalk. mGluR8 is sumoylated at its C-terminal K882 site (Tang et al., 2005). Other group III mGluRs (mGluR4/6/7) and GluK7 may also undergo reliable sumoylation (Wilkinson et al., 2008).

A striatum-enriched small G-protein ras homolog enriched in striatum (Rhes) has recently been found to act as a physiological regulator of protein sumoylation (Subramaniam et al., 2010). Rhes-deleted mice exhibited marked and selective reduction of protein sumoylation in the striatum (Subramaniam et al., 2009). Rhes expression is regulated by dopamine signals. Dopamine depletion reduced its mRNA and protein expression in the rat striatum (Harrison and LaHoste, 2006; Harrison et al., 2008). However, dopamine stimulation by acute amphetamine did not alter Rhes mRNA levels in the striatum (Schwendt and McGinty, 2010).

At present, sumoylation in drug action almost represents an uncharted area. It is possible that sumoylation of synaptic proteins including glutamate receptors is regulated by drug exposure. Altered sumoylation may contribute to the regulation of striatal signaling and drug addiction.

CONCLUSION

Glutamate receptors have long been appreciated to be subject to various post-translational modifications. Extensive studies have established glutamate receptors as direct substrates of protein kinases for robust phosphorylation at serine, threonine, or tyrosine

residues. Such phosphorylation is important for regulating trafficking and function of modified receptors. Reversible palmitoylation also occurs to glutamate receptor cysteine residues, which like phosphorylation regulates expression and activity of the receptor. Ubiquitination and sumoylation at lysine residues are other types of glutamate receptor modifications. These modifications control degradation or endocytosis of surface receptors and thus adjust surface expression levels of modified receptors. All these types of modifications are regulated by cellular and synaptic signals. In response to psychostimulants, glutamate receptors in striatal neurons show marked and dynamic changes in phosphorylation and palmitoylation. Even though limited studies have been attempted at present, emerging evidence indicates the linkage between these protein modifications and persistent drug-related plasticity at both receptor and behavioral levels.

In addition to phosphorylation, palmitoylation, ubiquitination and sumoylation summarized in this review, other types of protein modification may be substantial for the regulation of glutamate receptors. These potential modifications include nitrosylation (cysteine), sulfhydration (cysteine), acetylation (lysine), etc. (Sen and Snyder, 2010). Recently, these modifications are appreciated to impact far more proteins than expected and could influence glutamate receptors or their adaptor proteins as prominently and efficiently as phosphorylation. Additionally, different modifications are thought to interact with each other, especially when modifications occur at the same or proximal sites. This could pair two different modifications as a reciprocal process, such as palmitoylation and nitrosylation on cysteines or ubiquitination and sumoylation on lysines. It is anticipated that modification biology will grow rapidly and more modification models on glutamate receptors will be characterized *in vivo*. This will ultimately inform glutamate receptor modifications as to their clinical relevance, and assist in our understanding the pathogenesis of psychiatric illnesses and in developing therapeutic agents for drug addiction.

ACKNOWLEDGMENTS

The work by the authors discussed in this article was supported by NIH R01 DA010355 and R01 MH061469.

REFERENCES

- Ahmadian, G., Ju, W., Liu, L., Wyszynski, M., Lee, S. H., Dunah, A. W., Taghibiglou, C., Wang, Y., Lu, J., Wong, T. P., Sheng, M., and Wang, Y. T. (2004). Tyrosine phosphorylation of GluR2 is required for insulin-stimulated AMPA receptor endocytosis and LTD. *EMBO J.* 23, 1040–1050.
- Airas, J. M., Beta, H., and El Far, O. (2001). PKC phosphorylation of a conserved serine residue in the C-terminus of group III metabotropic glutamate receptors inhibits calmodulin binding. *FEBS Lett.* 494, 60–63.
- Alaluf, S., Mulvihill, E. R., and McIlhinney, R. A. (1995). The metabotropic glutamate receptor mGluR4, but not mGluR1 alpha, is palmitoylated when expressed in BHK cells. *J. Neurochem.* 64, 1548–1555.
- Anderson, S. M., Famous, K. R., Sadri-Vakili, G., Kumaresan, V., Schmidt, H. D., Bass, C. E., Terwilliger, E. F., Cha, J. H., and Pierce, R. C. (2008). CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. *Nat. Neurosci.* 11, 344–353.
- Ba, M., Kong, M., Yang, H., Ma, G., Lu, G., Chen, S., and Liu, Z. (2006). Changes in subcellular distribution and phosphorylation of GluR1 in lesioned striatum of 6-hydroxydopamine-lesioned and 1-dopa-treated rats. *Neurochem. Res.* 31, 1337–1347.
- Bachtell, R. K., Choi, K. H., Simmons, D. L., Falcon, E., Monteggia, L. M., Neve, R. L., and Self, D. W. (2008). Role of GluR1 expression in nucleus accumbens neurons in cocaine sensitization and cocaine-seeking behavior. *Eur. J. Neurosci.* 27, 2229–2240.
- Banke, T. G., Bowie, D., Lee, H. K., Haganir, R. L., Schousboe, A., and Traynelis, S. F. (2000). Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. *J. Neurosci.* 20, 89–102.
- Barria, A., Derkach, V., and Soderling, T. (1997). Identification of the Ca²⁺/calmodulin-dependent protein kinase II regulatory phosphorylation site in the α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor. *J. Biol. Chem.* 272, 32727–32730.
- Bernard, V., Somogyi, P., and Bolam, J. P. (1997). Cellular, subcellular, and subsynaptic distribution of AMPA-type glutamate receptor subunits in the neostriatum of the rat. *J. Neurosci.* 17, 819–833.
- Bingol, B., and Schuman, E. M. (2004). A proteasome-sensitive connection between PSD-95 and GluR1 endocytosis. *Neuropharmacology* 47, 755–763.
- Bird, M. K., and Lawrence, A. J. (2009). Group I metabotropic glutamate receptors: involvement in drug-seeking and drug-induced plasticity. *Curr. Mol. Pharmacol.* 2, 83–94.
- Boehm, J., Kang, M. G., Johnson, R. C., Esteban, J., Haganir, R. L., and Malinow, R. (2006). Synaptic incorporation of AMPA receptors during LTP is controlled by a PKC phosphorylation site on GluR1. *Neuron* 51, 213–225.
- Boudreau, A. C., and Wolf, M. E. (2005). Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J. Neurosci.* 25, 9144–9151.

- Bowers, M. S., Chen, B. T., and Bonci, A. (2010). AMPA receptor synaptic plasticity induced by psychostimulants: the past, present, and therapeutic future. *Neuron* 67, 11–24.
- Cai, Z., Saugstad, J. A., Sorensen, S. D., Ciombar, K. J., Zhang, C., Schaffhauser, H., Hubalek, F., Pohl, J., Duvoisin, R. M., and Conn, P. J. (2001). Cyclic AMP-dependent protein kinase phosphorylates group III metabotropic glutamate receptors and inhibits their function as presynaptic receptors. *J. Neurochem.* 78, 756–766.
- Carvalho, A. L., Duarte, C. B., and Carvalho, A. P. (2000). Regulation of AMPA receptors by phosphorylation. *Neurochem. Res.* 25, 1245–1255.
- Cha, X. Y., Pierce, R. C., Kalivas, P. W., and Mackler, S. A. (1997). NAC-1, a rat brain mRNA, is increased in the nucleus accumbens three weeks after chronic cocaine self-administration. *J. Neurosci.* 17, 6864–6871.
- Chao, S. Z., Lu, W., Lee, H. K., Haganir, R. L., and Wolf, M. E. (2002). D(1) dopamine receptor stimulation increases GluR1 phosphorylation in postnatal nucleus accumbens cultures. *J. Neurochem.* 81, 984–992.
- Chen, B. S., Braud, S., Badger, J. D. II, Isaac, J. T., and Roche, K. W. (2006). Regulation of NR1/NR2C NMDA receptors by phosphorylation. *J. Biol. Chem.* 281, 16583–16590.
- Chen, B. S., and Roche, K. W. (2009). Growth factor-dependent trafficking of cerebellar NMDA receptors via protein kinase B/Akt phosphorylation of NR2C. *Neuron* 62, 471–478.
- Chen, H., and Manev, H. (2010). Effects of minocycline on cocaine sensitization and phosphorylation of GluR1 receptors in 5-lipoxygenase deficient mice. *Neuropharmacology*. PMID: 20868701. [Epub ahead of print].
- Chen, Q., Veenman, C. L., and Reiner, A. (1996). Cellular expression of ionotropic glutamate receptor subunits on specific striatal neuron types and its implication for striatal vulnerability in glutamate receptor-mediated excitotoxicity. *Neuroscience* 73, 715–731.
- Chen, Q., Zhu, X., Zhang, Y., Wetsel, W. E., Lee, T. H., and Zhang, X. (2010). Integrin-linked kinase is involved in cocaine sensitization by regulating PSD-95 and synapsin I expression and GluR1 Ser845 phosphorylation. *J. Mol. Neurosci.* 40, 284–294.
- Chung, H. J., Xia, J., Scannevin, R. H., Zhang, X., and Haganir, R. L. (2000). Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. *J. Neurosci.* 20, 7258–7267.
- Colledge, M., Snyder, E. M., Crozier, R. A., Soderling, J. A., Jin, Y., Langeberg, L. K., Lu, H., Bear, M. F., and Scott, J. D. (2003). Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. *Neuron* 40, 595–607.
- Conn, P. J., and Pin, J. P. (1997). Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.* 37, 205–237.
- Conrad, K. L., Tseng, K. Y., Uejima, J. L., Reimers, J. M., Heng, L. J., Shaham, Y., Marinelli, M., and Wolf, M. E. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature* 454, 118–121.
- Cull-Candy, S., Brickley, S., and Farrant, M. (2001). NMDA receptor subunits: diversity, development and disease. *Curr. Opin. Neurobiol.* 11, 327–335.
- Delawary, M., Tezuka, T., Kiyama, Y., Yokoyama, K., Inoue, T., Hattori, S., Hashimoto, R., Umemori, H., Manabe, T., Yamamoto, T., and Nakazawa, T. (2010). NMDAR2B tyrosine phosphorylation regulates anxiety-like behavior and CRF expression in the amygdala. *Mol. Brain* 3, 37.
- Delgado, J. Y., Coba, M., Anderson, C. N., Thompson, K. R., Gray, E. E., Heusner, C. L., Martin, C. C., Grant, S. G., and O'Dell, T. J. (2007). NMDA receptor activation dephosphorylates AMPA receptor glutamate receptor 1 subunits at threonine 840. *J. Neurosci.* 27, 13210–13221.
- Derkach, V., Barria, A., and Soderling, T. R. (1999). Ca²⁺/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc. Natl. Acad. Sci. U.S.A.* 96, 3269–3274.
- Dingledine, R., Borges, K., Bowie, D., and Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacol. Rev.* 51, 7–61.
- Dudman, J. T., Eaton, M. E., Rajadhyaksha, A., Macias, W., Taher, M., Barczak, A., Kameyama, K., Haganir, R., and Konradi, C. (2003). Dopamine D1 receptors mediate CREB phosphorylation via phosphorylation of the NMDA receptor at Ser897-NR1. *J. Neurochem.* 87, 922–934.
- Dunah, A. W., Sirianni, A. C., Fienberg, A. A., Bastia, E., Schwarzschild, M. A., and Standaert, D. G. (2004). Dopamine D1-dependent trafficking of striatal N-methyl-D-aspartate glutamate receptors requires Fyn protein tyrosine kinase but not DARPP-32. *Mol. Pharmacol.* 65, 121–129.
- Dunah, A. W., and Standaert, D. G. (2001). Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. *J. Neurosci.* 21, 5546–5558.
- Dunah, A. W., and Standaert, D. G. (2003). Subcellular segregation of distinct heteromeric NMDA glutamate receptors in the striatum. *J. Neurochem.* 85, 935–943.
- Dunah, A. W., Wang, Y., Yasuda, R. P., Kameyama, K., Haganir, R. L., Wolfe, B. B., and Standaert, D. G. (2000). Alterations in subunit expression, composition, and phosphorylation of striatal N-methyl-D-aspartate glutamate receptors in a rat 6-hydroxydopamine model of Parkinson's disease. *Mol. Pharmacol.* 57, 342–352.
- Edwards, S., Graham, D. L., Bachtell, R. K., and Self, D. W. (2007). Region-specific tolerance to cocaine-regulated cAMP-dependent protein phosphorylation following chronic self-administration. *Eur. J. Neurosci.* 25, 2201–2213.
- Edwards, S., Graham, D. L., Whisler, K. N., and Self, D. W. (2009). Phosphorylation of GluR1, ERK, and CREB during spontaneous withdrawal from chronic heroin self-administration. *Synapse* 63, 224–235.
- Ehlers, M. D. (2003). Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat. Neurosci.* 6, 231–242.
- El-Husseini, A.-D., and Bredt, D. S. (2002). Protein palmitoylation: a regulator of neuronal development and function. *Nat. Rev. Neurosci.* 3, 791–802.
- Estaban, J. A., Shi, H., Wilson, C., Nuriya, M., Haganir, R. L., and Malinow, R. (2003). PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nat. Neurosci.* 6, 136–143.
- Famous, K. R., Kumaresan, V., Sadri-Vakili, G., Schmidt, H. D., Mierke, D. F., Cha, J. H., and Pierce, R. C. (2008). Phosphorylation-dependent trafficking of GluR2-containing AMPA receptors in the nucleus accumbens plays a critical role in the reinstatement of cocaine seeking. *J. Neurosci.* 28, 11061–11070.
- Fox, C. J., Russell, K., Titterness, A. K., Wang, Y. T., and Christie, B. R. (2007). Tyrosine phosphorylation of the GluR2 subunit is required for long-term depression of synaptic efficacy in young animals in vivo. *Hippocampus* 17, 600–605.
- Francesconi, A., and Duvoisin, R. M. (2000). Opposing effects of protein kinase C and protein kinase A on metabotropic glutamate receptor signaling: selective desensitization of the inositol trisphosphate/Ca²⁺ pathway by phosphorylation of the receptor-G protein-coupling domain. *Proc. Natl. Acad. Sci. U.S.A.* 97, 6185–6190.
- Geiss-Friedlander, R., and Melchior, F. (2007). Concepts in sumoylation: a decade on. *Nat. Rev. Mol. Cell Biol.* 8, 947–956.
- Gereau, R. W. IV, and Heinemann, S. F. (1998). Role of protein kinase C phosphorylation in rapid desensitization of metabotropic glutamate receptor 5. *Neuron* 20, 143–151.
- Goebel-Goody, S. M., Davies, K. D., Alvestad Linger, R. M., Freund, R. K., and Browning, M. D. (2009). Phospho-regulation of synaptic and extrasynaptic N-methyl-D-aspartate receptors in adult hippocampal slices. *Neuroscience* 158, 1446–1459.
- Guo, C., and Wolf, M. E. (2008). Dopamine receptors regulate NMDA receptor surface expression in prefrontal cortex neurons. *J. Neurochem.* 106, 2489–2501.
- Hakansson, K., Galdi, S., Hendrick, J., Snyder, G., Greengard, P., and Fisone, C. (2006). Regulation of phosphorylation of the GluR1 AMPA receptor by dopamine D2 receptors. *J. Neurochem.* 96, 482–488.
- Hallett, P. J., Spoelgen, R., Hyman, B. T., Standaert, D. G., and Dunah, A. W. (2006). Dopamine D1 activation potentiates striatal NMDA receptors by tyrosine phosphorylation-dependent subunit trafficking. *J. Neurosci.* 26, 4690–4700.
- Harrison, L. M., and LaHoste, G. J. (2006). Rhes, the Ras homolog enriched in striatum, is reduced under conditions of dopamine supersensitivity. *Neuroscience* 137, 483–492.
- Harrison, L. M., LaHoste, G. J., and Ruskin, D. N. (2008). Ontogeny and dopaminergic regulation in brain of Ras homolog enriched in striatum (Rhes). *Brain Res.* 1245, 16–25.
- Hayashi, T., and Haganir, R. L. (2004). Tyrosine phosphorylation and regulation of the AMPA receptor by SRC family tyrosine kinases. *J. Neurosci.* 24, 6152–6160.
- Hayashi, T., Rumbaugh, G., and Haganir, R. L. (2005). Differential regulation of AMPA receptor subunit trafficking by palmitoylation of two distinct sites. *Neuron* 47, 709–723.
- Hayashi, T., Thomas, G. M., and Haganir, R. L. (2009). Dual palmitoylation of NR2 subunits regulates NMDA receptor trafficking. *Neuron* 64, 213–226.
- Hemby, S. E., Tang, W., Muly, E. C., Kuhar, M. J., Howell, L., and Mash, D. C. (2005). Cocaine-induced alterations in nucleus accumbens ionotropic glutamate receptor subunits in human and non-human primates. *J. Neurochem.* 95, 1785–1793.
- Heun, P. (2007). SUMO organization of the nucleus. *Curr. Opin. Cell Biol.* 19, 350–355.
- Hisatsune, C., Umemori, H., Inoue, T., Michikawa, T., Kohda, K., Mikoshiba, K., and Yamamoto, T. (1997). Phosphorylation-dependent regulation of N-methyl-D-aspartate

- receptors by calmodulin. *J. Biol. Chem.* 272, 20805–20810.
- Hyman, S. E., Malenka, R. C., and Nestler, E. J. (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu. Rev. Neurosci.* 29, 565–598.
- Jurd, R., Thornton, C., Wang, J., Luong, K., Phamluong, K., Kharazia, V., Gibb, S. L., and Ron, D. (2008). Mind bomb-2 is an E3 ligase that ubiquitinates the *N*-methyl-D-aspartate receptor NR2B subunit in a phosphorylation-dependent manner. *J. Biol. Chem.* 283, 301–310.
- Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nat. Rev. Neurosci.* 10, 561–572.
- Kato, A., Rouach, N., Nicoll, R. A., and Bredt, D. S. (2005). Activity-dependent NMDA receptor degradation mediated by retrotranslocation and ubiquitination. *Proc. Natl. Acad. Sci. U.S.A.* 102, 5600–5605.
- Kawabata, S., Tsutsumi, R., Kohara, A., Yamaguchi, T., Nakanishi, S., and Okada, M. (1996). Control of calcium oscillations by phosphorylation of metabotropic glutamate receptors. *Nature* 383, 89–92.
- Kim, S. M., Ahn, S. M., Go, B. S., Wang, J. Q., and Choe, E. S. (2009). Alterations in AMPA receptor phosphorylation in the rat striatum following acute and repeated cocaine administration. *Neuroscience* 163, 618–626.
- Kim, C. H., Braud, S., Isaac, J. T., and Roche, K. W. (2005). Protein kinase C phosphorylation of the metabotropic glutamate receptor mGluR5 on Serine 839 regulates Ca²⁺ oscillations. *J. Biol. Chem.* 280, 25409–25415.
- Kim, C. H., Lee, J., Lee, J. Y., and Roche, K. W. (2008). Metabotropic glutamate receptors: phosphorylation and receptor signaling. *J. Neurosci. Res.* 86, 1–10.
- Landwehrmeyer, G. B., Standaert, D. G., Testa, C. M., Penny, J. B. Jr., and Young, A. B. (1995). NMDA receptor subunit mRNA expression by projection neurons and interneurons in rat striatum. *J. Neurosci.* 15, 5297–5307.
- Lau, L. F., and Haganir, R. L. (1995). Differential tyrosine phosphorylation of *N*-methyl-D-aspartate receptor subunits. *J. Biol. Chem.* 270, 20036–20041.
- Li, B., Davidze, N., Barengolts, D., Probst, N., Sphicas, E., Apicella, A. J., Malinow, R., and Emamian, E. S. (2009). NMDA receptor phosphorylation at a site affected in schizophrenia controls synaptic and behavioral plasticity. *J. Neurosci.* 29, 11965–11972.
- Liao, G. Y., Wagner, D. A., Hsu, M. H., and Leonard, J. P. (2001). Evidence for direct protein kinase-C mediated modulation of *N*-methyl-D-aspartate receptor current. *Mol. Pharmacol.* 59, 960–964.
- Liu, X. Y., Chu, X. P., Mao, L. M., Wang, M., Lan, H. X., Li, M. H., Zhang, G. C., Parelkar, N. K., Fibuch, E. E., Haines, M., Neve, K. A., Liu, F., Xiong, Z. G., and Wang, J. Q. (2006). Modulation of D2R-NR2B interactions in response to cocaine. *Neuron* 52, 897–909.
- Liu, Z., Mao, L., Parelkar, N. K., Tang, Q., Samdani, S., and Wang, J. Q. (2004). Distinct expression of phosphorylated *N*-methyl-D-aspartate receptor NR1 subunits by projection neurons and interneurons in the striatum of normal and amphetamine-treated rats. *J. Comp. Neurol.* 474, 393–406.
- Lofitis, J. M., and Janowsky, A. (2002). Cocaine treatment- and withdrawal-induced alterations in the expression and serine phosphorylation of the NR1 NMDA receptor subunit. *Psychopharmacology* 164, 349–359.
- Mackler, S. A., Korutla, L., Cha, X. Y., Koebbe, M. J., Fournier, K. M., Bowers, M. S., and Kalivas, P. W. (2000). NAC-1 is a brain POZ/BTB protein that can prevent cocaine-induced sensitization in the rat. *J. Neurosci.* 20, 6210–6217.
- Mammen, A. L., Kameyama, K., Roche, K. W., and Haganir, R. L. (1997). Phosphorylation of the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II. *J. Biol. Chem.* 272, 32528–32533.
- Mangiavacchi, S., and Wolf, M. E. (2004). D1 dopamine receptor stimulation increases the rate of AMPA receptor insertion onto the surface of cultured nucleus accumbens neurons through a pathway dependent on protein kinase A. *J. Neurochem.* 88, 1261–1271.
- Mao, L. M., Liu, X. Y., Zhang, G. C., Chu, X. P., Fibuch, E. E., Wang, L. S., Liu, Z., and Wang, J. Q. (2008). Phosphorylation of group I metabotropic glutamate receptors (mGluR1/5) in vitro and in vivo. *Neuropharmacology* 55, 403–408.
- Mao, L. M., Wang, W., Chu, X. P., Zhang, G. C., Liu, X. Y., Yang, Y. J., Haines, M., Papasian, C. J., Fibuch, E. E., Buch, S., Chen, J. G., and Wang, J. Q. (2009). Stability of surface NMDA receptors controls synaptic and behavioral adaptations to amphetamine. *Nat. Neurosci.* 12, 602–610.
- Martin, L. J., Blackstone, C. D., Haganir, R. L., and Price, D. L. (1993). The striatal mosaic in primates: striosomes and matrix are differentially enriched in ionotropic glutamate receptor subunits. *J. Neurosci.* 13, 782–792.
- Martin, S., Nishimune, A., Mellor, J. R., and Henley, J. M. (2007). SUMOylation regulates kainite-receptor-mediated synaptic transmission. *Nature* 447, 321–325.
- Mattson, B. J., Bossert, J. M., Simmons, D. E., Nozaki, N., Nagarkar, D., Kreuter, J. D., and Hope, B. T. (2005). Cocaine-induced CREB phosphorylation in nucleus accumbens of cocaine-sensitized rats is enabled by enhanced activation of extracellular signal-related kinase, but not protein kinase A. *J. Neurochem.* 95, 1481–1494.
- Medler, K. F., and Bruch, R. C. (1999). Protein kinase C β and delta selectively phosphorylate odorant and metabotropic glutamate receptors. *Chem. Senses* 24, 295–299.
- Menegoz, M., Lau, L. F., Herve, D., Haganir, R. L., and Girault, J. A. (1995). Tyrosine phosphorylation of NMDA receptor in rat striatum: effects of 6-OH-dopamine lesions. *Neuroreport* 7, 125–128.
- Moriyoshi, K., Lijima, K., Fujii, H., Ito, H., Cho, Y., and Nakanishi, S. (2004). Seven in absentia homolog 1A mediates ubiquitination and degradation of group I metabotropic glutamate receptors. *Proc. Natl. Acad. Sci. U.S.A.* 101, 8614–8619.
- Moussawi, K., and Kalivas, P. W. (2010). Group II metabotropic glutamate receptors (mGlu2/3) in drug addiction. *Eur. J. Pharmacol.* 639, 115–122.
- Nakazawa, T., Komai, S., Tezuka, T., Hisatsune, C., Umemori, H., Semba, K., Mishina, M., Manabe, T., and Yamamoto, T. (2001). Characterization of Fyn-mediated tyrosine phosphorylation sites on GluR epsilon 2 (NR2B) subunit of the *N*-methyl-D-aspartate receptor. *J. Biol. Chem.* 276, 693–699.
- Noritake, J., Fukata, Y., Iwanaga, T., Naoki, H., Tsutsumi, R., Matsuda, N., Tani, H., Iwanari, H., Mochizuki, Y., Kodama, T., Matsuura, Y., Bredt, D., Hamakubo, T., and Fukata, M. (2009). Mobile DHHC palmitoylating enzyme mediates activity-sensitive synaptic targeting of PSD-95. *J. Cell Biol.* 186, 147–160.
- Omikumar, R. V., Melinda, O., Kiely, M. J., Rosenstein, A. J., Min, K. T., and Kennedy, M. B. (1996). Identification of a phosphorylation site for calcium/calmodulin-dependent protein kinase II in the NR2B subunit of the *N*-methyl-D-aspartate receptor. *J. Biol. Chem.* 271, 31670–31678.
- Orlando, L. R., Ayala, R., Kett, L. R., Curley, A. A., Duffner, J., Bragg, D. C., Tsai, L. H., Dunah, A. W., and Young, A. B. (2009). Phosphorylation of the homer-binding domain of group I metabotropic glutamate receptors by cyclin-dependent kinase 5. *J. Neurochem.* 110, 557–569.
- Orlando, L. R., Dunah, A. W., Standaert, D. G., and Young, A. B. (2002). Tyrosine phosphorylation of the metabotropic glutamate receptor mGluR5 in striatal neurons. *Neuropharmacology* 43, 161–173.
- Pickering, D. S., Taverna, F. A., Salter, M. W., and Hampson, D. R. (1995). Palmitoylation of the GluR6 kainate receptor. *Proc. Natl. Acad. Sci. U.S.A.* 92, 12090–12094.
- Planey, S. L., and Zacharias, D. A. (2009). Palmitoyl acyltransferases, their substrates, and novel assays to connect them. *Mol. Mem. Biol.* 26, 14–31.
- Price, C. J., Kim, P., and Raymond, L. A. (1999). D1 dopamine receptor-induced cyclic AMP-dependent protein kinase phosphorylation and potentiation of striatal glutamate receptors. *J. Neurochem.* 73, 2441–2446.
- Ratnam, J., and Teichberg, V. I. (2005). Neurofilament-light increases the cell surface expression of the *N*-methyl-D-aspartate receptor and prevents its ubiquitination. *J. Neurochem.* 92, 878–885.
- Ren, J., Gao, X., Jin, C., Zhu, M., Wang, X., Shaw, A., Wen, L., Yao, X., and Xue, Y. (2009). Systematic study of protein sumoylation: development of a site-specific predictor of SUMOsp 2.0. *Proteomics* 9, 3409–3412.
- Roche, K. W., O'Brien, R. J., Mammen, A. L., Bernhardt, J., and Haganir, R. L. (1996). Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* 16, 1179–1188.
- Rostas, J. A., Brent, V. A., Voss, K., Errington, M. L., Bliss, T. V., and Gurd, J. W. (1996). Enhanced tyrosine phosphorylation of the 2B subunit of the *N*-methyl-D-aspartate receptor in long-term potentiation. *Proc. Natl. Acad. Sci. U.S.A.* 93, 10452–10456.
- Salinas, G. D., Blair, L. A., Needleman, L. A., Gonzales, J. D., Chen, Y., Li, M., Singer, J. D., and Marshall, J. (2006). Actinfilin is a Cul3 substrate adaptor, linking GluR6 kainate receptor subunits to the ubiquitin-proteasome pathway. *J. Biol. Chem.* 281, 40164–40173.
- Sanz-Clemente, A., Matta, J. A., Isaac, J. T., and Roche, K. W. (2010). Casein kinase 2 regulates the NR2 subunit composition of synaptic NMDA receptors. *Neuron* 67, 984–996.
- Schaffhauser, H., Cai, Z., Hubalek, E., Macek, T. A., Pohl, J., Murphy, T. J., and Conn, P. J. (2000). cAMP-dependent protein kinase inhibits mGluR2 coupling to G-proteins by direct receptor phosphorylation. *J. Neurosci.* 20, 5663–5670.
- Scheschonka, A., Tang, Z., and Betz, H. (2007). Sumoylation in neurons: nuclear and synaptic roles? *Trends Neurosci.* 30, 85–91.
- Schmidt, H. D., and Pierce, R. C. (2010). Cocaine-induced neuroadaptations in glutamate transmission: potential therapeutic targets for craving and addiction. *Ann. N. Y. Acad. Sci.* 1187, 35–75.
- Schumann, J., Michaeli, A., and Yaka, R. (2009). Src-protein tyrosine kinases

- are required for cocaine-induced increase in the expression and function of the NMDA receptor in the ventral tegmental area. *J. Neurochem.* 108, 697–706.
- Schwendt, M., and McGinty, J. F. (2010). Amphetamine up-regulates activator of G-protein signaling 1 mRNA and protein levels in rat frontal cortex: the role of dopamine and glucocorticoid receptors. *Neuroscience* 168, 96–107.
- Sen, N., and Snyder, S. H. (2010). Protein modifications involved in neurotransmitter and gasotransmitter signaling. *Trends Neurosci.* 33, 493–502.
- Shen, H., Korutla, L., Champiaux, N., Toda, S., LaLumiere, R., Vallone, J., Klugmann, M., Blendy, J. A., Mackler, S. A., and Kalivas, P. W. (2007). NAC1 regulates the recruitment of the proteasome complex into dendritic spines. *J. Neurosci.* 27, 8903–8913.
- Snyder, G. L., Allen, P. B., Fienberg, A. A., Valle, C. G., Huganir, R. L., Nairn, A. C., and Greengard, P. (2000). Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. *J. Neurosci.* 20, 4480–4488.
- Subramaniam, S., Mealer, R. G., Sixt, K. M., Barrow, R. K., Usiello, A., and Snyder, S. H. (2010). Rhes, a physiologic regulator of sumoylation, enhances cross-sumoylation between the basic sumoylation enzymes E1 and Ubc9. *J. Biol. Chem.* 285, 20428–20432.
- Subramaniam, S., Sixt, K. M., Barrow, R., and Snyder, S. H. (2009). Rhes, a striatal specific protein, mediates mutant-huntingtin cytotoxicity. *Science* 324, 1327–1330.
- Suh, Y. H., Pelkey, K. A., Lavezzari, G., Roche, P. A., Huganir, R. L., McBain, C. J., and Roche, K. W. (2008). Corequirement of PICK1 binding and PKC phosphorylation for stable surface expression of the metabotropic glutamate receptor mGluR7. *Neuron* 58, 736–748.
- Sutton, M. A., Schmidt, E. F., Choi, K. H., Schad, C. A., Whisler, K., Simmons, D., Karanian, D. A., Monteggia, L. M., Neve, R. L., and Self, D. W. (2003). Extinction-induced up-regulation in AMPA receptors reduces cocaine-seeking behavior. *Nature* 421, 70–75.
- Suzuki, T., and Okumura-Noji, K. (1995). NMDA receptor subunits epsilon 1 (NR2A) and epsilon 2 (NR2B) are substrates for Fyn in the postsynaptic density fraction isolated from the rat brain. *Biochem. Biophys. Res. Commun.* 216, 582–588.
- Swayze, R. D., Lise, M. F., Levinson, J. N., Phillips, A., and El-Husseini, A. (2004). Modulation of dopamine mediated phosphorylation of AMPA receptors by PSD-95 and AKAP79/150. *Neuropharmacology* 47, 764–778.
- Swope, S. L., Moss, S. J., Raymond, L. A., and Huganir, R. L. (1999). Regulation of ligand-gated ion channels by protein phosphorylation. *Adv. Second Messenger Phosphoprotein Res.* 33, 49–78.
- Tang, Z., El Far, O., Betz, H., and Scheschonka, A. (2005). Pias1 interaction and sumoylation of metabotropic glutamate receptor 8. *J. Biol. Chem.* 280, 38153–38159.
- Taniguchi, S., Nakazawa, T., Tanimura, A., Kiyama, Y., Tezuka, T., Watabe, A. M., Katayama, N., Yokoyama, K., Inoue, T., Izumi-Nakaseko, H., Kakuta, S., Sudo, K., Iwakura, Y., Umemori, H., Inoue, T., Murphy, N. P., Hashimoto, K., Kano, M., Manabe, T., and Yamamoto, T. (2009). Involvement of NMDAR2A tyrosine phosphorylation in depression-related behaviour. *EMBO J.* 28, 3717–3729.
- Tingley, W. G., Ehlers, M. D., Kameyama, K., Doherty, C., Ptak, J. B., Riley, C. T., and Huganir, R. L. (1997). Characterization of protein kinase A and protein kinase C phosphorylation of the N-methyl-D-aspartate receptor NR1 subunit using phosphorylation site-specific antibodies. *J. Biol. Chem.* 272, 5157–5166.
- Tovar, K. R., and Westbrook, G. L. (1999). The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *J. Neurosci.* 19, 4180–4188.
- Tzschentke, T. M., and Schmidt, W. J. (2003). Glutamatergic mechanisms in addiction. *Mol. Psychiatry* 8, 373–382.
- Uchino, M., Sakai, N., Kashiwagi, K., Shirai, Y., Shinohara, Y., Hirose, K., Lino, M., Yamamura, T., and Saito, N. (2004). Isoform-specific phosphorylation of metabotropic glutamate receptor 5 by protein kinase C (PKC) blocks Ca²⁺ oscillation and oscillatory translocation of Ca²⁺-dependent PKC. *J. Biol. Chem.* 279, 2254–2261.
- Van Dolah, D. K., Mao, L. M., Shaffer, C., Guo, M. L., Fibuch, E. E., Chu, X. P., Buch, S., and Wang, J. Q. (2011). Reversible palmitoylation regulates surface stability of AMPA receptors in the nucleus accumbens in response to cocaine in vivo. *Biol. Psychiatry*. doi: 10.1016/j.physletb.2003.10.071. [Epub ahead of print].
- Wang, J., Lanfranco, M. F., Gibb, S. L., Yowell, Q. V., Carnicella, S., and Ron, D. (2010). Long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum play a crucial role in alcohol consumption and relapse. *J. Neurosci.* 30, 10187–10198.
- Wang, J. Q., Liu, X., Zhang, G., Parekar, N. K., Arora, A., Haines, M., Fibuch, E. E., and Mao, L. (2006). Phosphorylation of glutamate receptors: a potential mechanism for the regulation of receptor function and psychostimulant action. *J. Neurosci. Res.* 84, 1621–1629.
- Wang, Y. T., and Salter, M. W. (1994). Regulation of NMDA receptors by tyrosine kinases and phosphatases. *Nature* 369, 233–235.
- Wilkinson, K. A., Nishimune, A., and Henley, J. M. (2008). Analysis of SUMO-1 modification of neuronal proteins containing consensus SUMOylation motifs. *Neurosci. Lett.* 436, 239–244.
- Wu, H. Y., Hsu, F. C., Gleichman, A. J., Bacongus, L., Coulter, D. A., and Lynch, D. R. (2007). Fyn-mediated phosphorylation of NR2B Tyr-1336 controls calpain-mediated NR2B cleavage in neurons and heterologous systems. *J. Biol. Chem.* 282, 20075–20087.
- Wu, K., Len, G. W., McAuliffe, G., Ma, C., Tai, J. P., Xu, F., and Black, I. B. (2004). Brain-derived neurotrophic factor acutely enhances tyrosine phosphorylation of the AMPA receptor subunit GluR1 via NMDA receptor-dependent mechanisms. *Brain Res. Mol. Brain Res.* 130, 178–186.
- Xi, Z. X., Ramamoorthy, S., Baker, D. A., Shen, H., Samuvel, D. J., and Kalivas, P. W. (2002). Modulation of group II metabotropic glutamate receptor signaling by chronic cocaine. *J. Pharmacol. Exp. Ther.* 303, 608–615.
- Xu, F., Plummer, M. R., Len, G. W., Nakazawa, T., Yamamoto, T., Black, I. B., and Wu, K. (2006). Brain-derived neurotrophic factor rapidly increases NMDA receptor channel activity through Fyn-mediated phosphorylation. *Brain Res.* 1121, 22–34.
- Xu, J., Litterst, C., Georgakopoulos, A., Zaganas, I., and Robakis, N. K. (2009). Peptide EphB2/CTF2 generated by the gamma-secretase processing of EphB2 receptor promotes tyrosine phosphorylation and cell surface localization of N-methyl-D-aspartate receptors. *J. Biol. Chem.* 284, 27220–27228.
- Yao, W. D., Gainetdinov, R. R., Arbuckle, M. I., Sotnikova, T. D., Cyr, M., Beaulieu, J. M., Torres, G. E., Grant, S. G., and Caron, M. G. (2004). Identification of PSD-95 as a regulator of dopamine-mediated synaptic and behavioral plasticity. *Neuron* 41, 625–638.
- Yi, J. J., and Ehlers, M. D. (2005). Ubiquitin and protein turnover in synaptic function. *Neuron* 47, 629–632.
- Zhang, X., Lee, T. H., Davidson, C., Lazarus, C., Wetsel, W. C., and Ellinwood, E. H. (2007). Reversal of cocaine-induced behavioral sensitization and associated phosphorylation of the NR2B and GluR1 subunits of the NMDA and AMPA receptors. *Neuropsychopharmacology* 32, 377–387.
- Zhao, J. (2007). Sumoylation regulates diverse biological processes. *Cell. Mol. Life Sci.* 64, 3017–3033.
- Zheng, F., Gingrich, M. B., Traynelis, S. F., and Conn, P. J. (1998). Tyrosine kinase potentiates NMDA receptor currents by reducing tonic zinc inhibition. *Nat. Neurosci.* 1, 185–191.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 18 January 2011; paper pending published: 07 February 2011; accepted: 03 March 2011; published online: 17 March 2011.

Citation: Mao L-M, Guo M-L, Jin D-Z, Fibuch EE, Choe ES and Wang JQ (2011) Post-translational modification biology of glutamate receptors and drug addiction. *Front. Neuroanat.* 5:19. doi: 10.3389/fnana.2011.00019

Copyright © 2011 Mao, Guo, Jin, Fibuch, Choe and Wang. This is an open-access article subject to an exclusive license agreement between the authors and Frontiers Media SA, which permits unrestricted use, distribution, and reproduction in any medium, provided the original authors and source are credited.



The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons

Mary Kay Lobo and Eric J. Nestler*

Fishberg Department of Neuroscience, Friedman Brain Institute, Mount Sinai School of Medicine, New York, NY, USA

Edited by:

Emmanuel Valjent, Université Montpellier 1 & 2, France

Reviewed by:

Bruce Thomas Hope, National Institute on Drug Abuse, USA
John Neumaier, University of Washington, USA

*Correspondence:

Eric J. Nestler, Department of Neuroscience, Friedman Brain Institute, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1065, New York, NY 10029-6574, USA.
e-mail: eric.nestler@mssm.edu

The striatum plays a key role in mediating the acute and chronic effects of addictive drugs, with drugs of abuse causing long-lasting molecular and cellular alterations in both dorsal striatum and nucleus accumbens (ventral striatum). Despite the wealth of research on the biological actions of abused drugs in striatum, until recently, the distinct roles of the striatum's two major subtypes of medium spiny neurons (MSNs) in drug addiction remained elusive. Recent advances in cell-type-specific technologies, including fluorescent reporter mice, transgenic, or knockout mice, and viral-mediated gene transfer, have advanced the field toward a more comprehensive understanding of the two MSN subtypes in the long-term actions of drugs of abuse. Here we review progress in defining the distinct molecular and functional contributions of the two MSN subtypes in mediating addiction.

Keywords: medium spiny neurons, addiction, nucleus accumbens, cell-type-specific, D₁+ MSNs, D₂+ MSNs, cocaine, dopamine

INTRODUCTION

Drugs of abuse exert potent molecular and cellular alterations in both dorsal striatum (dStr) and ventral striatum (nucleus accumbens, NAc), and many of these changes occur in medium spiny neurons (MSNs), the principal projection neurons in dStr and NAc, which account for 90–95% of all neurons in these regions. However, researchers have until recently been unable to clearly define the differential role of the two MSN subtypes in addiction-related phenomena. The two MSN subtypes are differentiated by their enrichment of dopamine receptor 1 (D₁) or dopamine receptor 2 (D₂) as well as several other genes (Gerfen and Young, 1988; Gerfen et al., 1990; Le Moine et al., 1990, 1991; Bernard et al., 1992; Ince et al., 1997; Lobo et al., 2006, 2007; Heiman et al., 2008; gensat.org) and by their distinct projections through the cortico-basal ganglia pathway (the direct vs. indirect pathways; Gerfen, 1984, 1992). Early work suggested that drugs of abuse exert most influence on the D₁+ MSNs, with the use of numerous dopamine receptor agonists and antagonists providing important insight into the functional and molecular roles of each MSN in drug reward behaviors (Self, 2010). However, current cell-type-specific methodologies, including fluorescent reporter mice that express GFP under D₁ or D₂ bacterial artificial chromosomes (BACs; Gong et al., 2003; Valjent et al., 2009; gensat.org), conditional mouse models such as the use of tetracycline-regulated inducible transgenic mice (Chen et al., 1998; Kelz et al., 1999), and transgenic mice expressing Cre-recombinase using D₁ or D₂ BACs, yeast artificial chromosomes (YACs), or knock-in mice (Gong et al., 2007; Lemberger et al., 2007; Heusner et al., 2008; Parkitna et al., 2009; Valjent et al., 2009; Bateup et al., 2010; Lobo et al., 2010; gensat.org) as well as cell-type-specific viral-mediated gene transfer (Cardin et al., 2010; Hikida et al., 2010; Lobo et al., 2010; Ferguson et al., 2011), have provided profound new insight into

the precise molecular underpinnings of each MSN subtype and their regulation by drugs of abuse (Table 1).

Recent findings support the conclusion of a more predominate role for D₁+ MSNs in producing the reinforcing and sensitizing effect of drugs of abuse, with most robust molecular changes occurring in these MSNs. For instance, acute exposure to psychostimulants potently induces numerous signaling molecules including FosB, ERK, c-Fos, and Zif268 in the D₁+ MSNs, while repeated cocaine preferentially induces Δ FosB and alters GABA receptor and other ion channel subunits in this cell-type as well (Robertson et al., 1991; Young et al., 1991; Berretta et al., 1992; Cenci et al., 1992; Moratalla et al., 1992; Hope et al., 1994; Bertran-Gonzalez et al., 2008; Heiman et al., 2008). Furthermore, disrupting or over-expressing specific molecules, such as Δ FosB, DARPP-32, or Nr3c1 (the glucocorticoid receptor), in D₁+ MSNs typically mimics the drug-related behaviors observed when these alterations are made in a non-cell-type-specific manner, while disrupting such genes in D₂+ MSNs often causes an opposite response (Fienberg et al., 1998; Kelz et al., 1999; Deroche-Gamonet et al., 2003; Zachariou et al., 2006; Ambroggi et al., 2009; Bateup et al., 2010). Nonetheless, we cannot rule out an important contribution of the D₂+ MSNs in adaptations to drugs of abuse, because cocaine exposure alters gene expression in both MSN subtypes (Heiman et al., 2008) and D₂-receptor agonists and antagonists exert potent effects in behavioral assays (Self, 2010). Indeed, recent findings show that molecular signaling adaptations in D₂+ MSNs potently modify an animal's behavioral response to drugs of abuse (Lobo et al., 2010). The latter findings showed that loss of TrkB (the receptor for BDNF) in D₂+ MSNs results in similar behavioral responses to cocaine as total TrkB knockout from the NAc, showing for the first time a selective dominant role for a molecular pathway in D₂+ MSNs in mediating the effects of drugs of abuse.

Table 1 | Effects of cell-type-specific genetic manipulation in D₁+ and D₂+ MSNs in drug addiction models.

Gene/function manipulated	Cell-type	Method	Effects mediated by drugs of abuse	References
NR1 (NMDA) subunit	D ₁	Mutated NR1 knocked into the D ₁ locus	Diminished cocaine sensitization and CPP	Heusner and Palmiter (2005)
NR1 (NMDA) subunit mGluR5	D ₁	D ₁ -Cre knockout	Attenuated amphetamine sensitization	Beutler et al. (2011)
	D ₁	mGluR5 shRNA driven under D ₁ BAC	Attenuated cue-induced cocaine seeking	Novak et al. (2010)
Acetylcholine muscarinic receptor 4 (M ₄)	D ₁	D ₁ -Cre knockout	Enhanced behavioral sensitization to cocaine and amphetamine	Jeon et al. (2010)
Cannabinoid receptor 1 (CB ₁)	D ₁	D ₁ -Cre knockout	Blunted response to THC	Monory et al. (2007)
Glucocorticoid receptor nuclear receptor 3c1 (Nr3c1)	D ₁	D ₁ -Cre knockout	Diminished cocaine self-administration	Ambroggi et al. (2009)
BDNF receptor (TrkB)	D ₁	D ₁ -Cre knockout	Enhanced cocaine sensitization and CPP	Lobo et al. (2010)
BDNF receptor (TrkB)	D ₂	D ₂ -Cre knockout	Diminished cocaine sensitization and CPP	Lobo et al. (2010)
c-Fos	D ₁	D ₁ -Cre knockout	Diminished cocaine sensitization, diminished cocaine extinction	Zhang et al. (2006)
AFosB	D ₁	Overexpression with tetracycline inducible D ₁ mouse line	Enhanced morphine CPP, enhanced morphine tolerance, diminished morphine analgesia, enhanced cocaine sensitization and CPP	Zachariou et al. (2006), Kelz et al. (1999)
DARPP-32	D ₁	D ₁ -Cre knockout	Diminished cocaine sensitization	Bateup et al. (2010)
DARPP-32	D ₂	D ₂ -Cre knockout	Enhanced cocaine sensitization	Bateup et al. (2010)
ChR2, light-activated neuronal activity	D ₁	Conditional AAV viruses + D ₁ -Cre	Enhanced cocaine CPP	Lobo et al. (2010)
ChR2, light-activated neuronal activity	D ₂	Conditional AAV viruses + D ₂ -Cre	Diminished cocaine sensitization	Lobo et al. (2010)
Tetanus toxin light chain inhibited synaptic transmission	D ₁	Overexpressed with AAV virus with substance P promoter	Diminished cocaine CPP and sensitization	Hikida et al. (2010)
Tetanus toxin light chain inhibited synaptic transmission	D ₂	Overexpressed with AAV virus with enkephalin promoter	No change in CPP, slight decrease in sensitization	Hikida et al. (2010)
hM ₄ D DREADD inhibit neuron firing	D ₁	Overexpressed with HSV virus with dynorphin promoter	Diminished amphetamine sensitization	Ferguson et al. (2011)
hM ₄ D DREADD inhibit neuron firing	D ₂	Overexpressed with HSV virus with enkephalin promoter	Enhanced amphetamine sensitization	Ferguson et al. (2011)
Inducible diphtheria toxin receptor – ablates cells	D ₂	Overexpressed with A _{2A} -Cre	Enhanced amphetamine CPP	Durieux et al. (2009)

Finally, recent literature reveals that the two MSNs exert antagonistic effects in drug-related behaviors, where activation of D₁+ MSNs or inhibition of D₂+ MSNs enhances an animal's sensitivity to a drug of abuse (Hikida et al., 2010; Lobo et al., 2010;

Ferguson et al., 2011). These findings are consistent with opposing roles of the two MSNs and their direct vs. indirect pathways in the basal ganglia in motor behaviors (Alexander et al., 1986; Albin et al., 1989; Graybiel, 2000; Kravitz et al., 2010). This recent

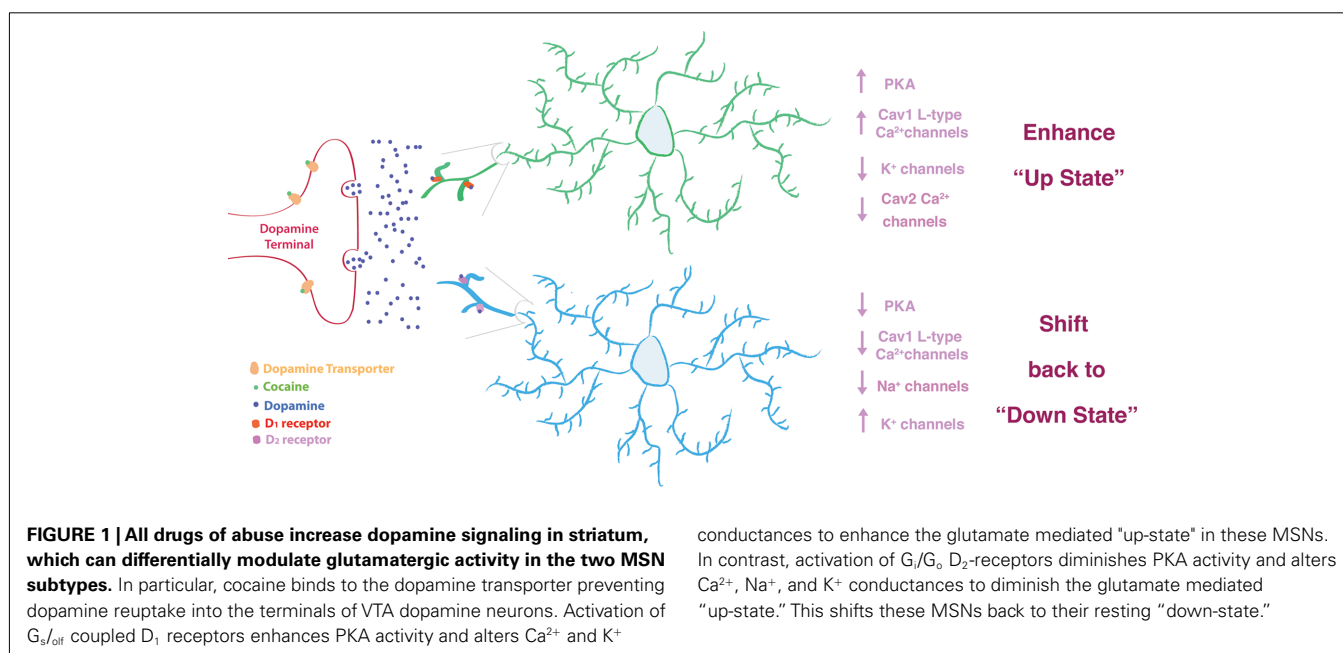
literature is in accord with the general idea that dopaminergic neurotransmission, which is activated by all drugs of abuse, facilitates glutamatergic activation of D₁+ MSNs while inhibiting glutamatergic activation of D₂+ MSNs through its actions on D₁ vs. D₂ dopamine receptors (**Figure 1**). In this review, we address the current knowledge of the distinct molecular signaling exhibited by these two MSN subtypes in relation to their functional roles and responses to drugs of abuse.

DOPAMINE RECEPTOR SIGNALING IN D₁ VS. D₂ MSNs

As already noted, all drugs of abuse activate dopaminergic input to the NAc and related limbic brain regions (Volkow et al., 2004; Wise, 2004; Nestler, 2005). For instance, psychostimulants such as cocaine or amphetamine act directly on the dopaminergic reward pathway by interfering with the dopamine transporter: cocaine blocks the transporter and amphetamine reverses the transporter, both actions resulting in a build up of dopamine in the synapse which can activate downstream dopamine receptors on target neurons (**Figure 1**). The two MSNs are most notably differentiated by their enrichment of D₁ vs. D₂-receptors although single-cell RT-PCR studies reveal that D₁+ MSNs express low levels of the D₂-like receptor, D₃ and D₂+ MSNs express low levels of the D₁-like receptor, D₅ (Surmeier et al., 1996). The two MSNs require glutamatergic innervation to drive neural activity; dopamine oppositely modulates these functional responses via stimulation of distinct dopamine receptor subtypes: by positively modulating excitatory glutamatergic input through D₁ receptor signaling via G_s or G_o_{lf}, which stimulates adenylyl cyclase leading to increased PKA activity, whereas dopamine negatively modulates this input through D₂-receptor signaling via G_i and G_o which inhibit adenylyl cyclase causing decreased PKA activity (Surmeier et al., 2007; Gerfen and Surmeier, 2011). In reality, each receptor exerts complex effects on many additional downstream signaling pathways. At rest, the two MSN subtypes are generally inhibited, they are in what researchers

have termed the down-state. Excitatory glutamatergic synaptic activity can release the MSNs from this down-state and shift them into a more depolarized state (the up-state). Dopamine oppositely modulates the excitatory glutamatergic shift to the up-state. D₁ activation of PKA enhances Cav1 L-type Ca²⁺ channel activity, decreases somatic K⁺ channel activity, and downregulates Cav2 Ca²⁺ channels that control activation of Ca²⁺ dependent, small-conductance K⁺ (SK) channels, resulting in increased spiking in these MSNs (Surmeier et al., 2007; Gerfen and Surmeier, 2011). In contrast, D₂ signaling inhibits the up-state transition, thereby preventing increased spiking, via reduction of Cav1 L-type Ca²⁺ channel activity and Nav1 Na⁺ channel activity while increasing K⁺ channel currents (Surmeier et al., 2007; Gerfen and Surmeier, 2011; **Figure 1**). Such opposite alterations in the two MSNs suggest that increased dopamine signaling elicited by drugs of abuse should enhance glutamatergic activation of D₁+ MSNs and reduce glutamatergic activation of D₂+ MSNs. In reality, such responses are far more varied and complex for reasons that remain poorly understood. This topic will be addressed further below.

The role of dopamine receptors in drug abuse is complex and often elusive (Self, 2010). There is an abundance of literature on the role of D₁ and D₂-receptor agonists and antagonists in modulating rewarding properties and self-administration of drugs of abuse, however, the results differ depending on the type of agonist/antagonist used, the type of delivery (systemic vs. brain region-specific), and the timing of the treatment (Self, 2010). Such results are further confounded by non-striatal specific effects, such as the contribution of pre-synaptic D₂-receptors from the VTA or presence of D₁ receptors in many other limbic regions, and the lack of specificity of the agonists/antagonists utilized as well as the expression of D₁-like and D₂-like receptors in both MSN subtypes as noted earlier. In general, it is thought that D₁ receptors play a more predominant role in the



primary rewarding properties of drugs of abuse, whereas D₂-receptors play a role in drug seeking mechanisms (Self et al., 1996; Self, 2010). Studies with D₁ receptor and D₂-receptor knock-out mice provide some insight into the role of these receptors in the two MSNs. D₁ knockout mice show a blunted induction of immediate early genes (IEGs) c-Fos and Zif268 in response to cocaine, a diminished response to psychostimulant-induced locomotor activity but with no alterations in cocaine-conditioned place preference (CPP) – an indirect measure of drug reward, and diminished cocaine self-administration and ethanol consumption (Miner et al., 1995; Drago et al., 1996; Crawford et al., 1997; El-Ghundi et al., 1998; Caine et al., 2007). D₂ knockout mice display diminished rewarding effects to opiates and cocaine as well as decreased ethanol consumption but no reduction in cocaine taking (Maldonado et al., 1997; Cunningham et al., 2000; Risinger et al., 2000; Caine et al., 2002; Chausmer et al., 2002; Elmer et al., 2002; Welter et al., 2007). Such data support important roles for D₁ and D₂-receptors in the two MSNs in multiple aspects of drug abuse, however, the knockouts lack striatal specificity and occur early in development, thus one cannot rule out other brain regions and cell-types and developmental factors in mediating these behaviors. Finally, decreased levels of D₂/D₃ receptors in striatum, as visualized by brain imaging, has become a common marker of addiction in human patients especially during periods of withdrawal (Volkow et al., 2009). Rodents receiving viral-mediated gene transfer of D₂-receptors to the NAc display attenuated cocaine self-administration and ethanol consumption (Thanos et al., 2004, 2008). These studies were not performed in a cell-type-specific manner, so we cannot rule out the possible effect of D₂-receptor overexpression influencing D₁+ MSNs. This collection of data emphasizes the need to move to more selective approaches, including cell-type-specific, region-specific, and even temporally specific manipulations of the dopamine receptors to better elucidate their functional roles in the two MSN subtypes in drug addiction.

Finally, it has been reported recently that D₂-GFP homozygote BAC transgenic mice display increased expression levels of the D₂-receptor in striatum and enhanced behavioral sensitivity and dopamine signaling to D₂ agonists. Moreover, both homozygotes and hemizygotes exhibit blunted behavioral responses to cocaine (Kramer et al., 2011). This study highlights the need to perform thorough characterization of D₁ and D₂ fluorescent reporter and Cre driver lines. However, the majority of the data collected in this study used homozygotes, which is not the ideal experimental genotype since 5–10% of transgene integrations result in insertional mutations (Meisler, 1992); therefore, the hemizygote genotype is the more reliable experimental genotype. Additionally, this study did not use littermate wildtype controls but used controls on a similar background (Swiss Webster) obtained from Taconic, while their transgenic lines were obtained from GENSAT and MMRRRC. Finally, another group has shown normal cocaine locomotor behavioral responses in D₂-GFP hemizygotes (Kim et al., 2011). Thus, future studies using proper controls and proper genotypes must be performed to fully characterize the various cell-type-specific transgenic lines available.

GLUTAMATE AND GABA SIGNALING IN D₁ VS. D₂ MSNs

Medium spiny neurons receive glutamatergic input from multiple brain regions including prefrontal cortex, amygdala, and hippocampus, and GABAergic input from local interneurons and perhaps collateral inputs from other MSNs. Net excitatory and inhibitory regulation of MSNs is no doubt crucial in regulating the drug-addicted state, and there is now a growing literature on the complex ways in which drugs of abuse alter glutamatergic neurotransmission in particular in the NAc (Pierce et al., 1996; Thomas et al., 2001; Beurrier and Malenka, 2002; Kourrich et al., 2007; Bachtell and Self, 2008; Bachtell et al., 2008; Conrad et al., 2008; Kalivas, 2009; Wolf, 2010). Although MSNs are thought to primarily exist in an inhibited down-state under basal conditions with glutamate driving activity of both cell-types, there remains limited information with respect to distinct regulation occurring in D₁ vs. D₂ MSNs.

ΔFosB overexpression in D₁+ MSNs (see below for more details) enhances the rewarding effects of cocaine and increases levels of the Ca²⁺-impermeable glutamate receptor subunit, GluR2, in NAc. Furthermore, viral-mediated gene transfer of GluR2 to the NAc similarly enhances the rewarding effects of cocaine (Kelz et al., 1999). However, it is not known whether the induction of GluR2 seen in response to ΔFosB overexpression in D₁+ MSNs is also specific to these neurons, and the viral overexpression of GluR2 is not cell-type-specific, therefore we cannot infer direct conclusions about GluR2 function in these two MSNs in drug reward. Heusner and Palmiter (2005) assessed the role of NMDA glutamatergic conductance in cocaine behaviors by expressing an NR1 subunit, which contains a mutation in the pore that reduces calcium flux, selectively in D₁+ MSNs. This group showed that lack of NMDA conductance in D₁+ MSNs prevents cocaine-induced CPP and cocaine locomotor sensitization, highlighting the necessity for NMDA signaling in D₁+ MSNs for the rewarding and sensitizing effects of cocaine (Heusner and Palmiter, 2005). Furthermore, recently it was found that knocking out the NR1 subunit in D₁+ MSNs attenuates amphetamine sensitization and this phenotype was rescued by resupplying the NR1 subunit to D₁+ MSNs specifically in the NAc (Beutler et al., 2011). Finally, knockdown of the mGluR5 subunit, using RNA interference, in D₁+ MSNs has no effect on the initial rewarding properties of cocaine but diminishes the cue-induced reinstatement of cocaine seeking (Novak et al., 2010). While these data reveal compelling roles for glutamatergic signaling in D₁+ MSNs, future work is needed to study glutamatergic systems in D₂+ MSNs. Future research should also evaluate how modulation of these glutamate receptor subunits in the two MSN subtypes affects the structural synaptic changes observed in NAc after drugs of abuse (Dietz et al., 2009; Russo et al., 2010), particularly the dendritic alterations observed after cocaine exposure selectively in the D₁+ MSNs (Lee et al., 2006; Kim et al., 2011) which may be associated with the increase in miniature excitatory postsynaptic currents observed in D₁+ MSNs (Kim et al., 2011). Interestingly, ΔFosB induction in D₁+ MSNs has been related directly to such dendritic adaptations after chronic cocaine (Maze et al., 2010).

In contrast to glutamate, there is a lack of research on GABA function in the two MSNs in addiction models, which is surprising

considering both ethanol and benzodiazepines enhance the effects of GABA and the two MSNs receive dense GABAergic inputs as stated above. There is also considerable evidence pointing to enhanced inhibition in the NAc at least after chronic cocaine exposure (White et al., 1995; Peoples et al., 1998; Zhang et al., 1998; Thomas et al., 2001; Beurrier and Malenka, 2002). Heiman et al. (2008) performed high throughput genetic screening in the two MSNs after chronic cocaine exposure and, interestingly, the most altered biological process in the D₁+ MSNs was GABA signaling. In particular, there was potent upregulation of GABA_A receptor subunits Gabra1 and Gabra4 as well as the GABA_B receptor subunit Gabrb3, and this group found that chronic cocaine increases the frequency of small-amplitude GABAergic mini inhibitory postsynaptic currents (mIPSCs) in D₁+ MSNs (Heiman et al., 2008). On the other hand, another group recently showed that chronic cocaine results in an opposite response with decreased frequency and amplitude of mIPSCs in the D₁+ MSNs (Kim et al., 2011). However, the latter group did show diminished membrane excitability in the D₁+ MSNs after chronic cocaine, which could be a reflection of enhanced GABA tone and is consistent with the field's assessment of enhanced inhibition in the NAc after exposure to chronic cocaine. Furthermore, such differences between the two groups could simply be due to the timing of cocaine exposure and withdrawal. In general, there is a need to study glutamatergic and GABAergic function in the two MSNs in response to drugs of abuse and the field is now equipped with the resources that make such a cell-type- and region-specific study possible.

OTHER RECEPTOR SIGNALING IN D₁ VS. D₂ MSN SUBTYPES

The two MSNs are differentially enriched in other G-protein-coupled receptors in addition to dopamine receptors. D₁+ MSNs express higher levels of the acetylcholine muscarinic receptor 4 (M₄; Bernard et al., 1992; Ince et al., 1997) and D₂+ MSNs are enriched in both adenosine receptor 2A (A_{2A}; Schiffmann et al., 1991; Schiffmann and Vanderhaeghen, 1993) and G-protein-coupled receptor 6 (Gpr6; Lobo et al., 2007; gensat.org). M₄ is coupled to G_{i/o}, which would produce an opposite response, compared to D₁ receptors, in D₁+ MSNs by inhibiting cAMP/PKA activity. Indeed, a D₁+ MSN selective M₄ knockout displayed enhanced behavioral sensitization to cocaine and amphetamine (Jeon et al., 2010). Furthermore, recent studies using a designer receptor exclusively activated by a synthetic drug (DREADDs) showed that activation of the DREADD Gi/o-coupled human M₄ receptor (hM₄D) in D₁+ MSNs diminished behavioral sensitization to amphetamine, with the opposite response seen in D₂+ MSNs (Ferguson et al., 2011). Such data reveal the antagonizing role of M₄ receptors in D₁+ MSNs in drug abuse. As well, since the hM₄D receptor potently inhibits these MSNs, the data provide insight into the effect of altered activity of these two MSNs in drug abuse, which will be discussed further below.

Both A_{2A} and Gpr6 are positively coupled to G_s/G_{olf} proteins, implicating their role in antagonizing the D₂-receptor in D₂+ MSNs. Indeed, stimulation of A_{2A} receptors has been shown to reduce both the development and expression of cocaine sensitization (Filip et al., 2006), impair the initiation of cocaine

self-administration (Knapp et al., 2001), and antagonize the reinstatement of cocaine seeking elicited by cocaine, D₂-receptor stimulation, or cocaine-conditioned cues (Bachtell and Self, 2009). As Gpr6 is also enriched in D₂+ MSNs (Lobo et al., 2007), its role in behavioral functions of the striatum should be evaluated. To date, it has been shown to influence instrumental learning (Lobo et al., 2007) but its role in drug abuse models is yet unknown.

The cannabinoid receptor 1 (CB1) is expressed ubiquitously throughout the central nervous system (Mackie, 2008), hence it is difficult to dissect the precise role of specific brain regions and cell-types in mediating Δ^9 -tetrahydrocannabinol (THC) addiction. Recently, deletion of CB1 from D₁+ MSNs was found to modestly affect behavioral responses to THC, including blunted effects in THC-induced hypolocomotion, hypothermia, and analgesia (Monory et al., 2007). It would be interesting to evaluate cannabinoid receptor function in D₂+ MSNs since these MSNs express endocannabinoid-mediated long-term depression (eCB-LTD), which requires dopamine D₂-receptor activation (Kreitzer and Malenka, 2007).

The glucocorticoid receptor, Nr3c1, is also broadly expressed in the CNS and periphery. Stress-induced glucocorticoid secretion can potentiate maladaptive behaviors including drug addiction (Frank et al., 2011). In particular, disrupting glucocorticoid signaling in D₁+ MSNs by deleting Nr3c1 diminished the motivation these mice display to self-administer cocaine, and this is consistent with previous data where Nr3c1 was deleted from the entire brain (Ambroggi et al., 2009). These data are consistent with other findings described in this review, showing a predominant role for D₁+ MSNs in mediating many of the effects of drugs of abuse.

Finally, we recently disrupted BDNF signaling in the two MSNs by deleting its TrkB receptor selectively from each MSN subtype. We observed opposite effects on cocaine-elicited behaviors: cocaine-induced locomotor activity and the induction of cocaine CPP were enhanced after TrkB deletion from D₁+ MSNs, but attenuated after deletion from D₂+ MSNs (Lobo et al., 2010). Interestingly, the deletion of TrkB from D₂+ MSNs mimics the effects of total deletion of TrkB from the NAc as well as disruption of BDNF signaling from the VTA (Horger et al., 1999; Graham et al., 2007, 2009; Bahi et al., 2008; Crooks et al., 2010). These findings thus show for the first time a predominant role of a signaling cascade in D₂+ MSNs in mediating the effects of a drug of abuse. The predominant role of D₂+ MSNs in mediating BDNF's effects on cocaine-elicited behaviors is not surprising considering both TrkB mRNA and protein are enriched in D₂+ MSNs (Lobo et al., 2010; Baydyuk et al., 2011). The behavioral changes observed in these mice were accompanied by enhanced neuronal activity in the D₂+ MSNs upon a selective knockout of TrkB. These findings prompted us to use optogenetic technology to selectively manipulate MSN activity in cocaine reward (see below).

TRANSCRIPTION FACTORS IN D₁ VS. D₂ MSNs

The most compelling evidence for the more robust role of D₁+ MSNs in drug abuse comes from literature evaluating induction of intracellular signaling molecules. As stated above, acute doses of psychostimulants induce IEG expression, including c-Fos, Zif268 (Egr1), and FosB primarily in D₁+ MSNs in NAc

and dStr (Robertson et al., 1991; Young et al., 1991; Berretta et al., 1992; Cenci et al., 1992; Moratalla et al., 1992; Bertran-Gonzalez et al., 2008). This induction requires activation of D₁ receptors, and the cell-type-specificity of the IEG induction in response to acute cocaine was recently confirmed using D₁-GFP and D₂-GFP reporter mice (Bertran-Gonzalez et al., 2008). Interestingly, the confirmation of cocaine's induction of c-Fos primarily in D₁-GFP throughout striatum with a small induction in D₂-GFP MSNs only in dStr was confirmed using a context-dependent paradigm (mice were injected in a novel environment outside of their home cage). Furthermore, a previous study using *in situ* hybridization in mice also showed induction of c-Fos in D₁+ and D₂+ MSNs in dStr, although in this study representative bar graphs show greater number of D₁+ c-Fos positive neurons (Ferguson et al., 2006). Interestingly, this study reveals significantly enhanced c-Fos induction in D₂+ MSNs in the dStr after loss of ERK1, which parallels our findings of enhanced c-Fos induction in D₂+ MSNs specifically in the NAc shell after disruption of BDNF signaling which is known to enhance ERK activity (Lobo et al., 2010). However, opposite behavioral responses to cocaine were observed in each study, which may reflect induction of c-Fos in D₂+ MSNs in dStr vs. NAc shell. Finally, previous literature using *in situ* hybridization/immunohistochemistry in rats has shown acute psychostimulants can induce c-Fos equally in both MSNs when the drug is given in a novel environment (Badiani et al., 1999; Uslaner et al., 2001a,b; Ferguson and Robinson, 2004) and chronic administration of amphetamine is reported to selectively induce c-Fos in D₂+ MSNs (Mattson et al., 2007). These different results could be a reflection of the experimental procedures used (*in situ* hybridization vs. GFP reporter mice) or even be due to the animal species used as the latter experiments used rats.

Recently, researchers genetically profiled the cocaine context-dependent, c-Fos activated neurons in rats using immunolabeled fluorescence activated cell sorting (FACS) and showed that the c-Fos+ neurons are enriched in a D₁+ MSN gene, prodynorphin (Pdyn), but have lower levels of D₂ and A_{2A}, both D₂+ MSN genes (Guez-Barber et al., 2011), suggesting that the c-Fos+ activated neurons consist primarily of D₁+ MSNs. Furthermore, this group previously showed that c-Fos expressing MSNs are important for this context-dependent sensitization, as ablation of these neurons abolishes this behavioral phenotype (Koya et al., 2009). Although previous data showed that the cocaine context-dependent induction of c-Fos occurs in both D₁+ and D₂+ MSNs in rats, the more recent results correspond to findings in which deletion of c-Fos selectively from D₁+ MSNs blunts cocaine-induced locomotor sensitization in mice (Zhang et al., 2006). Furthermore, this group found that deletion of c-Fos in D₁+ MSNs blunts the dendritic spine changes normally induced by cocaine in the NAc, indicating a role for c-Fos in mediating these synaptic plasticity changes. Finally, the group observed no change in the induction of cocaine CPP, but found that loss of c-Fos in D₁+ MSNs prevented extinction of cocaine CPP. Such data illustrate a dynamic role for c-Fos induction in D₁+ MSNs, however, one cannot rule out the differential effects at the behavioral level as being mediated by any of several other limbic brain regions that express the D₁ receptor.

Another IEG that has been extensively studied in the two MSN subtypes is FosB. Acute exposure to cocaine induces FosB in D₁+ MSNs (Berretta et al., 1992), whereas chronic exposure induces Δ FosB, a stable product of the FosB gene generated by alternative splicing (Hope et al., 1994; Nestler et al., 2001; Nestler, 2008), in D₁+ MSNs (Nye et al., 1995; Moratalla et al., 1996; Lee et al., 2006). Similar findings are observed with many other drugs of abuse as well as with natural rewards such as food, sex, and wheel running. For example, chronic wheel running, which is a natural reward (Iversen, 1993; Belke, 1997; Lett et al., 2000), induces Δ FosB in D₁+ MSNs but not D₂+ MSNs (Werme et al., 2002). To gain functional insight into the role of Δ FosB in the two MSNs, our group generated NSE-tTa lines, termed 11A and 11B, which direct transgene expression to either D₁+ or D₂+ MSNs, respectively (Chen et al., 1998; Kelz et al., 1999; Werme et al., 2002). Line 11A mice crossed with a Tet-Op Δ FosB line show increased responses to the rewarding and locomotor effects of cocaine (Kelz et al., 1999), which is consistent with Δ FosB induction in D₁+ MSNs (Nye et al., 1995; Moratalla et al., 1996). Furthermore, these same mice display increased morphine reward (evaluated by CPP) as well as diminished morphine analgesia and enhanced morphine tolerance, while the 11B Tet-Op Δ FosB mice show no change in morphine reward. Overexpression of a dominant negative antagonist of Δ FosB exerts effects opposite to those seen with Δ FosB, although this mouse model does not distinguish D₁ vs. D₂ MSNs (Peakman et al., 2003). Together, these data further supports the role of Δ FosB induction in D₁+ MSNs as an important molecular player in the rewarding properties of drugs of abuse (Zachariou et al., 2006). This phenomenon is also observed in other reward behaviors, in particular, wheel running: 11A Tet-Op Δ FosB mice display increased wheel running behavior, whereas 11B Tet-Op Δ FosB mice display diminished wheel running (Werme et al., 2002). The finding that Δ FosB induction in D₁ MSNs promotes reward is consistent with recent findings that such cell-type-selective induction also promotes resilience responses to chronic stress (Vialou et al., 2010). Finally, chronic cocaine induction of Δ FosB in D₁+ MSNs was shown to be accompanied by robust long-lasting increases in dendritic spine densities (Lee et al., 2006) and recently Δ FosB in the NAc was shown to be both necessary and sufficient in mediating the increased density of dendritic spines in this brain region (Maze et al., 2010). Such data support a role for Δ FosB in D₁+ MSNs in mediating the rewarding aspects of drugs of abuse and natural rewards as well as the accompanying structural plasticity changes. The data also suggest that induction of Δ FosB in D₂+ MSNs confers negative consequences to rewarding stimuli. Since Δ FosB induction in D₂+ MSNs is seen in response to chronic stress and antipsychotic drug exposure (Hiroi and Graybiel, 1996; Perrotti et al., 2004), further studies of the latter actions are needed.

OTHER INTRACELLULAR SIGNALING MOLECULES IN D₁ VS. D₂ MSNs

One signaling molecule that has been well studied in the two MSNs in the context of drug abuse is the protein kinase, ERK (extracellular signal related kinase). Acute or chronic exposure to cocaine induces phosphorylated ERK (pERK), the activated form of the protein, in the NAc and dStr in D₁+ MSNs using D₁-GFP and

D₂-GFP BAC transgenic reporter mice (Bertran-Gonzalez et al., 2008) and this response is mediated through D₁ receptors (Valjent et al., 2000; Lu et al., 2006). This group also showed that pMSK-1 (phospho-MAP and stress activated kinase-1) and histone H3, both targets of pERK signaling, are robustly induced in pERK containing D₁+ MSNs after acute cocaine exposure and modestly increased after chronic cocaine (Bertran-Gonzalez et al., 2008). pERK is also induced in response to chronic morphine, in particular, pERK is robustly induced in D₁+ MSNs and modestly induced in D₂+ MSNs in the NAc shell after withdrawal in response to the context-specific association with morphine (Borgkvist et al., 2008). The precise functional role of pERK in drug addiction remains to be determined. Pharmacological treatment with ERK inhibitors has been shown to decrease cocaine reward, however, a knockout of ERK1 potentiates cocaine reward, suggesting that ERK inhibitors may preferentially be affecting ERK2. Recently, we showed that optogenetic activation of D₁+ MSNs in the NAc, which increases an animal's rewarding responses to cocaine, potently reduces both pERK1 and pERK2. Future studies manipulating ERK expression in a cell-type-specific manner are necessary to fully address the functional role of ERK signaling in the two MSNs in drug abuse.

DARPP-32 is another signaling molecule that has been extensively studied in response to drugs of abuse. It is well known that acute psychostimulants lead to PKA phosphorylation of DARPP-32 at threonine 34 (T34), causing it to become a potent inhibitor of protein phosphatase 1 (PP-1), which regulates the phosphorylation state of many effector proteins, including transcription factors, ionotropic receptors, and ion channels (Greengard et al., 1999). However, until recently, it was unclear which MSN subtype mediates this biochemical change. Greengard et al. (1999) generated BAC transgenic mouse models that enable the evaluation of DARPP-32 phosphorylation in D₁+ or D₂+ MSNs by expressing tagged versions of DARPP-32 using D₁ or D₂ BACs allowing for immunoprecipitation of DARPP-32 from each MSN subtype. These studies demonstrated that acute cocaine treatment increases T34 phosphorylation in D₁+ MSNs and induces phosphorylation of threonine 75 (T75) by Cdk5, which inhibits PKA signaling, selectively in D₂+ MSNs (Bateup et al., 2008). Finally this group showed that deletion of DARPP-32 from each MSN subtype using D₁-Cre and D₂-Cre BAC transgenic mice results in opposite regulation of cocaine-induced locomotor activity (Bateup et al., 2010). Loss of DARPP-32 from D₁+ MSNs diminished the locomotor effects of cocaine, which mimics previous data evaluating a total DARPP-32 knockout (Fienberg et al., 1998), whereas loss of DARPP-32 from D₂+ MSNs enhanced cocaine locomotor responses. Such data provide concrete evidence for differential roles of DARPP-32 in the two MSNs in response to drugs of abuse and illustrate the importance of cell-type-specific methods to fully understand the contribution of these two neuronal types in drug addiction.

MODULATING ACTIVITY OF D₁ OR D₂ MSNs

Directly modulating the activity of the two MSN subtypes has recently provided novel insight into the molecular and functional role of D₁ and D₂ MSNs in addiction. We used optogenetic tools combined with a conditional (i.e., Cre-dependent)

adeno-associated viral (AAV) vector expressing the blue light-activated cation channel, channelrhodopsin-2 (ChR2). We injected the vector, or a control, into the NAc of D₁-Cre or D₂-Cre BAC transgenic mice and then stimulated the injected region with blue light to selectively activate D₁+ vs. D₂+ MSNs in the context of cocaine CPP. We found that activation of D₁+ MSNs potentiates induction of cocaine CPP, whereas activation of D₂+ MSNs inhibits this induction (Lobo et al., 2010). As noted previously, we observed the same behavioral effects when TrkB was deleted selectively from these MSN subtypes: enhanced cocaine CPP and locomotor activity after TrkB deletion from D₁+ MSNs, and reduced cocaine CPP and locomotor activity after TrkB deletion from D₂+ MSNs. The likely common action of TrkB knockout and optogenetic stimulation in D₂+ MSNs is their increased activity, since deletion of TrkB from these cells increases their electrical excitability. As mentioned earlier, we also found a robust reduction of pERK after TrkB deletion from D₁+ MSNs. pERK is a known downstream target of BDNF signaling, therefore, the shared behavioral effects observed after TrkB deletion from D₁+ MSNs and from optogenetic activation of these cells might be due to converging effects on pERK activity. However, future work is needed to determine the precise, shared molecular underpinnings that govern the behavioral effects seen after disruption of BDNF signaling and optogenetic control of these two neuronal subtypes.

Other groups have used different tools to modulate activity of the two MSNs in drug abuse models. Hikida et al. (2010) used AAV vectors to express tetracycline-repressive transcription factor (tTa) using the substance P (a D₁+ MSN gene) or enkephalin (a D₂+ MSN gene) promoters. These vectors were injected into the NAc of mice, in which tetanus toxin light chain (TN) – a bacterial toxin that cleaves the synaptic vesicle-associated protein, VAMP2 – was controlled by the tetracycline-responsive element, to selectively abolish synaptic transmission in each MSN subtype. Consistent with our optogenetic approach, these data showed a role of D₁+ MSN activity in enhancing cocaine CPP as well as cocaine-induced locomotor activity, since abolishing synaptic transmission in D₁+ MSNs diminished both behavioral effects. In contrast to the optogenetic studies, the authors found no alterations in cocaine CPP after abolishing synaptic transmission in D₂+ MSNs, but did observe reduced cocaine-induced locomotor activity in response to the first two cocaine exposures. Interestingly, this group showed that inactivation of the D₂+ MSNs played a more profound role in mediating aversive behaviors.

As stated earlier, Ferguson et al. (2011) used herpes simplex virus (HSV) vectors to express an engineered GPCR (a G_{i/o}-coupled human muscarinic M₄ designer receptor exclusively activated by a designer drug, hM₄D) that is activated by an otherwise pharmacologically inert ligand using enkephalin and dynorphin promoters to selectively silence D₁+ or D₂+ MSNs in the dStr. The authors showed that transiently disrupting D₂+ MSN activity in dStr facilitated amphetamine sensitization, whereas decreasing excitability of D₁+ MSNs impaired the persistence of amphetamine-induced sensitization. Finally, abolishing D₂+ MSNs in the NAc at adult ages using diphtheria toxin receptor enhances the rewarding effect of amphetamine (Durieux et al., 2009). Such data are in accordance with our optogenetic findings,

and together implicate opposite roles of D₁+ vs. D₂+ MSNs in drug addiction, with D₁+ MSNs promoting both reward and sensitizing responses to psychostimulants and D₂+ MSNs dampening these behaviors.

FUTURE DIRECTIONS

The field has made tremendous advances toward understanding the selective role of the D₁+ and D₂+ MSN subtypes in NAc and dStr in mediating the effects of drugs of abuse. In particular, recently developed tools that enable the selective manipulation of these cell-types have played a predominant role in obtaining the majority of this information. What are the next steps? Since the underlying molecular adaptations in drug addiction models are not static, but very dynamic, it is crucial to develop the capability to selectively manipulate signaling molecules of interest in D₁+ vs. D₂+ MSNs in a temporally precise way. DREADDs and optogenetic tools can help with this time scale manipulation. DREADD ligands can be administered at different time courses throughout

drug behavioral paradigms to parcel out the selective role of signaling receptors in the two MSNs in drug models. Optogenetic tools in particular provide an extremely powerful means to temporally regulate not only neuronal activity but G-protein-coupled receptor signaling using OptoXRs (Airan et al., 2009), glutamatergic signaling (Volgraf et al., 2006; Numano et al., 2009), GABAergic signaling, and even certain intracellular signaling molecules (Wu et al., 2009; Hahn and Kuhlman, 2010). Ultimately, it may be possible to extend these capabilities to optogenetic regulation of transcriptional activity. Likewise, optogenetic tools are making it possible for the first time to study the influence of specific inputs to striatum and to determine whether such inputs impinge in selective ways on D₁+ vs. D₂+ MSNs (Higley and Sabatini, 2010). The ability to control such signaling and molecular properties with great temporal resolution will allow major steps to be made toward a more comprehensive understanding of the two MSN subtypes, and other cell subtypes in NAc and dStr, in mediating the time course and different phases of drug addiction.

REFERENCES

- Airan, R. D., Thompson, K. R., Fenno, L. E., Bernstein, H., and Deisseroth, K. (2009). Temporally precise in vivo control of intracellular signalling. *Nature* 458, 1025–1029.
- Albin, R. L., Young, A. B., and Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends Neurosci.* 12, 366–375.
- Alexander, G. E., Delong, M. R., and Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu. Rev. Neurosci.* 9, 357–381.
- Ambroggi, F., Turiault, M., Milet, A., Deroche-Gamonet, V., Parnaud, S., Balado, E., Barik, J., Van Der Veen, R., Maroteaux, G., Lemberger, T., Schütz, G., Lazar, M., Marinelli, M., Piazza, P. V., and Tronche, F. (2009). Stress and addiction: glucocorticoid receptor in dopaminergic neurons facilitates cocaine seeking. *Nat. Neurosci.* 12, 247–249.
- Bachtell, R. K., Choi, K. H., Simmons, D. L., Falcon, E., Monteggia, L. M., Neve, R. L., and Self, D. W. (2008). Role of GluR1 expression in nucleus accumbens neurons in cocaine sensitization and cocaine-seeking behavior. *Eur. J. Neurosci.* 27, 2229–2240.
- Bachtell, R. K., and Self, D. W. (2008). Renewed cocaine exposure produces transient alterations in nucleus accumbens AMPA receptor-mediated behavior. *J. Neurosci.* 28, 12808–12814.
- Bachtell, R. K., and Self, D. W. (2009). Effects of adenosine A2A receptor stimulation on cocaine-seeking behavior in rats. *Psychopharmacology (Berl.)* 206, 469–478.
- Badiani, A., Oates, M. M., Day, H. E., Watson, S. J., Akil, H., and Robinson, T. E. (1999). Environmental modulation of amphetamine-induced c-fos expression in D1 versus D2 striatal neurons. *Behav. Brain Res.* 103, 203–209.
- Bahi, A., Boyer, F., Chandrasekar, V., and Dreyer, J. L. (2008). Role of accumbens BDNF and TrkB in cocaine-induced psychomotor sensitization, conditioned-place preference, and reinstatement in rats. *Psychopharmacology (Berl.)* 199, 169–182.
- Bateup, H. S., Santini, E., Shen, W., Birnbaum, S., Valjent, E., Surmeier, D. J., Fisone, G., Nestler, E. J., and Greengard, P. (2010). Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14845–14850.
- Bateup, H. S., Svenningsson, P., Kuroiwa, M., Gong, S., Nishi, A., Heintz, N., and Greengard, P. (2008). Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. *Nat. Neurosci.* 11, 932–939.
- Baydyuk, M., Nguyen, M. T., and Xu, B. (2011). Chronic deprivation of TrkB signaling leads to selective late-onset nigrostriatal dopaminergic degeneration. *Exp. Neurol.* 228, 118–125.
- Belke, T. W. (1997). Running and responding reinforced by the opportunity to run: effect of reinforcer duration. *J. Exp. Anal. Behav.* 67, 337–351.
- Bernard, V., Normand, E., and Bloch, B. (1992). Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. *J. Neurosci.* 12, 3591–3600.
- Berretta, S., Robertson, H. A., and Graybiel, A. M. (1992). Dopamine and glutamate agonists stimulate neuron-specific expression of Fos-like protein in the striatum. *J. Neurophysiol.* 68, 767–777.
- Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamalas, M., Herve, D., Valjent, E., and Girault, J. A. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J. Neurosci.* 28, 5671–5685.
- Beurrier, C., and Malenka, R. C. (2002). Enhanced inhibition of synaptic transmission by dopamine in the nucleus accumbens during behavioral sensitization to cocaine. *J. Neurosci.* 22, 5817–5822.
- Beutler, L. R., Wanat, M. J., Quintana, A., Sanz, E., Bamford, N. S., Zweifel, L. S., and Palmiter, R. D. (2011). Balanced NMDA receptor activity in dopamine D1 receptor (D1R)- and D2R-expressing medium spiny neurons is required for amphetamine sensitization. *Proc. Natl. Acad. Sci. U.S.A.* 108, 4206–4211.
- Borgkvist, A., Valjent, E., Santini, E., Herve, D., Girault, J. A., and Fisone, G. (2008). Delayed, context- and dopamine D1 receptor-dependent activation of ERK in morphine-sensitized mice. *Neuropharmacology* 55, 230–237.
- Caine, S. B., Negus, S. S., Mello, N. K., Patel, S., Bristow, L., Kulagowski, J., Vallone, D., Saiardi, A., and Borrelli, E. (2002). Role of dopamine D2-like receptors in cocaine self-administration: studies with D2 receptor mutant mice and novel D2 receptor antagonists. *J. Neurosci.* 22, 2977–2988.
- Caine, S. B., Thomsen, M., Gabriel, K. I., Berkowitz, J. S., Gold, L. H., Koob, G. F., Tonegawa, S., Zhang, J., and Xu, M. (2007). Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. *J. Neurosci.* 27, 13140–13150.
- Cardin, J. A., Carlen, M., Meletis, K., Knoblich, U., Zhang, F., Deisseroth, K., Tsai, L. H., and Moore, C. I. (2010). Targeted optogenetic stimulation and recording of neurons in vivo using cell-type-specific expression of channelrhodopsin-2. *Nat. Protoc.* 5, 247–254.
- Cenci, M. A., Campbell, K., Wictorin, K., and Bjorklund, A. (1992). Striatal c-fos induction by cocaine or apomorphine occurs preferentially in output neurons projecting to the substantia nigra in the rat. *Eur. J. Neurosci.* 4, 376–380.
- Chausmer, A. L., Elmer, G. I., Rubinstein, M., Low, M. J., Grandy, D. K., and Katz, J. L. (2002). Cocaine-induced locomotor activity and cocaine discrimination in dopamine D2 receptor mutant mice. *Psychopharmacology (Berl.)* 163, 54–61.
- Chen, J., Kelz, M. B., Zeng, G., Sakai, N., Steffen, C., Shockett, P. E., Picciotto, M. R., Duman, R. S., and Nestler, E. J. (1998). Transgenic animals with inducible, targeted gene expression in brain. *Mol. Pharmacol.* 54, 495–503.

- Conrad, K. L., Tseng, K. Y., Uejima, J. L., Reimers, J. M., Heng, L. J., Shaham, Y., Marinelli, M., and Wolf, M. E. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature* 454, 118–121.
- Crawford, C. A., Drago, J., Watson, J. B., and Levine, M. S. (1997). Effects of repeated amphetamine treatment on the locomotor activity of the dopamine D1A-deficient mouse. *Neuroreport* 8, 2523–2527.
- Crooks, K. R., Kleven, D. T., Rodriguez, R. M., Wetsel, W. C., and McNamara, J. O. (2010). TrkB signaling is required for behavioral sensitization and conditioned place preference induced by a single injection of cocaine. *Neuropharmacology* 58, 1067–1077.
- Cunningham, C. L., Howard, M. A., Gill, S. J., Rubinstein, M., Low, M. J., and Grandy, D. K. (2000). Ethanol-conditioned place preference is reduced in dopamine D2 receptor-deficient mice. *Pharmacol. Biochem. Behav.* 67, 693–699.
- Deroche-Gamonet, V., Sillaber, I., Aouizerate, B., Izawa, R., Jaber, M., Ghozland, S., Kellendonk, C., Le Moal, M., Spanagel, R., Schutz, G., Tronche, F., and Piazza, P. V. (2003). The glucocorticoid receptor as a potential target to reduce cocaine abuse. *J. Neurosci.* 23, 4785–4790.
- Dietz, D. M., Dietz, K. C., Nestler, E. J., and Russo, S. J. (2009). Molecular mechanisms of psychostimulant-induced structural plasticity. *Pharmacopsychiatry* 42(Suppl. 1), S69–S78.
- Drago, J., Gerfen, C. R., Westphal, H., and Steiner, H. (1996). D1 dopamine receptor-deficient mouse: cocaine-induced regulation of immediate-early gene and substance P expression in the striatum. *Neuroscience* 74, 813–823.
- Durieux, P. F., Bearzatto, B., Guiducci, S., Buch, T., Waisman, A., Zoli, M., Schiffmann, S. N., and De Kerchove D'Exaerde, A. (2009). D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat. Neurosci.* 12, 393–395.
- El-Ghundi, M., George, S. R., Drago, J., Fletcher, P. J., Fan, T., Nguyen, T., Liu, C., Sibley, D. R., Westphal, H., and O'Dowd, B. F. (1998). Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. *Eur. J. Pharmacol.* 353, 149–158.
- Elmer, G. I., Pieper, J. O., Rubinstein, M., Low, M. J., Grandy, D. K., and Wise, R. A. (2002). Failure of intravenous morphine to serve as an effective instrumental reinforcer in dopamine D2 receptor knock-out mice. *J. Neurosci.* 22, RC224.
- Ferguson, S. M., Eskenazi, D., Ishikawa, M., Wanat, M. J., Phillips, P. E., Dong, Y., Roth, B. L., and Neumaier, J. F. (2011). Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nat. Neurosci.* 14, 22–24.
- Ferguson, S. M., Fasano, S., Yang, P., Brambilla, R., and Robinson, T. E. (2006). Knockout of ERK1 enhances cocaine-evoked immediate early gene expression and behavioral plasticity. *Neuropsychopharmacology* 31, 2660–2668.
- Ferguson, S. M., and Robinson, T. E. (2004). Amphetamine-evoked gene expression in striatopallidal neurons: regulation by corticostriatal afferents and the ERK/MAPK signaling cascade. *J. Neurochem.* 91, 337–348.
- Fienberg, A. A., Hiroi, N., Mermelstein, P. G., Song, W., Snyder, G. L., Nishi, A., Cheramy, A., O'Callaghan, J. P., Miller, D. B., Cole, D. G., Corbett, R., Haile, C. N., Cooper, D. C., Onn, S. P., Grace, A. A., Ouimet, C. C., White, F. J., Hyman, S. E., Surmeier, D. J., Girault, J., Nestler, E. J., and Greengard, P. (1998). DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. *Science* 281, 838–842.
- Filip, M., Frankowska, M., Zaniowska, M., Przegalinski, E., Muller, C. E., Agnati, L., Franco, R., Roberts, D. C., and Fuxe, K. (2006). Involvement of adenosine A2A and dopamine receptors in the locomotor and sensitizing effects of cocaine. *Brain Res.* 1077, 67–80.
- Frank, M. G., Watkins, L. R., and Maier, S. F. (2011). Stress- and glucocorticoid-induced priming of neuroinflammatory responses: potential mechanisms of stress-induced vulnerability to drugs of abuse. *Brain Behav. Immun.* 25, S21–S28.
- Gerfen, C. R. (1984). The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. *Nature* 311, 461–464.
- Gerfen, C. R. (1992). The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. *Annu. Rev. Neurosci.* 15, 285–320.
- Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J. Jr., and Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250, 1429–1432.
- Gerfen, C. R., and Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. *Annu. Rev. Neurosci.* 34, 441–466.
- Gerfen, C. R., and Young, W. S. III. (1988). Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Res.* 460, 161–167.
- Gong, S., Doughty, M., Harbaugh, C. R., Cummins, A., Hatten, M. E., Heintz, N., and Gerfen, C. R. (2007). Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J. Neurosci.* 27, 9817–9823.
- Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E., and Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425, 917–925.
- Graham, D. L., Edwards, S., Bachtell, R. K., Dileone, R. J., Rios, M., and Self, D. W. (2007). Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat. Neurosci.* 10, 1029–1037.
- Graham, D. L., Krishnan, V., Larson, E. B., Graham, A., Edwards, S., Bachtell, R. K., Simmons, D., Gent, L. M., Berton, O., Bolanos, C. A., Dileone, R. J., Parada, L. F., Nestler, E. J., and Self, D. W. (2009). Tropomyosin-related kinase B in the mesolimbic dopamine system: region-specific effects on cocaine reward. *Biol. Psychiatry* 65, 696–701.
- Graybiel, A. M. (2000). The basal ganglia. *Curr. Biol.* 10, R509–R511.
- Greengard, P., Allen, P. B., and Nairn, A. C. (1999). Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* 23, 435–447.
- Guez-Barber, D., Fanous, S., Golden, S. A., Schrama, R., Koya, E., Stern, A. L., Bossert, J. M., Harvey, B. K., Picciotto, M. R., and Hope, B. T. (2011). FACS identifies unique cocaine-induced gene regulation in selectively activated adult striatal neurons. *J. Neurosci.* 31, 4251–4259.
- Hahn, K. M., and Kuhlman, B. (2010). Hold me tightly LOV. *Nat. Methods* 7, 595, 597.
- Heiman, M., Schaefer, A., Gong, S., Peterson, J. D., Day, M., Ramsey, K. E., Suarez-Farinas, M., Schwarz, C., Stephan, D. A., Surmeier, D. J., Greengard, P., and Heintz, N. (2008). A translational profiling approach for the molecular characterization of CNS cell types. *Cell* 135, 738–748.
- Heusner, C. L., Beutler, L. R., Houser, C. R., and Palmiter, R. D. (2008). Deletion of GAD67 in dopamine receptor-1 expressing cells causes specific motor deficits. *Genesis* 46, 357–367.
- Heusner, C. L., and Palmiter, R. D. (2005). Expression of mutant NMDA receptors in dopamine D1 receptor-containing cells prevents cocaine sensitization. *J. Neurosci.* 25, 6651–6657.
- Higley, M. J., and Sabatini, B. L. (2010). Competitive regulation of synaptic Ca²⁺ influx by D2 dopamine and A2A adenosine receptors. *Nat. Neurosci.* 13, 958–966.
- Hikida, T., Kimura, K., Wada, N., Funabiki, K., and Nakanishi, S. (2010). Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron* 66, 896–907.
- Hiroi, N., and Graybiel, A. M. (1996). Atypical and typical neuroleptic treatments induce distinct programs of transcription factor expression in the striatum. *J. Comp. Neurol.* 374, 70–83.
- Hope, B. T., Nye, H. E., Kelz, M. B., Self, D. W., Iadarola, M. J., Nakabeppu, Y., Duman, R. S., and Nestler, E. J. (1994). Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* 13, 1235–1244.
- Horger, B. A., Iyasere, C. A., Berhow, M. T., Messer, C. J., Nestler, E. J., and Taylor, J. R. (1999). Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J. Neurosci.* 19, 4110–4122.
- Ince, E., Ciliax, B. J., and Levey, A. I. (1997). Differential expression of D1 and D2 dopamine and m4 muscarinic acetylcholine receptor proteins in identified striatonigral neurons. *Synapse* 27, 357–366.
- Iversen, I. H. (1993). Techniques for establishing schedules with wheel running as reinforcement in rats. *J. Exp. Anal. Behav.* 60, 219–238.
- Jeon, J., Dencker, D., Wortwein, G., Woldbye, D. P., Cui, Y., Davis, A. A., Levey, A. I., Schutz, G., Sager, T. N., Mork, A., Li, C., Deng, C. X., Fink-Jensen, A., and Wess, J. (2010). A subpopulation of neuronal M4 muscarinic acetylcholine receptors plays a critical role in modulating dopamine-dependent behaviors. *J. Neurosci.* 30, 2396–2405.

- Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nat. Rev. Neurosci.* 10, 561–572.
- Kelz, M. B., Chen, J., Carlezon, W. A. Jr., Whisler, K., Gilden, L., Beckmann, A. M., Steffen, C., Zhang, Y. J., Marotti, L., Self, D. W., Tkatch, T., Baranaukas, G., Surmeier, D. J., Neve, R. L., Duman, R. S., Picciotto, M. R., and Nestler, E. J. (1999). Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature* 401, 272–276.
- Kim, J., Park, B. H., Lee, J. H., Park, S. K., and Kim, J. H. (2011). Cell type-specific alterations in the nucleus accumbens by repeated exposures to cocaine. *Biol. Psychiatry* 69, 1026–1034.
- Knapp, C. M., Foye, M. M., Cottam, N., Ciraulo, D. A., and Kornetsky, C. (2001). Adenosine agonists CGS 21680 and NECA inhibit the initiation of cocaine self-administration. *Pharmacol. Biochem. Behav.* 68, 797–803.
- Kourrich, S., Rothwell, P. E., Klug, J. R., and Thomas, M. J. (2007). Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. *J. Neurosci.* 27, 7921–7928.
- Koya, E., Golden, S. A., Harvey, B. K., Guez-Barber, D. H., Berkow, A., Simmons, D. E., Bossert, J. M., Nair, S. G., Uejima, J. L., Marin, M. T., Mitchell, T. B., Farquhar, D., Ghosh, S. C., Mattson, B. J., and Hope, B. T. (2009). Targeted disruption of cocaine-activated nucleus accumbens neurons prevents context-specific sensitization. *Nat. Neurosci.* 12, 1069–1073.
- Kramer, P. F., Christensen, C. H., Hazelwood, L. H., Dobi, A., Bock, R., Sibley, D. R., Mateo, Y., and Alvarez, V. A. (2011). Dopamine D2 receptor overexpression alters behavior and physiology in Drd2-EGFP mice. *J. Neurosci.* 31, 126–132.
- Kravitz, A. V., Freeze, B. S., Parker, P. R., Kay, K., Thwin, M. T., Deisseroth, K., and Kreitzer, A. C. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature* 466, 622–626.
- Kreitzer, A. C., and Malenka, R. C. (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature* 445, 643–647.
- Le Moine, C., Normand, E., and Bloch, B. (1991). Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. *Proc. Natl. Acad. Sci. U.S.A.* 88, 4205–4209.
- Le Moine, C., Normand, E., Guitteny, A. F., Fouque, B., Teoule, R., and Bloch, B. (1990). Dopamine receptor gene expression by enkephalin neurons in rat forebrain. *Proc. Natl. Acad. Sci. U.S.A.* 87, 230–234.
- Lee, K. W., Kim, Y., Kim, A. M., Helmin, K., Nairn, A. C., and Greengard, P. (2006). Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proc. Natl. Acad. Sci. U.S.A.* 103, 3399–3404.
- Lemberger, T., Parlato, R., Dassel, D., Westphal, M., Casanova, E., Turiault, M., Tronche, F., Schiffrmann, S. N., and Schutz, G. (2007). Expression of Cre recombinase in dopaminergic neurons. *BMC Neurosci.* 8, 4. doi: 10.1186/1471-2202-8-4
- Lett, B. T., Grant, V. L., Byrne, M. J., and Koh, M. T. (2000). Pairings of a distinctive chamber with the aftereffect of wheel running produce conditioned place preference. *Appetite* 34, 87–94.
- Lobo, M. K., Covington, H. E. III, Chaudhury, D., Friedman, A. K., Sun, H., Damez-Werno, D., Dietz, D. M., Zaman, S., Koo, J. W., Kennedy, P. J., Mouzon, E., Mogri, M., Neve, R. L., Deisseroth, K., Han, M. H., and Nestler, E. J. (2010). Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science* 330, 385–390.
- Lobo, M. K., Cui, Y., Ostlund, S. B., Balleine, B. W., and Yang, X. W. (2007). Genetic control of instrumental conditioning by striatopallidal neuron-specific SIP receptor Gpr6. *Nat. Neurosci.* 10, 1395–1397.
- Lobo, M. K., Karsten, S. L., Gray, M., Geschwind, D. H., and Yang, X. W. (2006). FACS-array profiling of striatal projection neuron subtypes in juvenile and adult mouse brains. *Nat. Neurosci.* 9, 443–452.
- Lu, L., Koya, E., Zhai, H., Hope, B. T., and Shaham, Y. (2006). Role of ERK in cocaine addiction. *Trends Neurosci.* 29, 695–703.
- Mackie, K. (2008). Cannabinoid receptors: where they are and what they do. *J. Neuroendocrinol.* 20(Suppl. 1), 10–14.
- Maldonado, R., Saiardi, A., Valverde, O., Samad, T. A., Roques, B. P., and Borrelli, E. (1997). Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. *Nature* 388, 586–589.
- Mattson, B. J., Crombag, H. S., Mitchell, T., Simmons, D. E., Kreuter, J. D., Morales, M., and Hope, B. T. (2007). Repeated amphetamine administration outside the home cage enhances drug-induced Fos expression in rat nucleus accumbens. *Behav. Brain Res.* 185, 88–98.
- Maze, I., Covington, H. E. III, Dietz, D. M., Laplant, Q., Renthal, W., Russo, S. J., Mechanic, M., Mouzon, E., Neve, R. L., Haggarty, S. J., Ren, Y., Sampath, S. C., Hurd, Y. L., Greengard, P., Tarakhovskiy, A., Schaefer, A., and Nestler, E. J. (2010). Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science* 327, 213–216.
- Meisler, M. H. (1992). Insertional mutation of “classical” and novel genes in transgenic mice. *Trends Genet.* 8, 341–344.
- Miner, L. L., Drago, J., Chamberlain, P. M., Donovan, D., and Uhl, G. R. (1995). Retained cocaine conditioned place preference in D1 receptor deficient mice. *Neuroreport* 6, 2314–2316.
- Monory, K., Blaudzun, H., Massa, F., Kaiser, N., Lemberger, T., Schutz, G., Wotjak, C. T., Lutz, B., and Marsicano, G. (2007). Genetic dissection of behavioural and autonomic effects of Delta(9)-tetrahydrocannabinol in mice. *PLoS Biol.* 5, e269. doi: 10.1371/journal.pbio.0050269
- Moratala, R., Robertson, H. A., and Graybiel, A. M. (1992). Dynamic regulation of NGFI-A (zif268, egr1) gene expression in the striatum. *J. Neurosci.* 12, 2609–2622.
- Moratala, R., Vallejo, M., Elibol, B., and Graybiel, A. M. (1996). D1-class dopamine receptors influence cocaine-induced persistent expression of Fos-related proteins in striatum. *Neuroreport* 8, 1–5.
- Nestler, E. J. (2005). Is there a common molecular pathway for addiction? *Nat. Neurosci.* 8, 1445–1449.
- Nestler, E. J. (2008). Review. Transcriptional mechanisms of addiction: role of DeltaFosB. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 3245–3255.
- Nestler, E. J., Barrot, M., and Self, D. W. (2001). DeltaFosB: a sustained molecular switch for addiction. *Proc. Natl. Acad. Sci. U.S.A.* 98, 11042–11046.
- Novak, M., Halbout, B., O'Connor, E. C., Rodriguez Parkitna, J., Su, T., Chai, M., Crombag, H. S., Bilbao, A., Spanagel, R., Stephens, D. N., Schutz, G., and Engblom, D. (2010). Incentive learning underlying cocaine-seeking requires mGluR5 receptors located on dopamine D1 receptor-expressing neurons. *J. Neurosci.* 30, 11973–11982.
- Numano, R., Szobota, S., Lau, A. Y., Gorostiza, P., Volgraf, M., Roux, B., Trauner, D., and Isacoff, E. Y. (2009). Nanosculpting reversed wavelength sensitivity into a photoswitchable iGluR. *Proc. Natl. Acad. Sci. U.S.A.* 106, 6814–6819.
- Nye, H. E., Hope, B. T., Kelz, M. B., Iadarola, M., and Nestler, E. J. (1995). Pharmacological studies of the regulation of chronic FOS-related antigen induction by cocaine in the striatum and nucleus accumbens. *J. Pharmacol. Exp. Ther.* 275, 1671–1680.
- Parkitna, J. R., Engblom, D., and Schutz, G. (2009). Generation of Cre recombinase-expressing transgenic mice using bacterial artificial chromosomes. *Methods Mol. Biol.* 530, 325–342.
- Peakman, M. C., Colby, C., Perrotti, L. I., Tekumalla, P., Carle, T., Ulery, P., Chao, J., Duman, C., Steffen, C., Monteggia, L., Allen, M. R., Stock, J. L., Duman, R. S., Mcneish, J. D., Barrot, M., Self, D. W., Nestler, E. J., and Schaeffer, E. (2003). Inducible, brain region-specific expression of a dominant negative mutant of c-Jun in transgenic mice decreases sensitivity to cocaine. *Brain Res.* 970, 73–86.
- Peoples, L. L., Uzwiak, A. J., Guyette, F. X., and West, M. O. (1998). Tonic inhibition of single nucleus accumbens neurons in the rat: a predominant but not exclusive firing pattern induced by cocaine self-administration sessions. *Neuroscience* 86, 13–22.
- Perrotti, L. I., Hadeishi, Y., Ulery, P. G., Barrot, M., Monteggia, L., Duman, R. S., and Nestler, E. J. (2004). Induction of deltaFosB in reward-related brain structures after chronic stress. *J. Neurosci.* 24, 10594–10602.
- Pierce, R. C., Bell, K., Duffy, P., and Kalivas, P. W. (1996). Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. *J. Neurosci.* 16, 1550–1560.
- Risinger, F. O., Freeman, P. A., Rubinstein, M., Low, M. J., and Grandy, D. K. (2000). Lack of operant ethanol self-administration in dopamine D2 receptor knockout mice. *Psychopharmacology (Berl.)* 152, 343–350.
- Robertson, H. A., Paul, M. L., Moratala, R., and Graybiel, A. M. (1991). Expression of the immediate early gene c-fos in basal ganglia: induction by dopaminergic drugs. *Can. J. Neurol. Sci.* 18, 380–383.

- Russo, S. J., Dietz, D. M., Dumitriu, D., Morrison, J. H., Malenka, R. C., and Nestler, E. J. (2010). The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci.* 33, 267–276.
- Schiffmann, S. N., Libert, F., Vassart, G., and Vanderhaeghen, J. J. (1991). Distribution of adenosine A2 receptor mRNA in the human brain. *Neurosci. Lett.* 130, 177–181.
- Schiffmann, S. N., and Vanderhaeghen, J. J. (1993). Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. *J. Neurosci.* 13, 1080–1087.
- Self, D. W. (2010). “Dopamine receptor subtypes in reward and relapse,” in *The Dopamine Receptors*, ed. K. A. Neve (New York, NY: Humana Press), 479–523.
- Self, D. W., Barnhart, W. J., Lehman, D. A., and Nestler, E. J. (1996). Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. *Science* 271, 1586–1589.
- Surmeier, D. J., Ding, J., Day, M., Wang, Z., and Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci.* 30, 228–235.
- Surmeier, D. J., Song, W. J., and Yan, Z. (1996). Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. *J. Neurosci.* 16, 6579–6591.
- Thanos, P. K., Michaelides, M., Umegaki, H., and Volkow, N. D. (2008). D2R DNA transfer into the nucleus accumbens attenuates cocaine self-administration in rats. *Synapse* 62, 481–486.
- Thanos, P. K., Taintor, N. B., Rivera, S. N., Umegaki, H., Ikari, H., Roth, G., Ingram, D. K., Hitzemann, R., Fowler, J. S., Gately, S. J., Wang, G. J., and Volkow, N. D. (2004). DRD2 gene transfer into the nucleus accumbens core of the alcohol preferring and nonpreferring rats attenuates alcohol drinking. *Alcohol. Clin. Exp. Res.* 28, 720–728.
- Thomas, M. J., Beurrier, C., Bonci, A., and Malenka, R. C. (2001). Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat. Neurosci.* 4, 1217–1223.
- Uslaner, J., Badiani, A., Day, H. E., Watson, S. J., Akil, H., and Robinson, T. E. (2001a). Environmental context modulates the ability of cocaine and amphetamine to induce c-fos mRNA expression in the neocortex, caudate nucleus, and nucleus accumbens. *Brain Res.* 920, 106–116.
- Uslaner, J., Badiani, A., Norton, C. S., Day, H. E., Watson, S. J., Akil, H., and Robinson, T. E. (2001b). Amphetamine and cocaine induce different patterns of c-fos mRNA expression in the striatum and subthalamic nucleus depending on environmental context. *Eur. J. Neurosci.* 13, 1977–1983.
- Valjent, E., Bertran-Gonzalez, J., Herve, D., Fisone, G., and Girault, J. A. (2009). Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci.* 32, 538–547.
- Valjent, E., Corvol, J. C., Pages, C., Besson, M. J., Maldonado, R., and Caboche, J. (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J. Neurosci.* 20, 8701–8709.
- Vialou, V., Robison, A. J., Laplant, Q. C., Covington, H. E. III, Dietz, D. M., Ohnishi, Y. N., Mouzon, E., Rush, A. J. III, Watts, E. L., Wallace, D. L., Iniguez, S. D., Ohnishi, Y. H., Steiner, M. A., Warren, B. L., Krishnan, V., Bolanos, C. A., Neve, R. L., Ghose, S., Berton, O., Tamminga, C. A., and Nestler, E. J. (2010). DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nat. Neurosci.* 13, 745–752.
- Volgraf, M., Gorostiza, P., Numano, R., Kramer, R. H., Isacoff, E. Y., and Trauner, D. (2006). Allosteric control of an ionotropic glutamate receptor with an optical switch. *Nat. Chem. Biol.* 2, 47–52.
- Volkow, N. D., Fowler, J. S., Wang, G. J., Baler, R., and Telang, F. (2009). Imaging dopamine's role in drug abuse and addiction. *Neuropharmacology* 56(Suppl. 1), 3–8.
- Volkow, N. D., Fowler, J. S., Wang, G. J., and Swanson, J. M. (2004). Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Mol. Psychiatry* 9, 557–569.
- Welter, M., Vallone, D., Samad, T. A., Meziane, H., Usiello, A., and Borrelli, E. (2007). Absence of dopamine D2 receptors unmasks an inhibitory control over the brain circuitries activated by cocaine. *Proc. Natl. Acad. Sci. U.S.A.* 104, 6840–6845.
- Werme, M., Messer, C., Olson, L., Gilden, L., Thoren, P., Nestler, E. J., and Brene, S. (2002). Delta FosB regulates wheel running. *J. Neurosci.* 22, 8133–8138.
- White, F. J., Hu, X. T., Zhang, X. F., and Wolf, M. E. (1995). Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. *J. Pharmacol. Exp. Ther.* 273, 445–454.
- Wise, R. A. (2004). Dopamine, learning and motivation. *Nat. Rev. Neurosci.* 5, 483–494.
- Wolf, M. E. (2010). Regulation of AMPA receptor trafficking in the nucleus accumbens by dopamine and cocaine. *Neurotox. Res.* 18, 393–409.
- Wu, Y. I., Frey, D., Lungu, O. I., Jaehrig, A., Schlichting, I., Kuhlman, B., and Hahn, K. M. (2009). A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* 461, 104–108.
- Young, S. T., Porrino, L. J., and Iadarola, M. J. (1991). Cocaine induces striatal c-fos-immunoreactive proteins via dopaminergic D1 receptors. *Proc. Natl. Acad. Sci. U.S.A.* 88, 1291–1295.
- Zachariou, V., Bolanos, C. A., Selley, D. E., Theobald, D., Cassidy, M. P., Kelz, M. B., Shaw-Lutchman, T., Berton, O., Sim-Selley, L. J., Dileone, R. J., Kumar, A., and Nestler, E. J. (2006). An essential role for DeltaFosB in the nucleus accumbens in morphine action. *Nat. Neurosci.* 9, 205–211.
- Zhang, J., Zhang, L., Jiao, H., Zhang, Q., Zhang, D., Lou, D., Katz, J. L., and Xu, M. (2006). c-Fos facilitates the acquisition and extinction of cocaine-induced persistent changes. *J. Neurosci.* 26, 13287–13296.
- Zhang, X. F., Hu, X. T., and White, F. J. (1998). Whole-cell plasticity in cocaine withdrawal: reduced sodium currents in nucleus accumbens neurons. *J. Neurosci.* 18, 488–498.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 12 May 2011; paper pending published: 31 May 2011; accepted: 05 July 2011; published online: 18 July 2011.
Citation: Lobo MK and Nestler EJ (2011) The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. *Front. Neuroanat.* 5:41. doi: 10.3389/fnana.2011.00041
Copyright © 2011 Lobo and Nestler. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Targeting neuronal populations of the striatum

Pierre F. Durieux, Serge N. Schiffmann and Alban de Kerchove d'Exaerde*

Laboratory of Neurophysiology, School of Medicine, Université Libre de Bruxelles, Brussels, Belgium

Edited by:

Emmanuel Valjent, Université Montpellier 1 and 2, France

Reviewed by:

Charles R. Gerfen, National Institutes of Health, USA

Helen Bateup, Harvard Medical School, USA

***Correspondence:**

Alban de Kerchove d'Exaerde,
Laboratory of Neurophysiology,
Université Libre de Bruxelles,
Campus Erasme CP 601, Building C
Room 3.145, 808 Route de Lennik,
B-1070 Brussels, Belgium.
e-mail: adekerch@ulb.ac.be

The striatum is critically involved in motor and motivational functions. The dorsal striatum, caudate–putamen, is primarily implicated in motor control and the learning of habits and skills, whereas the ventral striatum, the nucleus accumbens, is essential for motivation and drug reinforcement. The GABA medium-sized spiny neurons (MSNs, about 95% of striatal neurons), which are targets of the cerebral cortex and the midbrain dopaminergic neurons, form two pathways. The dopamine D₁ receptor-positive (D₁R) striatonigral MSNs project to the medial globus pallidus and substantia nigra pars reticulata (direct pathway) and co-express D₁R and substance P, whereas dopamine D₂ receptor-positive (D₂R) striatopallidal MSNs project to the lateral globus pallidus (indirect pathway) and co-express D₂R, adenosine A_{2A} receptor (A_{2A}R) and enkephalin (Enk). The specific role of the two efferent pathways in motor and motivational control remained poorly understood until recently. Indeed, D₁R striatonigral and D₂R striatopallidal neurons, are intermingled and morphologically indistinguishable, and, hence, cannot be functionally dissociated with techniques such as chemical lesions or surgery. In view of the still debated respective functions of projection D₂R striatopallidal and D₁R striatonigral neurons and striatal interneurons, both in motor control and learning but also in more cognitive processes such as motivation, the present review sum up the development of new models and techniques (bacterial artificial chromosome transgenesis, optogenetic, viral transgenesis) allowing the selective targeting of these striatal neuronal populations in adult animal brain to understand their specific roles.

Keywords: striatum, medium-sized spiny neurons, interneurons, transgenesis, BAC

INTRODUCTION

The striatum represents the main input nucleus of the basal ganglia, a system of subcortical nuclei critically involved in motor control and motivational processes and altered in several conditions such as Parkinson's and Huntington's diseases or drug addiction and schizophrenia (Nestler, 2005; Graybiel, 2008; Kreitzer and Malenka, 2008). The projection neurons of the striatum are GABAergic (γ -aminobutyric acid) medium-sized spiny neurons (MSNs), which account for the large majority of striatal neurons (95% in rodents; Tepper et al., 2007). The MSNs are subdivided into two subpopulations that form two main efferent pathways. The striatonigral MSNs mainly project to the entopeduncular nucleus (EP or medial globus pallidus) and substantia nigra *pars reticulata* SNr (direct pathway) and express dopamine D₁ receptor (D₁R, *drd1a*), M4 muscarinic acetylcholine receptor (*chrm4*) and substance P neuropeptide (SP) whereas striatopallidal MSNs mostly project to the lateral globus pallidus (LGP; indirect pathway) and co-express dopamine D₂ receptor (D₂R, *drd2*), adenosine A_{2A} receptor (A_{2A}R) and enkephalin (Enk; Young and Bonner, 1986; Gerfen et al., 1990; Graybiel, 1990; Schiffmann and Vanderhaegen, 1993). Striatal interneurons, which account for a small proportion of striatal neurons (2–3% in rodent and possibly up to 20% in primates; Tepper and Bolam, 2004), are phenotypically diverse and consist of four different populations: one cholinergic and three GABAergic. The cholinergic interneurons are giant aspiny neurons (with a somatic diameter that can

be in excess of 40 μ m) expressing the choline acetyltransferase (ChAT; Kawaguchi et al., 1995). The three subtypes of GABAergic interneurons can be distinguished neurochemically: two express either the calcium binding protein parvalbumin or calretinin, and one co-expresses the peptides somatostatin and neuropeptide Y (NPY) as well as the enzyme neuronal nitric oxide synthase (nNOS; Tepper and Bolam, 2004). Based on the origin of cortical glutamatergic and midbrain dopaminergic (DA) afferents, the striatum can be functionally divided into dorsal and ventral subregions. The dorsal striatum is thought to be involved mostly in motor behaviors, while ventral striatum (or nucleus accumbens, NAc) is crucial for motivational processes (Robbins and Everitt, 1996; Groenewegen, 2003; Nestler, 2005).

The striatal neurons are mosaically distributed through all the striatum (Bolam et al., 2000) and cannot be targeted with techniques such as electrolysis or surgery. Selective modulations of striatal subpopulation activity by drugs remains difficult since most of pharmacological agents have multiple targets widely distributed in the brain. Moreover, striatal MSNs are similar in number, in size, and shape. Because of these technical limitations, the deciphering of striatum cell-type specific functions remained unsatisfactory for decades. In this paper, we will review the different strategies developed to target intrinsic striatal neuronal subtypes, with an emphasis on the most recent state of the art genetic tools and the major functional processes illuminated in these models.

FIRST EXPERIMENTAL TOOLS TO TARGET STRIATAL SUBPOPULATIONS

Because striatal neuronal types differ one from each other notably according to their gene expression pattern, expression of a particular gene can lead to selective susceptibility to a given pharmacological agent. Then, initial approaches attempting to target neuronal population in the striatum consisted in using toxic compound with a relative specificity for a neuronal subtype in wildtype animals. For instance, stereotaxic injection of the aziridinium ion of ethylcholine (AF64A; Mantione et al., 1981) in the rat striatum led to a desired and expected decrease in ChAT activity but with a concomitant loss of glutamate decarboxylase (GAD) and tyrosine hydroxylase (TH) activities (Sandberg et al., 1984), suggesting a toxic effect against striatal cholinergic neurons as well as other neuronal populations. Intraventricular administration of an immunotoxin composed of a monoclonal antibody directed to the low-affinity rat nerve growth factor (NGF) receptor (192 IgG) chemically linked to the ribosome inactivating protein saporin (192 IgG-saporin; Wiley et al., 1991) was shown to destroy cholinergic neurons in the basal forebrain but not in the striatum, perhaps due to the low NGF receptor expression level in the latter cells (Book et al., 1992; Leanza et al., 1995). A similar immunotoxin-based strategy was developed to target striatal neurons expressing high levels of tachykinin (NK-1) receptor, ablating both NPY and ChAT expressing striatal interneurons (Saka et al., 2002).

In other experiments, neurotoxic non-selective agents were injected in target nuclei of the striatum efferent populations (i.e., the SNr or the LGP) to selectively kill the striatonigral or the striatopallidal neurons by axonal transport (Harrison et al., 1990; Hervé et al., 1993; Roberts et al., 1993). While this strategy can lead to modest reduction of striatopallidal or striatonigral neuron number (Roberts et al., 1993), it lacks striatum specificity since the neurotoxin can be axonally transported from the injection site to multiple areas.

To target a specific neuronal population, either to ablate these cells or to inactivate a specific gene within them, the genetic targeting is the most promising approach given the large number of available tools (inducible systems, specific recombination) and regulatory sequences. The first success to target a striatal cell population by classical additional transgenesis was obtained by Nestler and colleagues (Chen et al., 1998) on the striatonigral neurons using a 1.8-kb fragment of the NSE (neuronal specific enolase) promoter to express the tetracycline transactivator (tTA) of the tetracycline (Tet) system. The Tet system is based on two elements. On one hand, the transcriptional transactivator tTA (tetracycline transactivator) is under the control of a cell-specific promoter. On the other hand, the gene necessary to affect the target neuronal population is under the control of the TRE (tetracycline-responsive element) promoter that binds tTA and will be, therefore, regulated by the presence or absence of tetracycline (Gossen and Bujard, 2002). Despite the fact that NSE is not specifically expressed in striatonigral neurons, 1 the 12 lines obtained, the line A, expressed tTA in these neurons. The specificity of this cell expression pattern is due to the insertion site of the transgene in the genome. Using this transgenic line,

Nestler and colleagues have demonstrated that overexpression of the transcription factor Δ FosB in striatonigral neurons increases the responsiveness of mice to the rewarding effect of cocaine (Kelz et al., 1999; Colby et al., 2003) as well as the rewarding effect and physical dependence to morphine (Zachariou et al., 2006).

The second striatal cell population successfully targeted by classical additional transgenesis were the cholinergic interneurons. Nakanishi and colleagues ablated them by immunotoxin-mediated cell targeting (IMTC) which allows a conditional and time controlled destruction of neurons (Kaneko et al., 2000). A transgene was made containing the alpha subunit of human interleukin-2 receptor (hIL-2R α) under the control of the mouse 18.3 kb 5'-upstream genomic sequence containing the first and second exons of the mGluR2 gene. Out of the 14 transgenic lines obtained, two lines (IG16 and IG17) expressed hIL-2R α specifically in striatal cholinergic interneurons (Kaneko et al., 2000). The ablation of these cholinergic interneurons was achieved by means of the stereotaxic injection of a monoclonal antibody against hIL-2R α fused with a truncated *Pseudomonas* exotoxin. The study of these mice unraveled the concerted and adapted interactions between acetylcholine and dopamine transmissions in the basal ganglia during motor behavior (Kaneko et al., 2000) the cocaine-elicited behavior (Hikida et al., 2001) and procedural learning (Kitabatake et al., 2003).

KNOCK-IN STRATEGIES

The most accurate method to preserve all regulatory elements and sequences allowing the exact spatial-temporal expression of a gene is to insert a transgene into the native locus of the gene of interest by homologous recombination (Knock-in). Again, the first population targeted by this method were the striatonigral neurons. A transgene composed of a LoxP-NEO/STOP-LoxP cassette and the diphtheria toxin A-chain gene was inserted into the *drd1a* gene (Drago et al., 1998). After crossing these knock-in mice with EIIa/Cre mice, expressing Cre recombinase in the fertilized oocyte, the resulting pups express the diphtheria toxin A-chain under the control of *drd1a* promoter. Although most pups died in the first postnatal week, some mice survived to P19 and displayed bradykinesia, dystonia and reduced striatal volume. These mice did not express D₁R, substance P and dynorphin mRNAs in the striatum but had normal levels of D₂R and enkephalin mRNAs (Drago et al., 1998). To circumvent the short lifespan and potential developmental compensation of these mice, Drago and colleagues (Gantois et al., 2007) crossed the knock-in mice with calcium/calmodulin-dependent protein kinase II α /Cre mice (Casanova et al., 2001). The resulting mice express Cre in the forebrain starting from 1 to 2 weeks after birth. These mice are viable and displayed, among other, hindlimb dystonia and locomotor hyperactivity. An important limitation of these mice to decipher the role of the striatonigral neurons results from the destruction of other populations of neurons expressing *drd1a* in the forebrain (Weiner et al., 1991).

Palmiter's group targeted the expression of Cre recombinase in *drd1a* neurons by a knock-in strategy by insertion in the *drd1a* loci (Heusner et al., 2008). These *drd1a*-Cre knock-in mice have

been crossed with *Gad1* floxed mice to selectively reduce GABA synthesis in striatonigral neurons (Heusner et al., 2008). The resulting mice showed altered motor skill learning in some behavioral test such as rotarod (Heusner et al., 2008). Using the same knock-in mice, this group inactivated the NR1 subunit of the glutamate NMDA receptor in *drd1a* neurons and demonstrated the impairment of amphetamine sensitization and conditioned place preference (CPP) in the mutant mice (Beutler et al., 2011). To decipher the role of these neurons in amphetamine-induced plastic phenomena and to bypass the fact that *drd1a* gene is not only expressed in striatonigral neurons they re-expressed NR1 selectively in the *drd1a* neurons of the NAc by Cre/lox viral strategy. NR1 re-expression rescued amphetamine sensitization, demonstrating that striatonigral neurons in the NAc are sufficient to retain this behavioral effect (Beutler et al., 2011).

An IMTC (see above) of striatal *drd2* neurons has been undertaken by a knock-in strategy in *drd2* loci (Sano et al., 2003). One week after intrastratial immunotoxin treatment and ablation of *drd2* striatal neurons, mice had spontaneous hyperactivity (Sano et al., 2003). However, *drd2* is expressed not only in striatopallidal neurons but also in striatal cholinergic interneurons and in dopaminergic neurons of the SNc and VTA projecting to striatal regions (Weiner et al., 1991). In this model, the intrastratial injection of the immunotoxin preserved the DA neurons and destroyed the striatopallidal neurons and cholinergic interneurons (Sano et al., 2003).

Knock-in parvalbumin (PV)-Cre mice were generated by insertion of an internal ribosome entry site (IRES)-Cre cassette 3' to the translational stop codon of the *Pvalb* locus allowing a bicistronic translation (Hippenmeyer et al., 2005). Striatal Cre recombinase expression in these *Pvalb*-IRES-Cre mice was not described (Hippenmeyer et al., 2005), but preliminary results obtained with a LacZ reporter strain (Soriano, 1999) indicate a very low colocalization of PV immunostaining and LacZ expression (PFD et al., unpublished data). More recently, Madisen et al. (2009) generated a *Pvalb*-2A-Cre knock-in line with a 2A-Cre sequence (mediating bicistronic translation in a mechanism different from the IRES) inserted 3' to the PV stop codon. While *Pvalb*-2A-Cre mice, as compared to *Pvalb*-IRES-Cre mice, exhibited higher level of Cre recombination in brain areas such as cerebral cortex or thalamus, Cre recombination in the striatum was not investigated (Madisen et al., 2009).

While the knock-in strategy is the best strategy to mimic the exact expression of a gene, it has the potentially important limitation that the insertion of the transgene causes the loss of one of the two loci of the target gene. In fact, there are several reports on the effects of *drd1a* or *drd2* heterozygosity. For example, acoustic startle reactivity (Ralph-Williams et al., 2002) and dopamine-elicited late-phase LTP and facilitation of the LTD in the medial prefrontal cortex are significantly altered in *drd1a* +/- mice (Huang et al., 2004). In the case of *drd2*, locomotor activity is determined by gene dosage (Kelly et al., 1998) and non-selective attention is different in *drd2* +/- and *drd2* ++ mice (Vallone et al., 2002).

BAC STRATEGIES

In late 1990s, the possibility to manipulate bacterial artificial chromosome (BAC) containing large fragments of genomic mouse

DNA (100–250 kb) to obtain transgenic mice allowed to combine the advantages of classical transgenesis and Knock-in strategy (Heintz, 2001). Firstly, the use of large genomic DNA fragments increases the possibility to preserve the key regulatory elements of a gene to allow an accurate expression *in vivo*. Secondly, BAC constructs insert at random locations throughout the genome and the two loci of the gene of interest are then more likely to be preserved as compare to a knock-in approach. Thirdly, since the site of insertion of the transgene could maintain only part of the native expression profile of the targeted gene, it is possible to select transgenic lines that target specific cell subpopulations or selected brain nuclei.

To target the neuronal population of the striatum the two main transgenes used were fluorescent proteins (GFP or dTomato) and Cre recombinase (Valjent et al., 2009; see **Table 1** for the different cell-type targeting tools). It is worth to mention that the fluorescent proteins are mainly used to target neuronal populations in adult mice to characterize them. Thus it is important that the regulatory sequence used in the BAC mimics the adult *in vivo* expression in the transgenic mice. In contrast, for Cre recombinase expression, transgenic mice are made for mating with floxed mice, and the resulting mice recapitulate the complete developmental profile (before and after birth) of the targeted gene that could be different from adult expression profile only.

MEDIUM SPINY NEURON TARGETING

BAC-EGFP for D₁R- and D₂R-MSN visualization

In 2003, the GENSAT project published transgenic BAC reporter mouse lines in which the enhanced green fluorescent protein (EGFP) gene is selectively expressed in a large variety of central nervous system cell-types including the D₁R or D₂R expressing cells (*drd1a*-EGFP and *drd2*-EGFP; Gong et al., 2003). Extensive characterization of *drd1a*-EGFP and *drd2*-EGFP lines (Bertran-Gonzalez et al., 2008; Matamalas et al., 2009) revealed that EGFP expression patterns correlate with previous *in situ* hybridization or immunohistochemical studies (Hersch et al., 1995; Le Moine and Bloch, 1995) showing that D₁R and D₂R are expressed in distinct striatal neuronal populations. In the *drd1a*-EGFP strain, fluorescence was restricted to the striatonigral neuron population while both striatopallidal MSNs and cholinergic interneurons were EGFP-positive in the *drd2*-EGFP mice (Bertran-Gonzalez et al., 2008). Other reporter BAC strains were also generated to target the striatonigral neurons as *chrm4*-EGFP line (Gong et al., 2003) and the red fluorophore strain *drd1a*-tdTomato (Shuen et al., 2008).

These reporter mice represent a powerful tool for identifying each MSN-subtype in multiple experimental paradigms using fixed tissue preparations, fluorescence-activated cell sorting (FACS) or *ex vivo* electrophysiological recordings (Day et al., 2006, 2008; Lee et al., 2006; Lobo et al., 2006; Wang et al., 2006; Kreitzer and Malenka, 2007; Shen et al., 2007, 2008; Bertran-Gonzalez et al., 2008; Gertler et al., 2008; Matamalas et al., 2009; Tecuapetla et al., 2009; Grueter et al., 2010). For instance, they allowed to show that DA depletion induces a loss of spines and glutamatergic synapses selectively on striatopallidal neurons (Day et al., 2006) and that striatopallidal neurons selectively express

Table 1 | Distinct strategies to target the subpopulations of the striatum.

	Classical transgenesis or knock-in	BAC	Other
D ₁ R striatonigral neurons	NSE-tTA (Chen et al., 1998) <i>drd1a</i> -tox176 (Drago et al., 1998) <i>drd1a</i> -Cre (Heusner et al., 2008)	<i>drd1a</i> -EGFP, <i>Chrm4</i> -EGFP (Gong et al., 2003) <i>drd1a</i> -tdTomato (Shuen et al., 2008) <i>drd1a</i> -Cre (YAC) (Mantamadiotis et al., 2002) <i>drd1a</i> -Cre (EY262 line) (Gong et al., 2007) <i>drd1a</i> -DARPP-32/Flag (Bateup et al., 2008) <i>drd1a</i> -EGFP-L10A (Heiman et al., 2008) <i>drd1a</i> -iRNA (mGluR5 knock-down) (Novak et al., 2010)	Volkensin toxin (Harrison et al., 1990) PPTA-tTA AAV (Hikida et al., 2010) pDyn-hM ₄ D HSV (Ferguson et al., 2011)
D ₂ R striatopallidal neurons	<i>drd2</i> -hIL2R α (Sano et al., 2003)	<i>drd2</i> -EGFP (Gong et al., 2003) <i>drd2</i> -Cre (ER44 line) (Gong et al., 2007) <i>drd2</i> -DARPP-32/Myc (Bateup et al., 2008) <i>drd2</i> -EGFP-L10a (Heiman et al., 2008) <i>adora2a</i> -Cre (Durieux et al., 2009)	OX7-saporin toxin (Roberts et al., 1993) PPE-tTA AAV (Hikida et al., 2010) pEnk-hM ₄ D HSV (Ferguson et al., 2011)
ChAT interneurons	mGluR2-hIL2R α (Kaneko et al., 2000)	<i>Chat</i> -EGFP (Gong et al., 2003) <i>Chat</i> -Cre (GM24 and GM60) (Gong et al., 2007)	AF64A toxin (Mantione et al., 1981) SP-PE35 toxin (Saka et al., 2002)
NPY-NO interneurons	n/a	<i>Npy</i> -Cre (DeFalco et al., 2001) <i>Npy</i> -Tau-Sapphire-GFP (Pinto et al., 2004; Roseberry et al., 2004) <i>Npy</i> -hrGFP (van den Pol et al., 2009) <i>Nos1</i> -EGFP, <i>Npy</i> -Cre (RH26-CRE and RH28-CRE) (http://www.gensat.org)	SP-PE35 toxin (Saka et al., 2002)
PV-interneurons	<i>Pvalb</i> -IRES-Cre (Hippenmeyer et al., 2005) <i>Pvalb</i> -2A-Cre (Madisen et al., 2009)	<i>Pvalb</i> -EGFP (Meyer et al., 2002) <i>Pvalb</i> -EGFP (Gong et al., 2003) <i>Pvalb</i> -Cre (Tanahira et al., 2009)	n/a
CR interneurons	n/a	n/a	n/a

n/a: not available.

an endocannabinoid-mediated long-term depression (eCB-LTD) that is disrupted in DA-depleted animals (Kreitzer and Malenka, 2007). Specific expression profiles of D₁R and D₂R MSNs were determined using FACS assays coupled to microarray analysis in *drd1a*-, *drd2*-, and *chrm4*-EGFP mice (Lobo et al., 2006), identifying multiple new genes preferentially expressed in each MSN-subtype.

Recent report pointed out major abnormalities in *drd2*-EGFP mice from GENSAT (Kramer et al., 2010). Alvarez and colleagues showed that, as compare to WT mice, *drd2*-EGFP mice exhibit a D₂R overexpression (i.e., increase in striatal D₂R binding and D₂R mRNA levels), enhanced electrophysiological responses to D₂R activation in midbrain dopaminergic neurons as well as impaired DA clearance after NAc stimulation or cocaine administration (Kramer et al., 2010). Behaviorally, *drd2*-EGFP mice display hyperactivity in novel environment, hypersensitivity to D₂R-like agonists and deficient acute and chronic response to cocaine (Kramer et al., 2010).

These results highlight the importance of extensive characterization and adequate controls when using transgenic mice in neuroscience research.

Cre BAC for manipulation of striatonigral and striatopallidal neurons or genes to shed light on their physiological roles

The first attempt to use a large mouse genomic fragment (140 kb) of *drd1a* gene has been undertaken with a yeast artificial chromosome (YAC) to unravel the role of cAMP responsive element binding protein (CREB) and cAMP responsive element modulatory protein (CREM) in striatal neurodegeneration (Mantamadiotis et al., 2002). The extensive characterization of these mice showed that the transgene has a spatiotemporal expression pattern that closely recapitulated the pattern of *drd1a* gene but is expressed in most dopaminergic neurons of the striatum and not only in the striatonigral neurons (Lemberger et al., 2007).

Having generated a large library of BAC-GFP mice, the GENSAT project then proceeded to generate a BAC-Cre mice library (Gong et al., 2007). In a first study, Greengard and colleagues used the *drd1a*-Cre (EY262 line) and *drd2*-Cre (ER44 line) mice to demonstrate that deletion of the histone methylase GLP/G9a led to de-repression of non-neuronal genes in each MSN populations. The study showed an altered locomotor response to pharmacological agents that target striatonigral and striatopallidal neurons in the conditional KO mice (Schaefer

et al., 2009). In a second study, they crossed the same lines of *drd1a*-Cre and *drd2*-Cre mice with dopamine- and cAMP-regulated phosphoprotein Mr 32 kDa (DARPP-32) floxed mice. Striatonigral DARPP-32-deleted mice show a decrease in basal and cocaine-induced locomotion and an abolition of L-DOPA-induced dyskinesia. Conversely, striatopallidal DARPP-32-deleted mice have increased locomotor activity and reduced haloperidol-induced catalepsy (Bateup et al., 2010). In this last study, they clearly showed that the deletions of DARPP-32 in striatonigral or striatopallidal neurons are specific. However, since DARPP-32 is only expressed in MSNs, these experiments do not rule out that, *drd2*-Cre mice, Cre recombinase is also expressed in cholinergic interneurons and/or DA neurons as shown for D₂-GFP (Matamales et al., 2009).

In parallel with the GENSAT BAC-Cre, other striatopallidal-Cre mice have been developed by choosing the *adora2a* gene (adenosine A_{2A} receptor) which is expressed in striatopallidal neurons but not in other striatal neuronal populations or in the presynaptic DA neurons (Durieux et al., 2009). The characterization of mice resulting from the crossing between *adora2a*-Cre mice and inducible diphtheria toxin receptor (iDTR) mice (Buch et al., 2005) after striatal diphtheria toxin injection have demonstrated that only striatopallidal neurons were targeted. The resulting mice show an increase in spontaneous locomotor activity after a whole striatum striatopallidal neuron ablation and an increase of amphetamine CPP after ventral striatum striatopallidal neuron ablation, thus demonstrating the *in vivo* roles of striatopallidal neurons in both locomotion and drug reinforcement inhibition (Durieux et al., 2009).

The most recent advances in this domain have been obtained by combining BAC-Cre expressing mice with optogenetics, allowing control of neuron-type activity with high temporal and spatial resolutions (Kravitz et al., 2010; Lobo et al., 2010). In this paradigm, two types of membrane light-sensitive proteins are used to respectively activate or silence neurons: the channelrhodopsin-2 (ChR2) cation channel and the halorhodopsin (NpHR) chloride pump respectively (Boyden et al., 2005; Zhang et al., 2007). Blue light induces a conformational change that opens ChR2 pore which rapidly and reversibly depolarizes the cell membrane and triggers action potentials (Boyden et al., 2005), while yellow light activates NpHR, which generates a chloride flow and causes rapid and reversible cell membrane hyperpolarization that prevents action potentials (Zhang et al., 2007).

Stereotactic injections of Cre-dependent ChR2 or NpHR adeno associated virus (AAV) in Cre lines lead to cell-type specific expressions of the light-sensitive proteins and optical stimulation is applied locally, through an optical fiber coupled to a diode laser, inserted in the brain area of interest (Sohal et al., 2009; Carter et al., 2010).

A first study using striatal BAC-Cre strains has demonstrated that bilateral excitation of dorsomedial striatal neurons of *drd1a*-Cre mice injected with AAV-DIO-ChR2-YFP (in which Cre-dependent expression of ChR2 is coupled to the yellow fluorescent protein, YFP) causes reduced freezing and increased locomotion (Kravitz et al., 2010). The same dorsomedial neuron excitation of *drd2*-Cre mice injected with AAV-DIO-ChR2-YFP increases freezing, and bradykinesia, and decreases locomotor

initiation. In a mouse model of Parkinson's disease (injection of 6-hydroxydopamine in dorsomedial striatum), dorsomedial excitation of striatonigral neurons rescued these deficits (Kravitz et al., 2010). They showed that the AAV recombined mainly in striatonigral and striatopallidal neurons, respectively, by demonstrating the presence of YFP staining in the targets nuclei of the two neuronal populations (SNr and GP, respectively). However, they did not specify which *drd1a*-Cre (EY217, EY242, EY262, EY266, FK150, and FK164) and *drd2*-Cre (ER43 and ER44) GENSAT lines were used and this could be of importance because the Cre expression profiles are different in these lines. Yet, some striatal PV and NPY interneurons are also targeted in *drd1a*-Cre and ChAT interneurons are targeted in *drd2*-Cre mice (Kravitz et al., 2010). Finally, DA neurons are not targeted in *drd2*-Cre mice by these stereotaxic injections of AAV-DIO-ChR2-YFP in the dorsomedial striatum.

The second optogenetic study using also the *drd1a*-Cre and *drd2*-Cre GENSAT mice and AAV-DIO-ChR2-YFP, targets the NAc to unravel the role of direct and indirect pathways (Lobo et al., 2010). Illumination of NAc neurons in *drd2*-Cre mice injected with AAV-DIO-ChR2-YFP suppresses cocaine reward whereas the opposite effect is found by illumination of the neurons in the NAc of *drd1a*-Cre mice injected with AAV-DIO-ChR2-YFP (Lobo et al., 2010). These results mimic those obtained in Trkb (the brain-derived neurotrophic factor receptor) floxed mice with the same *drd2*-Cre and *drd1a*-Cre mice (Lobo et al., 2010).

It is important to conclude by noting that currently GENSAT offers six different *drd1a*-Cre lines and two *drd2*-Cre lines¹ with variable Cre recombinase expression profile that potentially allow to target different populations and subregions in the striatum. It is worth also to note that the Cre recombinase expression in the BAC-Cre mice is generally evaluated by crossing them with reporter strains as Rosa26 Lox-Stop-Lox EGFP; meaning that only the spatiotemporal profile of the Cre recombinase can be deduced and not its the level of expression.

Others transgene-BAC

drd1a and *drd2* BAC have been used to express other transgenes than eGFP and Cre recombinase. Indeed, to unravel the specific role of DARPP-32 in striatonigral and striatopallidal neurons Greengard and colleagues have generated two different BAC transgenic mice: one containing a bicistronic transgene composed of a C-terminal Flag-tagged DARPP-32 and a Venus fluorescent protein under the control of the *drd1a* promoter on a BAC and the other containing the bicistronic transgene composed of a C-terminal Myc-tagged DARPP-32 and enhanced cyan fluorescent protein (Bateup et al., 2008). Characterization of these two transgenic mice show that 36.7% of striatal cells expressed GFP mRNA in *drd1a*-DARPP-32/Flag mice and 41.4% in *drd2*-DARPP-32/Myc mice and that the expression is specific to striatonigral or striatopallidal neurons, respectively (Bateup et al., 2008). These transgenic mice have allowed to demonstrate that cocaine increases T-34 phosphorylation of DARPP-32 specifically in striatonigral neurons whereas haloperidol induces the

¹<http://www.gensat.org/cre.jsp>

same type of phosphorylation selectively in striatopallidal neurons (Bateup et al., 2008).

Greengard, Heintz, and colleagues have also generated two other mouse lines with *drd1a* or *drd2* BAC that allow to purify polysomal mRNAs of specific neuronal populations to identify molecular changes in normal condition or after cocaine injection (Heiman et al., 2008). With this purpose, a fusion of EGFP to the N terminal of the large subunit ribosomal protein L10a was inserted under the control of the promoter of either *drd1a* or *drd2* in an appropriate BAC. The *drd1a*-EGFP-L10a line (CP73) showed expression of EGFP in the dorsal and ventral striatum, olfactory bulb, olfactory tubercle, and cortical layers five and six. The *drd2*-EGFP-L10a line (CP101) showed expression of EGFP in the dorsal and ventral striatum, olfactory tubercle, and hippocampus, as well as in the substantia nigra pars compacta and ventral tegmental area. Enkephalin immunostaining in the striatum colocalized with EGFP in *drd2*-EGFP-L10a mice but not in *drd1a*-EGFP-L10a. Polysomal mRNAs of isolated and homogenized striata were immunoprecipitated with magnetic beads coated with an antibody against EGFP on each of this two lines and translational profiling was determined by microarrays. Around 70 additional striatopallidal-enriched transcripts and more than 150 additional striatonigral-enriched transcripts with respect to the FACS-isolated MSN study (Lobo et al., 2006) were identified by this method (Heiman et al., 2008). The same analysis was performed after acute or chronic cocaine injection and hundreds of genes whose expression was increased or decreased in each cell-type were identified (Heiman et al., 2008).

Another study has used a *drd1a* BAC to express a short hairpin RNA to knock-down mGluR5 (Novak et al., 2010). GFP was introduced in tandem with two microRNAs against mGluR5 to track the expression of the sh-RNA. The GFP was expressed in 53% of striatal neurons that correspond to DARPP-32 neurons that do not express enkephalin, thus striatonigral neurons as expected (Novak et al., 2010). Moreover, the mRNA level of mGluR5 decreases significantly only in the striatum. These transgenic mice show impairment in the reinstatement of cocaine-seeking induced by a cocaine-paired stimulus due to a deficit in specific incentive learning processes (Novak et al., 2010).

TARGETING OF STRIATAL INTERNEURONS

GENSAT project generated choline acetyltransferase (ChAT)-EGFP (Gong et al., 2003) and ChAT-Cre (Gong et al., 2007) mice. Two *Chat* BAC-Cre transgenic lines (GM24 and GM60) showed brain Cre recombination that matched the expression of *Chat* in the adult, while the GM53 founder exhibited Cre recombination only in brainstem and spinal cord motor neurons (see text footnote 1). The GM24 strain was recently used in an optogenetic paradigm showing that ChAT interneurons in the NAc could be activated by cocaine and that silencing this drug-induced activity during cocaine exposure disrupted cocaine place preference conditioning (Witten et al., 2010).

Enhanced green fluorescent protein expression in parvalbumin (PV) interneurons of the striatum was initially obtained in a BAC line (*Pvalb*-EGFP; Meyer et al., 2002) with 96% of colocalization, calculated as the number of EGFP-positive cells stained with PV antibody. Using these *Pvalb*-EGFP mice, Freiman et al. (2006)

found that activation of CB1 cannabinoid receptors led to presynaptic inhibition of PV-interneurons to MSN neurotransmission. *Pvalb*-EGFP mice were also developed by the GENSAT project (Gong et al., 2003) in which EGFP detection in the striatum seems to match the endogenous pattern of *Pvalb* expression but was not extensively characterized.

A *Pvalb*-Cre BAC strain, with a Cre recombinase DNA inserted into the *Pvalb* start codon, was generated by Tanahira et al. (2009), in which the Cre expression seems to match the PV expression in basal ganglia, including the caudate nucleus and the putamen (determined by Cre and PV double immunohistochemistry).

Npy-Cre BAC mice were generated by Friedman and colleagues at the Rockefeller University (DeFalco et al., 2001) to trace hypothalamic NPY neuron connections after stereotactic injection of a Cre-dependent GFP virus. Extensive characterization of Cre expression with a reporter strain was not performed in these mice and, notably, presence of Cre recombinase in the striatum remains elusive. Recently, the GENSAT project generated *Npy*-Cre mice (founders RH26-CRE and RH28-CRE) exhibiting a Cre expression pattern spatially similar to NPY (see text footnote 1) but Cre and NPY colocalization needs to be performed in these mice.

Friedman's laboratory also developed *Npy*-Tau-Sapphire-GFP BAC mice to investigate functions of NPY-positive neurons in the arcuate nucleus of the hypothalamus (Pinto et al., 2004; Roseberry et al., 2004). Unfortunately, the GFP expression in these mice appeared lower than revealed by histochemistry (van den Pol et al., 2009), and recent study showed morphological and electrophysiological pathological abnormalities in *Npy*-Tau-Sapphire-GFP interneurons compared with WT NPY interneurons that could be due to the overexpression of tau-coupled reporter constructs (Rancillac et al., 2010). Another group generated a BAC *Npy*-human codon corrected *Renilla* (hr)-GFP transgenic mice, with a GFP from a type of soft coral (sea pansy *Renilla*) that seems to be more stable and brighter than other green fluorescent protein (van den Pol et al., 2009). In these *Npy*-hrGFP mice, the caudate-putamen and the NAc contained scattered intensely GFP-positive cells (van den Pol et al., 2009) and colocalization of NPY and GFP in the striatum was confirmed by Partridge et al. (2009) that used these mice to specify properties of synaptic connections onto striatal NPY interneurons.

Finally, the GENSAT project also generated BAC lines in which GFP is under control of the neuronal nitric oxide synthase (nNOS) promoter (*Nos1*)². However, none of the *Nos1*-EGFP lines generated show EGFP in striatal nNOS interneurons.

To date, to the best of our knowledge, no genetic tool has been developed to target selectively the striatal calretinin-positive interneurons.

VIRAL TARGETING

Recently, two papers have targeted striatonigral and striatopallidal neurons in mice and rats by using viral vectors (Hikida et al., 2010; Ferguson et al., 2011). To target mice striatonigral neurons, the −1525 to +543 residues of the mouse *PPTA* gene (Substance P) have been inserted in front of Flag-tagged tTA in

²<http://www.gensat.org>

an AAV vector whereas the −1834 to +148 residues of the mouse *Penk* gene (Enkephalin) have been used to target the striatopallidal neurons (Hikida et al., 2010). The AAVs have been injected in transgenic mice (TN) containing the TRE–GFP–tetanus toxin transgene allowing that cause the inhibition of neurotransmission in neurons expressing tTA when doxycycline is withdrawn. The TN mice bilaterally injected into 11 sites of the striatum with AAV–PPTA–tTA showed an exclusive GFP immunostaining in 74.2% of SP-positive cells whereas in TN mice injected with AAV–PPE–tTA 71.1% of ENK-containing neurons were immunostained by GFP, demonstrating a specific targeting of striatonigral and striatopallidal neurons, respectively (Hikida et al., 2010). Surprisingly, despite these percentage of targeted neurons targeted in either direct or indirect pathways whose neurotransmission was abolished, the resulting mice did not show a decrease or increase of spontaneous locomotor activity (Hikida et al., 2010) in contrast to the results obtained with BAC (Durieux et al., 2009; Bateup et al., 2010; Kravitz et al., 2010). TN mice bilaterally injected into four sites of the NAc with the transgenic AAV that results in the blockade of the direct pathway showed a decrease in cocaine-induced CPP (Hikida et al., 2010) in accordance with the results obtained in BAC mice using optogenetic approaches (Lobo et al., 2010). No difference in cocaine-induced CCP was found when the indirect pathway was inhibited (Hikida et al., 2010) in contrast with the results obtained with BAC technology (Durieux et al., 2009; Lobo et al., 2010). These differences could be explained by the different proportion of neurons that were targeted by the two approaches.

In rats, the availability of transgenic tools is much more limited than in mice. A very recent study reports the genetic targeting of both MSN populations. The −2609 to +52 residues of the rat *Penk* gene (Enkephalin) and the −1858 to +135 residues of the rat *Pdyn* gene (Dynorphin) were inserted in herpes simplex virus (HSV) in front of an hemagglutinin-tagged engineered GPCR (derived from the $G_{i/o}$ -coupled human muscarinic M_4 , hM₄D, activated by an inert ligand, CNO; Ferguson et al., 2011). Administration of CNO induced the activation of Kir3 channel in neurons expressing

hM₄D, resulting in membrane hyperpolarization, and transient neuronal silencing. In rats infused into one site of the dorsal striatum with pEnk–hM₄D HSV, 90% of hemagglutinin cells were Enk-positive and 6% were SP-positive whereas with pDyn–hM₄D HSV 95% of hemagglutinin cells were substance P positive and 5% were Enk-positive (Ferguson et al., 2011). The percentage of Enk- or SP- positive neurons infected in the dorsal striatum by these HSV vectors was unknown. CNO treatment decreased the number of evoked action potentials in hM₄D-expressing neurons. In pEnk–hM₄D rats, CNO treatment facilitated the development of a robust amphetamine sensitization in contrast with pDyn–hM₄D rats (Ferguson et al., 2011).

CONCLUSION AND PERSPECTIVES

The recent advances in genetic targeting have allowed to address a lot of important physiological issues in the striatum which were unattainable in the past. However each of these approaches (Knock-in, BAC, Virus...) needs an extensive characterization of the resulting transgenic mice to avoid some overstated conclusions. The optogenetics by its flexibility and its time resolution will certainly allow to solve new important questions in striatal physiology. Moreover, a very recent advance in optogenetic tools (Ye et al., 2011) has demonstrated the possibility to control transcription of specific transgenes by light. This additional progress will allow not only to control the activity of a neuronal population but also to control the expression of a specific gene in this neuronal population. A next important step will be to export all these tools to other models than mouse, as rat and monkey, where more elaborated behavioral paradigms are possible.

ACKNOWLEDGMENTS

Pierre F. Durieux is Research Fellow of the FRS–FNRS (chargé de recherche FNRS) and AdKdE Research Associate of the FRS–FNRS (Belgium). This study was supported by FMRE (Belgium), FRS–FNRS (Belgium), FER from ULB, Action de Recherche Concertée from the CFWB.

REFERENCES

- Bateup, H. S., Santini, E., Shen, W., Birnbaum, S., Valjent, E., Surmeier, D. J., Fisone, G., Nestler, E. J., and Greengard, P. (2010). Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14845–14850.
- Bateup, H. S., Svenningsson, P., Kuroiwa, M., Gong, S., Nishi, A., Heintz, N., and Greengard, P. (2008). Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. *Nat. Neurosci.* 11, 932–939.
- Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamalas, M., Hervé, D., Valjent, E., and Girault, J. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J. Neurosci.* 28, 5671–5685.
- Beutler, L. R., Wanat, M. J., Quintana, A., Sanz, E., Bamford, N. S., Zweifel, L. S., and Palmiter, R. D. (2011). Balanced NMDA receptor activity in dopamine D1 receptor (D1R)- and D2R-expressing medium spiny neurons is required for amphetamine sensitization. *Proc. Natl. Acad. Sci. U.S.A.* 108, 4206–4211.
- Bolam, J., Hanley, J., and Booth, P. (2000). Synaptic organisation of the basal ganglia. *J. Anat.* 196, 527–542.
- Book, A. A., Wiley, R. G., and Schweitzer, J. B. (1992). Specificity of 192 IgG-saporin for NGF receptor-positive cholinergic basal forebrain neurons in the rat. *Brain Res.* 590, 350–355.
- Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8, 1263–1268.
- Buch, T., Heppner, F. L., Tertilt, C., Heinen, T. J. A. J., Kremer, M., Wunderlich, F. T., Jung, S., and Waisman, A. (2005). A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. *Nat. Methods* 2, 419–426.
- Carter, M. E., Yizhar, O., Chikahisa, S., Nguyen, H., Adamantidis, A., Nishino, S., Deisseroth, K., and de Lecea, L. (2010). Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat. Neurosci.* 13, 1526–1533.
- Casanova, E., Fehsenfeld, S., Manta-madiotis, T., Lemberger, T., Greiner, E., Stewart, A. F., and Schütz, G. (2001). A CamKIIalpha iCre BAC allows brain-specific gene inactivation. *Genesis* 31, 37–42.
- Chen, J., Kelz, M. B., Zeng, G., Sakai, N., Steffen, C., Shockett, P. E., Picciotto, M. R., Duman, R. S., and Nestler, E. J. (1998). Transgenic animals with inducible, targeted gene expression in brain. *Mol. Pharmacol.* 54, 495–503.
- Colby, C. R., Whisler, K., Steffen, C., Nestler, E. J., and Self, D. W. (2003). Striatal cell type-specific overexpression of DeltaFosB enhances incentive for cocaine. *J. Neurosci.* 23, 2488–2493.
- Day, M., Wang, Z., Ding, J., An, X., Ing-ham, C. A., Shering, A. F., Wokosin, D., Ilijic, E., Sun, Z., Sampson, A. R., Mugnaini, E., Deutch, A. Y., Sesack, S. R., Arbuthnott, G. W., and Surmeier, D. J. (2006). Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nat. Neurosci.* 9, 251–259.

- Day, M., Wokosin, D., Plotkin, J. L., Tian, X., and Surmeier, D. J. (2008). Differential excitability and modulation of striatal medium spiny neuron dendrites. *J. Neurosci.* 28, 11603–11614.
- DeFalco, J., Tomishima, M., Liu, H., Zhao, C., Cai, X., Marth, J. D., Enquist, L., and Friedman, J. M. (2001). Virus-assisted mapping of neural inputs to a feeding center in the hypothalamus. *Science* 291, 2608–2613.
- Drago, J., Padungchaichot, P., Wong, J. Y., Lawrence, A. J., McManus, J. F., Sumarsono, S. H., Natoli, A. L., Lakso, M., Wreford, N., Westphal, H., Kola, I., and Finkelstein, D. I. (1998). Targeted expression of a toxin gene to D1 dopamine receptor neurons by cre-mediated site-specific recombination. *J. Neurosci.* 18, 9845–9857.
- Durieux, P. F., Bearzatto, B., Guiducci, S., Buch, T., Waisman, A., Zoli, M., Schiffmann, S. N., and de Kerchove d'Exaerde, A. (2009). D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat. Neurosci.* 12, 393–395.
- Ferguson, S. M., Eskenazi, D., Ishikawa, M., Wanat, M. J., Phillips, P. E. M., Dong, Y., Roth, B. L., and Neumaier, J. F. (2011). Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nat. Neurosci.* 14, 22–24.
- Freiman, I., Anton, A., Monyer, H., Urbanski, M. J., and Szabo, B. (2006). Analysis of the effects of cannabinoids on identified synaptic connections in the caudate-putamen by paired recordings in transgenic mice. *J. Physiol.* 575, 789–806.
- Gantois, I., Fang, K., Jiang, L., Babovic, D., Lawrence, A. J., Ferreri, V., Teper, Y., Jupp, B., Ziebell, J., Morganti-Kossmann, C. M., O'Brien, T. J., Nally, R., Schütz, G., Waddington, J., Egan, G. F., and Drago, J. (2007). Ablation of D1 dopamine receptor-expressing cells generates mice with seizures, dystonia, hyperactivity, and impaired oral behavior. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4182–4187.
- Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J. Jr., and Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250, 1429–1432.
- Gertler, T. S., Chan, C. S., and Surmeier, D. J. (2008). Dichotomous anatomical properties of adult striatal medium spiny neurons. *J. Neurosci.* 28, 10814–10824.
- Gong, S., Doughty, M., Harbaugh, C. R., Cummins, A., Hatten, M. E., Heintz, N., and Gerfen, C. R. (2007). Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J. Neurosci.* 27, 9817–9823.
- Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E., and Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425, 917–925.
- Gossen, M., and Bujard, H. (2002). Studying gene function in eukaryotes by conditional gene inactivation. *Annu. Rev. Genet.* 36, 153–173.
- Graybiel, A. (1990). Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci.* 13, 244–254.
- Graybiel, A. M. (2008). Habits, rituals, and the evaluative brain. *Annu. Rev. Neurosci.* 31, 359–387.
- Groenewegen, H. (2003). The basal ganglia and motor control. *Neural Plast.* 10, 107–120.
- Grueter, B. A., Brasnjo, G., and Malenka, R. C. (2010). Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens. *Nat. Neurosci.* 13, 1519–1525.
- Harrison, M. B., Wiley, R. G., and Wooten, G. F. (1990). Selective localization of striatal D1 receptors to striatonigral neurons. *Brain Res.* 528, 317–322.
- Heiman, M., Schaefer, A., Gong, S., Peterson, J. D., Day, M., Ramsey, K. E., Suarez-Farinas, M., Schwarz, C., Stephan, D. A., Surmeier, J. D., Greengard, P., and Heintz, N. (2008). A translational profiling approach for the molecular characterization of CNS cell types. *Cell* 135, 738–748.
- Heintz, N. (2001). BAC to the future: the use of bac transgenic mice for neuroscience research. *Nat. Rev. Neurosci.* 2, 861–870.
- Hersch, S., Ciliax, B., and Gutekunst, C. (1995). Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. *J. Neurosci.* 15, 5222–5237.
- Hervé, D., Lévi-Strauss, M., Marey-Semper, I., Verney, C., Tassin, J. P., Glowinski, J., and Girault, J. A. (1993). G(olf) and Gs in rat basal ganglia: possible involvement of G(olf) in the coupling of dopamine D1 receptor with adenylyl cyclase. *J. Neurosci.* 13, 2237–2248.
- Heusner, C. L., Beutler, L. R., Houser, C. R., and Palmiter, R. D. (2008). Deletion of GAD67 in dopamine receptor-1 expressing cells causes specific motor deficits. *Genesis* 46, 357–367.
- Hikida, T., Kaneko, S., Isobe, T., Kitabatake, Y., Watanabe, D., Pastan, I., and Nakanishi, S. (2001). Increased sensitivity to cocaine by cholinergic cell ablation in nucleus accumbens. *Proc. Natl. Acad. Sci. U.S.A.* 98, 13351–13354.
- Hikida, T., Kimura, K., Wada, N., Funabiki, K., and Nakanishi, S. (2010). Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron* 66, 896–907.
- Hippenmeyer, S., Vrieseling, E., Sgrist, M., Portmann, T., Laengle, C., Ladle, D. R., and Arber, S. (2005). A developmental switch in the response of DRG neurons to ETS transcription factor signaling. *PLoS Biol.* 3, e159. doi: 10.1371/journal.pbio.0030159
- Huang, Y., Simpson, E., Kellendonk, C., and Kandel, E. R. (2004). Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3236–3241.
- Kaneko, S., Hikida, T., Watanabe, D., Ichinose, H., Nagatsu, T., Kreitman, R. J., Pastan, I., and Nakanishi, S. (2000). Synaptic integration mediated by striatal cholinergic interneurons in basal ganglia function. *Science* 289, 633–637.
- Kawaguchi, Y., Wilson, C. J., Augood, S. J., and Emon, P. C. (1995). Striatal interneurons: chemical, physiological and morphological characterization. *Trends Neurosci.* 18, 527–535.
- Kelly, M. A., Rubinstein, M., Phillips, T. J., Lessov, C. N., Burkhardt-Kasch, S., Zhang, G., Bunzow, J. R., Fang, Y., Gerhardt, G. A., Grandy, D. K., and Low, M. J. (1998). Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. *J. Neurosci.* 18, 3470–3479.
- Kelz, M. B., Chen, J., Carlezon, W. A., Whisler, K., Gilden, L., Beckmann, A. M., Steffen, C., Zhang, Y. J., Marotti, L., Self, D. W., Tkatch, T., Baranaukas, G., Surmeier, D. J., Neve, R. L., Duman, R. S., Picciotto, M. R., and Nestler, E. J. (1999). Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature* 401, 272–276.
- Kitabatake, Y., Hikida, T., Watanabe, D., Pastan, I., and Nakanishi, S. (2003). Impairment of reward-related learning by cholinergic cell ablation in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 100, 7965–7970.
- Kramer, P. E., Christensen, C. H., Hazelwood, L. A., Dobi, A., Bock, R., Sibley, D. R., Mateo, Y., and Alvarez, V. A. (2010). Dopamine D2 receptor overexpression alters behavior and physiology in Drd2-EGFP mice. *J. Neurosci.* 31, 126–132.
- Kravitz, A. V., Freeze, B. S., Parker, P. R. L., Kay, K., Thwin, M. T., Deisseroth, K., and Kreitzer, A. C. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature* 466, 622–626.
- Kreitzer, A. C., and Malenka, R. C. (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature* 445, 643–647.
- Kreitzer, A. C., and Malenka, R. C. (2008). Striatal plasticity and basal ganglia circuit function. *Neuron* 60, 543–554.
- Le Moine, C., and Bloch, B. (1995). D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. *J. Comp. Neurol.* 355, 418–426.
- Leanza, G., Nilsson, O. G., Wiley, R. G., and Björklund, A. (1995). Selective lesioning of the basal forebrain cholinergic system by intraventricular 192 IgG-saporin: behavioural, biochemical and stereological studies in the rat. *Eur. J. Neurosci.* 7, 329–343.
- Lee, K., Kim, Y., Kim, A. M., Helmin, K., Nairn, A. C., and Greengard, P. (2006). Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proc. Natl. Acad. Sci. U.S.A.* 103, 3399–3404.
- Lemberger, T., Parlato, R., Dassel, D., Westphal, M., Casanova, E., Turiault, M., Tronche, F., Schiffmann, S. N., and Schütz, G. (2007). Expression of Cre recombinase in dopaminergic neurons. *BMC Neurosci.* 8, 4. doi: 10.1186/1471-2202-8-4
- Lobo, M. K., Covington, H. E., Chaudhury, D., Friedman, A. K., Sun, H., Dames-Werno, D., Dietz, D. M., Zaman, S., Koo, J. W., Kennedy, P. J., Mouzon, E., Mogri, M., Neve, R. L., Deisseroth, K., Han, M. H., and Nestler, E. J. (2010). Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science* 330, 385–390.
- Lobo, M. K., Karsten, S. L., Gray, M., Geschwind, D. H., and Yang, X. W. (2006). FACS-array profiling of striatal projection neuron subtypes in juvenile and adult mouse brains. *Nat. Neurosci.* 9, 443–452.
- Madisen, L., Zwingman, T. A., Sunkin, S. M., Oh, S. W., Zariwala, H. A.,

- Gu, H., Ng, L. L., Palmiter, R. D., Hawrylycz, M. J., Jones, A. R., Lein, E. S., and Zeng, H. (2009). A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat. Neurosci.* 13, 133–140.
- Mantamadiotis, T., Lemberger, T., Bleckmann, S. C., Kern, H., Kretz, O., Villalba, A. M., Tronche, F., Kellendonk, C., Gau, D., Kapfhammer, J., Otto, C., Schmid, W., and Schütz, G. (2002). Disruption of CREB function in brain leads to neurodegeneration. *Nat. Genet.* 31, 47–54.
- Mantione, C. R., Fisher, A., and Hanin, I. (1981). The AF64a-treated mouse: possible model for central cholinergic hypofunction. *Science* 213, 579–580.
- Matamalas, M., Bertran-Gonzalez, J., Salomon, L., Degos, B., Deniau, J., Valjent, E., Hervé, D., and Girault, J. (2009). Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. *PLoS ONE* 4, e4770. doi: 10.1371/journal.pone.0004770
- Meyer, A. H., Katona, I., Blatow, M., Rozov, A., and Monyer, H. (2002). In vivo labeling of parvalbumin-positive interneurons and analysis of electrical coupling in identified neurons. *J. Neurosci.* 22, 7055–7064.
- Nestler, E. J. (2005). Is there a common molecular pathway for addiction? *Nat. Neurosci.* 8, 1445–1449.
- Novak, M., Halbout, B., O'Connor, E. C., Rodriguez Parkitna, J., Su, T., Chai, M., Crombag, H. S., Bilbao, A., Spanagel, R., Stephens, D. N., Schütz, G., and Engblom, D. (2010). Incentive learning underlying cocaine-seeking requires mGluR5 receptors located on dopamine D1 receptor-expressing neurons. *J. Neurosci.* 30, 11973–11982.
- Partridge, J. G., Janssen, M. J., Chou, D. Y. T., Abe, K., Zukowska, Z., and Vicini, S. (2009). Excitatory and inhibitory synapses in neuropeptide Y-expressing striatal interneurons. *J. Neurophysiol.* 102, 3038–3045.
- Pinto, S., Roseberry, A. G., Liu, H., Diano, S., Shanabrough, M., Cai, X., Friedman, J. M., and Horvath, T. L. (2004). Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science* 304, 110–115.
- Ralph-Williams, R. J., Lehmann-Masten, V., Otero-Corchon, V., Low, M. J., and Geyer, M. A. (2002). Differential effects of direct and indirect dopamine agonists on prepulse inhibition: a study in D1 and D2 receptor knock-out mice. *J. Neurosci.* 22, 9604–9611.
- Rancillac, A., Lainé, J., Perrenoud, Q., Geoffroy, H., Ferezou, I., Vitalis, T., and Rossier, J. (2010). Degenerative abnormalities in transgenic neocortical neuropeptide Y interneurons expressing tau-green fluorescent protein. *J. Neurosci. Res.* 88, 487–499.
- Robbins, T. W., and Everitt, B. J. (1996). Neurobehavioural mechanisms of reward and motivation. *Curr. Opin. Neurobiol.* 6, 228–236.
- Roberts, R. C., Harrison, M. B., Francis, S. M., and Wiley, R. G. (1993). Differential effects of suicide transport lesions of the striatonigral or striatopallidal pathways on subsets of striatal neurons. *Exp. Neurol.* 124, 242–252.
- Roseberry, A. G., Liu, H., Jackson, A. C., Cai, X., and Friedman, J. M. (2004). Neuropeptide Y-mediated inhibition of proopiomelanocortin neurons in the arcuate nucleus shows enhanced desensitization in ob/ob mice. *Neuron* 41, 711–722.
- Saka, E., Iadarola, M., Fitzgerald, D. J., and Graybiel, A. M. (2002). Local circuit neurons in the striatum regulate neural and behavioral responses to dopaminergic stimulation. *Proc. Natl. Acad. Sci. U.S.A.* 99, 9004–9009.
- Sandberg, K., Hanin, I., Fisher, A., and Coyle, J. T. (1984). Selective cholinergic neurotoxin: AF64A's effects in rat striatum. *Brain Res.* 293, 49–55.
- Sano, H., Yasoshima, Y., Matsushita, N., Kaneko, T., Kohno, K., Pastan, I., and Kobayashi, K. (2003). Conditional ablation of striatal neuronal types containing dopamine D2 receptor disturbs coordination of basal ganglia function. *J. Neurosci.* 23, 9078–9088.
- Schaefer, A., Sampath, S. C., Intrator, A., Min, A., Gertler, T. S., Surmeier, D. J., Tarakhovskiy, A., and Greengard, P. (2009). Control of cognition and adaptive behavior by the GLP/G9a epigenetic suppressor complex. *Neuron* 64, 678–691.
- Schiffmann, S. N., and Vanderhaegen, J. J. (1993). Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. *J. Neurosci.* 13, 1080–1087.
- Shen, W., Flajolet, M., Greengard, P., and Surmeier, D. J. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. *Science* 321, 848–851.
- Shen, W., Tian, X., Day, M., Ulrich, S., Tkatch, T., Nathanson, N. M., and Surmeier, D. J. (2007). Cholinergic modulation of Kir2 channels selectively elevates dendritic excitability in striatopallidal neurons. *Nat. Neurosci.* 10, 1458–1466.
- Shuen, J. A., Chen, M., Gloss, B., and Calakos, N. (2008). Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. *J. Neurosci.* 28, 2681–2685.
- Sohal, V. S., Zhang, F., Yizhar, O., and Deisseroth, K. (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* 459, 698–702.
- Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat. Genet.* 21, 70–71.
- Tanahira, C., Higo, S., Watanabe, K., Tomioka, R., Ebihara, S., Kaneko, T., and Tamamaki, N. (2009). Parvalbumin neurons in the forebrain as revealed by parvalbumin-Cre transgenic mice. *Neurosci. Res.* 63, 213–223.
- Tecuapetla, F., Koós, T., Tepper, J. M., Kabbani, N., and Yeckel, M. F. (2009). Differential dopaminergic modulation of neostriatal synaptic connections of striatopallidal axon collaterals. *J. Neurosci.* 29, 8977–8990.
- Tepper, J. M., Abercrombie, E. D., and Bolam, J. P. (2007). Basal ganglia macrocircuits. *Prog. Brain Res.* 160, 3–7.
- Tepper, J. M., and Bolam, J. P. (2004). Functional diversity and specificity of neostriatal interneurons. *Curr. Opin. Neurobiol.* 14, 685–692.
- Valjent, E., Bertran-Gonzalez, J., Hervé, D., Fisone, G., and Girault, J. (2009). Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci.* 32, 538–547.
- Vallone, D., Pignatelli, M., Grammatikopoulos, G., Ruocco, L., Bozzi, Y., Westphal, H., Borrelli, E., and Sadile, A. G. (2002). Activity, non-selective attention and emotionality in dopamine D2/D3 receptor knock-out mice. *Behav. Brain Res.* 130, 141–148.
- van den Pol, A. N., Yao, Y., Fu, L., Foo, K., Huang, H., Coppari, R., Lowell, B. B., and Broberger, C. (2009). Neuromedin B and gastrin-releasing peptide excite arcuate nucleus neuropeptide Y neurons in a novel transgenic mouse expressing strong Renilla green fluorescent protein in NPY neurons. *J. Neurosci.* 29, 4622–4639.
- Wang, Z., Kai, L., Day, M., Ronesi, J., Yin, H. H., Ding, J., Tkatch, T., Lovinger, D. M., and Surmeier, D. J. (2006). Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. *Neuron* 50, 443–452.
- Weiner, D. M., Levey, A. I., Sunahara, R. K., Niznik, H. B., O'Dowd, B. F., Seeman, P., and Brann, M. R. (1991). D1 and D2 dopamine receptor mRNA in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* 88, 1859–1863.
- Wiley, R. G., Oeltmann, T. N., and Lappi, D. A. (1991). Immunolesioning: selective destruction of neurons using immunotoxin to rat NGF receptor. *Brain Res.* 562, 149–153.
- Witten, I. B., Lin, S., Brodsky, M., Prakash, R., Diester, I., Anikeeva, P., Gradinaru, V., Ramakrishnan, C., and Deisseroth, K. (2010). Cholinergic interneurons control local circuit activity and cocaine conditioning. *Science* 330, 1677–1681.
- Ye, H., Daooud-El Baba, M., Peng, R.-W., and Fussenegger, M. (2011). A synthetic optogenetic transcript device enhances blood-glucose homeostasis in mice. *Science* 332, 1565–1568.
- Young, W., and Bonner, T. (1986). Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. *Proc. Natl. Acad. Sci. U.S.A.* 83, 9827–9831.
- Zachariou, V., Bolanos, C. A., Selley, D. E., Theobald, D., Cassidy, M. P., Kelz, M. B., Shaw-Lutchman, T., Berton, O., Sim-Selley, L. J., Dileone, R. J., Kumar, A., and Nestler, E. J. (2006). An essential role for DeltaFosB in the nucleus accumbens in morphine action. *Nat. Neurosci.* 9, 205–211.
- Zhang, F., Wang, L. P., Brauner, M., Liewald, J. F., Kay, K., Watzke, N., Wood, P. G., Bamberg, E., Nagel, G., Gottschalk, A., and Deisseroth, K. (2007). Multimodal fast optical interrogation of neural circuitry. *Nature* 446, 633–639.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 26 May 2011; paper pending published: 17 June 2011; accepted: 03 July 2011; published online: 15 July 2011.

Citation: Durieux PF, Schiffmann SN and de Kerchove d'Exaerde A (2011) Targeting neuronal populations of the striatum. *Front. Neuroanat.* 5:40. doi: 10.3389/fnana.2011.00040

Copyright © 2011 Durieux, Schiffmann and de Kerchove d'Exaerde. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Striatal signaling in L-DOPA-induced dyskinesia: common mechanisms with drug abuse and long term memory involving D1 dopamine receptor stimulation

Mario Gustavo Murer¹ and Rosario Moratalla^{2,3*}

¹ Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

² Instituto Cajal, Consejo Superior Investigaciones Científicas, Madrid, Spain

³ Centro de Investigación Biomédica en Red, Enfermedades Neurodegenerativas, Instituto de Salud Carlos III, Madrid, Spain

Edited by:

Emmanuel Valjent, Université Montpellier 1&2, France

Reviewed by:

Riccardo Brambilla, Vita-Salute San Raffaele University, Italy
M. Angela Cenci, Lund University, Sweden

*Correspondence:

Rosario Moratalla, Instituto Cajal, Avenida Doctor Arce, 37, 20002 Madrid, Spain.
e-mail: moratalla@cajal.csic.es

Parkinson's disease is a common neurodegenerative disorder caused by the degeneration of midbrain substantia nigra dopaminergic neurons that project to the striatum. Despite extensive investigation aimed at finding new therapeutic approaches, the dopamine precursor molecule, 3,4-dihydroxyphenyl-L-alanine (L-DOPA), remains the most effective and commonly used treatment. However, chronic treatment and disease progression lead to changes in the brain's response to L-DOPA, resulting in decreased therapeutic effect and the appearance of dyskinesias. L-DOPA-induced dyskinesia (LID) interferes significantly with normal motor activity and persists unless L-DOPA dosages are reduced to below therapeutic levels. Thus, controlling LID is one of the major challenges in Parkinson's disease therapy. LID is the result of intermittent stimulation of supersensitive D1 dopamine receptors located in the very severely denervated striatal neurons. Through increased coupling to $G_{\alpha_{olf}}$, resulting in greater stimulation of adenylyl-cyclase, D1 receptors phosphorylate DARPP-32, and other protein kinase A targets. Moreover, D1 receptor stimulation activates extracellular signal-regulated kinase and triggers a signaling pathway involving mammalian target for rapamycin and modifications of histones that results in changes in translation, chromatin modification, and gene transcription. In turn, sensitization of D1 receptor signaling causes a widespread increase in the metabolic response to D1 agonists and changes in the activity of basal ganglia neurons that correlate with the severity of LID. Importantly, different studies suggest that dyskinesias may share mechanisms with drug abuse and long term memory involving D1 receptor activation. Here we review evidence implicating D1 receptor signaling in the genesis of LID, analyze mechanisms that may translate enhanced D1 signaling into dyskinetic movements, and discuss the possibility that the mechanisms underlying LID are not unique to the Parkinson's disease brain.

Keywords: L-DOPA-induced dyskinesia, D1 dopamine receptor, Parkinson's disease

Shortly after the recognition that L-DOPA in combination with a peripheral decarboxylase inhibitor has a dramatic therapeutic effect in Parkinson's disease, it became clear that the treatment was associated with major adverse effects like the appearance of severely incapacitating abnormal involuntary movements (Cotzias et al., 1969; Papavasiliou et al., 1972). This raised concern about the safety of the treatment and the consequences of beginning L-DOPA therapy early in the course of the disease (Fahn, 1996), but the fear that L-DOPA could be toxic was dispelled more than a decade ago (Murer et al., 1999; Olanow et al., 2004). Currently, the principal candidates for the mechanism underlying L-DOPA-induced dyskinesia (LID) are long term functional and structural changes induced in the dopamine-depleted striatum by dopamine-regulated signaling cascades (Jenner, 2008; Voon et al., 2009; Cenci and Konradi, 2010).

Clinical studies have identified several factors that increase the risk of LID: severity and/or duration of the disease, L-DOPA dose, and intermittent L-DOPA dosing (Nutt, 2000, 2008). Studies in

patients indicate that initiating therapy with a selective D2-like dopamine receptor agonist is associated with a very low risk of developing dyskinesia, whereas D1-like agonists are very dyskinesia-inducing (Rascol et al., 2001, 2006; Holloway et al., 2004) in agreement with recent findings in animal models of Parkinson's disease (Darmopil et al., 2009). Here we review animal studies investigating the mechanisms of LID, with a focus on rodent models and D1 receptor signaling mechanisms.

IS D1 RECEPTOR STIMULATION NECESSARY AND SUFFICIENT TO INDUCE DYSKINESIA?

Early studies in primates suggested that D1-like agonists are less likely than D2-like agonists to induce dyskinesia (Grondin et al., 1997; Goulet and Madras, 2000). Indeed, it has been proposed that stimulation of sensitized D2-like receptors may be necessary and sufficient to induce dyskinesia (Blanchet et al., 1995). However, based on the administration of selective D1- or D2-like receptor antagonists together with L-DOPA to MPTP-treated primates, it

was concluded that neither the beneficial motor effects of L-DOPA, nor LID, can be ascribed to stimulation of one receptor family alone (Grondin et al., 1999). Currently, there is plenty of evidence that the risk of dyskinesia is very low during chronic treatment with a selective D2-like agonist in patients who have never been exposed to L-DOPA (Damier et al., 2000; Holloway et al., 2004; Rascol et al., 2006).

The discovery that rodents with a nigrostriatal lesion develop LID provided a means to perform a non-pharmacological intervention to study the mechanisms underlying it. Rats and mice with unilateral nigrostriatal lesions induced by 6-hydroxydopamine (6-OHDA) develop abnormal involuntary movements resembling “peak-dose dyskinesia” after a few challenges with L-DOPA (Andersson et al., 1999; Delfino et al., 2004; Pavon et al., 2006). Pharmacological studies show that selective D1-like agonists have more powerful dyskinetogenic effects than D2-like agonists, and D1-like antagonists are more effective at inhibiting LID than D2-like antagonists (Taylor et al., 2005; Delfino et al., 2007; Westin et al., 2007). As in patients, *de novo* administration of a therapeutic dose of D2-like agonist produces mild or no dyskinesia in 6-OHDA rats, but previous exposure to L-DOPA increases the dyskinetogenic effect of selective D2 receptor stimulation (Delfino et al., 2004; Larramendy et al., 2008).

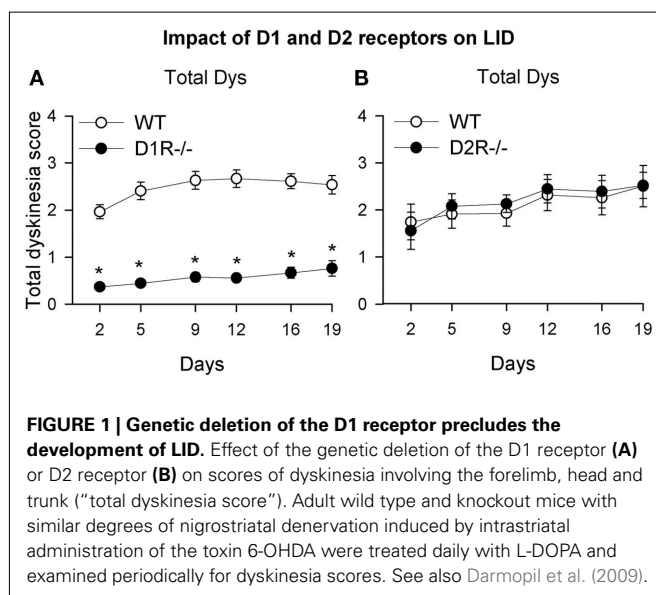
Powerful evidence supporting a role for the D1R subtype in LID has been provided in studies of genetically engineered mice lacking the D1 receptor (Darmopil et al., 2009). LID is completely blocked in D1 knockouts with severe striatal dopaminergic denervation induced by 6-OHDA (Figures 1 and 2). In contrast, D2R knockouts develop LID as easily as controls. Although the latter does not rule out a possible contribution of D3R to LID expression (Bézar et al., 2003), the fact that D1R knockouts do not show abnormal involuntary movements with prolonged L-DOPA treatment suggests that D1R stimulation is essential for the development of LID (Figures 1 and 2).

The possibility remains that the D3R, which is abnormally expressed in the dorsal striatum after repeated exposure to

levodopa, contributes to LID expression (Bézar et al., 2003). However, there is wide divergence between studies regarding the extent of D3R upregulation and the effects of D3R preferring antagonists and partial agonists on LID (Hurley et al., 1996; Bordet et al., 1997; Quik et al., 2000; Hsu et al., 2004; Visanji et al., 2009). Moreover, D1R stimulation induces a full repertoire of dyskinetic movements resembling peak-dose dyskinesia even in the presence of D3R antagonists (Kumar et al., 2009; Mela et al., 2010), suggesting that D3R stimulation is not an obligatory requirement for LID to occur. In summary, D1R stimulation seems to be necessary for LID development and sufficient to induce dyskinetic movements. This is in agreement with data showing that, in contrast to D2-like receptor agonists (most of which have a high affinity for the D3R – Rascol, 1999), D1-like receptor agonists are markedly dyskinetogenic in monkeys rendered parkinsonian by MPTP (Doucet et al., 1996) as well as in patients (Rascol et al., 2001). Additional mechanisms involving D2 family receptors may contribute to the expression of LID, especially when dyskinetic movements are induced by D2-like agonists in animals previously exposed to L-DOPA.

LID IS ASSOCIATED WITH CHANGES IN D1 RECEPTOR SIGNALING IN THE DOPAMINE-DEPLETED STRIATUM

Early studies showing that deletion of the D1R gene blocks control of striatal prodynorphin expression and motor behavior by cocaine and amphetamine called attention to D1R signaling in behavioral sensitization to psychostimulants (Moratalla et al., 1996a). This study also suggested involvement of the direct pathway in psychostimulant sensitization, because prodynorphin is a marker of those striatal “medium spiny” neurons (MSN) that contain the D1 receptor and project to the substantia nigra. In parallel, it has been shown that repeated administration of a D1-like receptor agonist induces dyskinesia and the expression of the transcription factors FosB/ Δ FosB in striatonigral MSNs (Doucet et al., 1996). Further investigation showed that LID in the 6-OHDA rat is associated with increased co-expression of FosB/ Δ FosB and prodynorphin in striatal neurons (Andersson et al., 1999; see also Sgambato-Faure et al., 2005; Pavon et al., 2006). In contrast to full-length FosB proteins, which behave as immediate early genes, its truncated forms called Δ FosB show high stability and persist for long periods (McClung et al., 2004). Although Δ FosB cannot be distinguished from FosB in immunocytochemical studies, Doucet et al. (1996), Andersson et al. (1999), and Pavon et al. (2006) used Western blotting to demonstrate that it is the truncated form, Δ FosB, which is up-regulated preferentially in LID. Importantly, Andersson et al. (1999) provided causal evidence linking Δ FosB upregulation to LID, as intrastriatal infusion of *fosB*/ Δ *fosB* antisense oligonucleotide inhibited the development of LID. Double *in situ* hybridization studies using 35S- and digoxigenin-labeled riboprobes showed that FosB/ Δ FosB induction occurred in striatal neurons that contain D1R (Pavon et al., 2006), extending previous findings showing co-expression of FosB/ Δ FosB with prodynorphin (Andersson et al., 1999). Interestingly, Δ FosB expression is inversely correlated with the abundance of dopamine fibers in the striatum and directly correlated with the severity of dyskinesia (Pavon et al., 2006), and it is blocked by pharmacological or genetic inactivation of the D1 receptor (Westin et al., 2007;



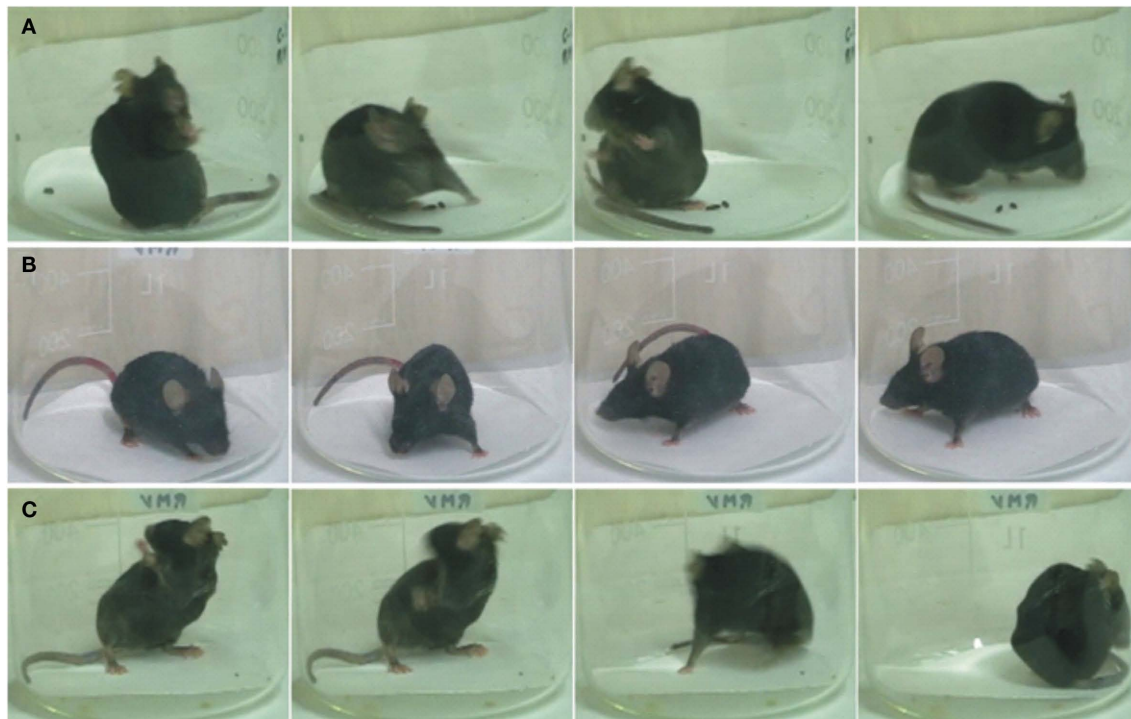


FIGURE 2 | L-DOPA does not induce dyskinesia in D1 receptor knockout mice with severe nigrostriatal denervation. Frames extracted from videos taken from representative 6-OHDA-lesioned wild type (A), D1^{-/-} (B), and

D2^{-/-} (C) mice, which have been treated with L-DOPA for 2 weeks. The sequence was taken 30 min after an L-DOPA challenge and the frames are 0.15 s apart from each other. Reproduced from Darmopil et al., 2009.

Darmopil et al., 2009). The correlation between Δ FosB induction and the severity of LID has been corroborated in the MPTP macaque model of Parkinson's disease, in which overexpression of a truncated form of JunD that acts as a dominant negative inhibitor of Δ FosB markedly reduces LID (Berton et al., 2009). Overall, these findings show that LID is related to specific D1R-dependent changes in gene expression in striatonigral MSNs neurons that selectively express dynorphin and D1 receptor.

The fact that L-DOPA administration to naive animals does not induce Δ FosB expression and LID suggests that denervation-induced supersensitivity of dopamine D1 receptors is at the core of the mechanism leading to LID. Although D1 receptor supersensitivity is well documented in Parkinson's disease, D1 receptor expression does not change dramatically after nigrostriatal lesion or repeated L-DOPA administration. However, D1 receptors may be redistributed preferentially to the postsynaptic membrane and cytoplasmic compartments involved in D1R trafficking during chronic L-DOPA administration (Muriel et al., 1999; Guigoni et al., 2007; Berthet et al., 2009). Moreover, D1 receptor supersensitivity in 6-OHDA rats and Parkinson's disease patients is related to increased levels of $G\alpha_{olf}$ (Corvol et al., 2004). Indeed, the ability of D1-like receptor agonists to recruit G proteins in striatal tissue is linearly related to dyskinesia scores in MPTP-lesioned monkeys (Aubert et al., 2005).

There is also evidence that the resulting increase in cAMP production is translated into effects on targets of the cAMP-dependent protein kinase (PKA), like the Thr34 site of DARPP-32. The

striatum contains higher levels of phospho[Thr34]-DARPP-32 in dyskinetic rats than in 6-OHDA rats that have not developed dyskinesia under L-DOPA treatment (Picconi et al., 2003). Moreover, levels of phospho[Thr34]-DARPP-32 correlate with the severity of dyskinesia in a mouse model of LID (Santini et al., 2007). Similar changes in DARPP-32 phosphorylation have more recently been found in MPTP-lesioned monkeys that have developed LID (Santini et al., 2010). Importantly, selective disruption of the DARPP-32 gene in striatonigral neurons reduces LID, whereas a similar gene disruption in striatopallidal neurons has no effect (Bateup et al., 2010).

Phosphorylation at another specific target of PKA, the Ser845 in the GluR1 subunit of AMPA receptors, is also increased in highly dyskinetic mice and MPTP-lesioned monkeys, but not in control parkinsonian animals that have received L-DOPA but did not develop substantial dyskinesia (Santini et al., 2007, 2010). Moreover, there is evidence that phosphorylation at Ser845 in GluR1 is modulated by DARPP-32. Phospho[Thr34]-DARPP-32 is a potent inhibitor of protein phosphatase-1 (PP-1), so, dephosphorylation of targets of PP-1 should be reduced in LID. In agreement, DARPP-32 knockouts not only exhibit reduced LID but also show reduced phosphorylation at Ser845 in GluR1 (Santini et al., 2007).

Molecular changes related to LID have been detected in other signaling cascades associated with dopamine D1/D5 receptor function, like the extracellular signal-regulated kinase (ERK) 1/2 cascade (Figure 3). Indeed, D1R stimulation induces ERK1/2 phosphorylation in the dopamine-intact (Valjent et al.,

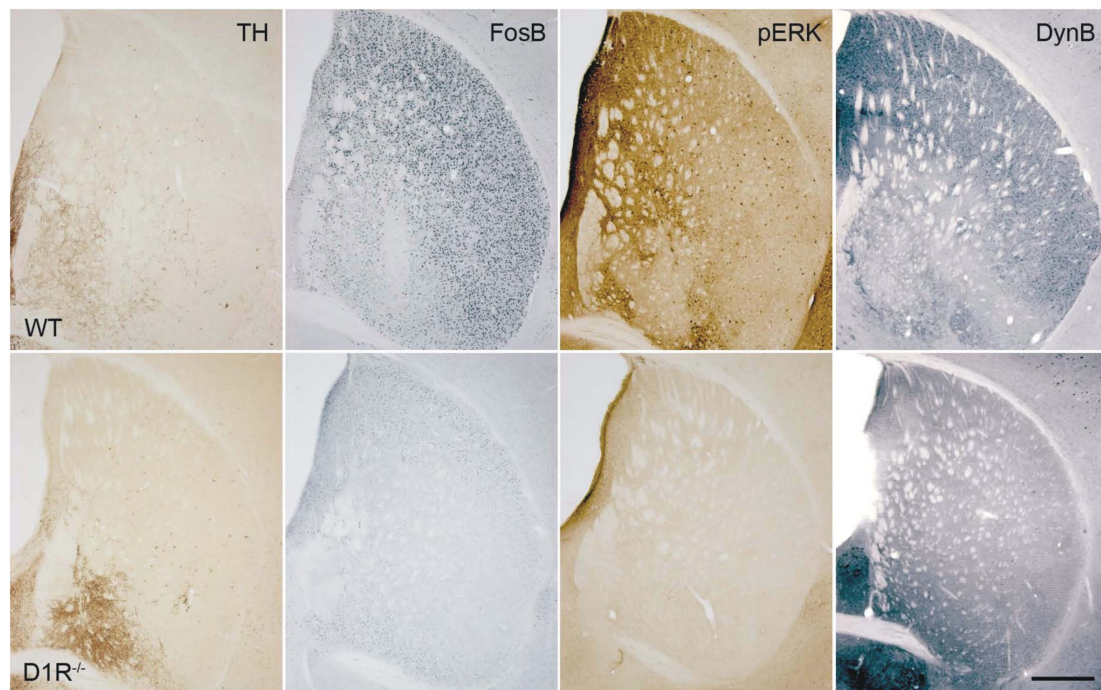


FIGURE 3 | Genetic inactivation of dopamine D1 receptor blocks striatal molecular changes induced by chronic L-DOPA administration in hemiparkinsonian mice.

Photomicrographs of adjacent coronal striatal sections from WT (top) and D1R^{-/-} (bottom) hemiparkinsonian mice treated with L-DOPA. Sections are

immunostained for TH, FosB, Dynorphin, and p-ERK. Chronic L-DOPA treatment induced marked FosB, Dynorphin, and p-ERK expression in the striatal areas that are devoid of TH-immunoreactive fibers in WT mice, but not in the striatum of D1R^{-/-} mice. Scale bar = 500 μ m.

2000; Mazzucchelli et al., 2002; Bami-Cherrier et al., 2005) and dopamine-depleted brain (Gerfen et al., 2002). More recent work established a correlation between ERK1/2 phosphorylation and LID (Pavon et al., 2006; Santini et al., 2007; Westin et al., 2007; Darmopil et al., 2009) and showed that pharmacological inhibition of the ERK1/2 signaling cascade reduces LID (Santini et al., 2007; Schuster et al., 2008; Lindgren et al., 2009). Induction of ERK1/2 phosphorylation by L-DOPA is blocked, in parallel with LID, by D1/D5 receptor antagonists (Westin et al., 2007; Santini et al., 2009a), and by genetic deletion of the specific D1 receptor subtype (Darmopil et al., 2009), further supporting the requirement for the D1R subtype and the involvement of the direct striatal outflow pathway. Finally, genetic interventions aimed at reducing the activity of Ras-GRF1, a neuronal specific activator of ERK signaling that mediates striatal ERK1/2 phosphorylation in response to D1/D5R agonists (Fasano et al., 2009), reduced LID in mice and monkeys (Fasano et al., 2010). As DARPP-32 knockouts also show reduced ERK1/2 phosphorylation, excessive inhibition of PP-1 by phospho[Thr34]-DARPP-32 may contribute to the higher levels of phospho-ERK1/2 in LID (Santini et al., 2007).

ENHANCED D1R SIGNALING INDUCES CHANGES IN TRANSLATION AND TRANSCRIPTION

It has been possible to follow the signaling cascade involved in LID downstream to translation and transcription mechanisms.

In the denervated striatum, but not in the normal striatum, L-DOPA administration induces phosphorylation of S6 kinase at a site specifically regulated by the mammalian target for rapamycin (mTOR). S6 kinase phosphorylates S6, a ribosomal protein, and the abundance of phosphorylated S6 kinase and S6 is linearly related to the severity of LID. L-DOPA-induced S6 phosphorylation takes place in MSNs of the direct pathway and is blocked by D1/D5R antagonists and rapamycin. Similar results were obtained with another target of mTOR, eukaryotic translation initiation factor 4E-binding protein (4E-BP), which when phosphorylated, allows formation of a multiprotein complex with a critical role in translation. Finally, inhibitors of the ERK signaling cascade prevent phosphorylation of targets of mTOR, and rapamycin, an inhibitor of mTOR signaling, reduces the development of LID (Santini et al., 2009b).

Changes downstream of the ERK1/2 signaling cascade have been detected in the MSN nucleus. Thr581 of the mitogen and stress-activated kinase-1 (MSK-1), a nuclear target of ERK1/2, and histone H3, which is modified in response to phospho[Thr581]MSK-1, are persistently activated in MSNs of the direct pathway in rodents that develop LID (Darmopil et al., 2009; Santini et al., 2009a). Induction of phospho-MSK-1 by L-DOPA was completely inhibited in animals pretreated with D1-like antagonists (Westin et al., 2007). Moreover, D1-like antagonists (Santini et al., 2009a) or genetic deletion of the D1R (Darmopil et al., 2009), reduce histone H3 activation in parallel with LID. Modified histone H3 is co-expressed with dynorphin and

FosB/ Δ FosB in MSNs in highly denervated striatal areas in the mouse model of LID, further supporting that histone H3 modification is related to transcriptional changes mediating LID (Darmopil et al., 2009).

Finally, some changes in transcription may be related to a switch in gene expression control from cAMP-response element binding transcription factor (CREB) dominance to Δ FosB dependence. In the intact striatum, prodynorphin transcription is mediated by CREB, but L-DOPA induction of prodynorphin transcription in parkinsonian rats is under the control of Δ FosB and JunD (Andersson et al., 2001).

CHANGES IN D2/D3 RECEPTOR-MEDIATED SIGNALING MAY CONTRIBUTE TO THE EXPRESSION OF LID

Different lines of evidence suggest that D2R modulation of striatopallidal neurons is not necessary for development of LID. In D2R knockouts with 6-OHDA striatal lesions LID develops with similar time course and intensity as in controls (Darmopil et al., 2009). Moreover, selective genetic deletion of DARPP-32 in striatopallidal neurons has no effect on the development of LID (Bateup et al., 2010). The above conclusion is reinforced by the finding that there is a very low risk of dyskinesia associated with administration of D2-like receptor agonists to patients or to drug-naïve animals with nigrostriatal lesions (Delfino et al., 2004; Holloway et al., 2004; Lundblad et al., 2005; Rascol et al., 2006). By contrast, D2R antagonists that produce tardive dyskinesia in patients, induce FosB/ Δ FosB expression in striatopallidal neurons (Hiroi and Graybiel, 1996; Hiroi et al., 2002; Grande et al., 2004).

Thus, it seems likely that D2R subtype regulation of striatopallidal neurons may modulate LID expression. This is illustrated in a study by Gold et al. (2007) aimed at modulating D2 receptor signaling in LID. Modifying the expression of regulator of G-protein signaling (RSG) 9-2, a protein that inhibits D2 receptor interaction with G proteins, regulated LID: RSG 9-2 knockouts showed increased susceptibility to LID, whereas overexpression of RSG 9-2 reduced LID in MPTP-lesioned monkeys, and 6-OHDA rats. However, the effects of modifying RSG 9-2 expression on motor function were not specific to LID, suggesting that striatal D2R subtype signaling allows an increase in motor output that results in more severe dyskinesia when the main LID signaling cascade, the direct striatonigral pathway, is activated.

On the other hand, the D3R may directly modulate the main LID signaling cascade. Repeated stimulation of D1R by L-DOPA or dopamine agonists in 6-OHDA rats leads to a progressive increase in the expression of D3R in direct pathway MSNs, which parallels sensitization of the rotational response to L-DOPA (Bordet et al., 1997, 2000; see however Hurley et al., 1996; Quik et al., 2000). Similar results were obtained in MPTP-lesioned monkeys that have developed LID; further study showed that pharmacological reduction of D3R activity reduced LID (Bézar et al., 2003; see however Kumar et al., 2009; Mela et al., 2010). When co-expressed in the same cell, D1R and D3R interact synergistically in the membrane, with D3R stimulation enhancing D1-like agonist affinity and capacity to stimulate adenylyl cyclase (Fiorentini et al., 2008; Marcellino et al., 2008). Recent work suggests that direct cross-talk between D1R and D3R in the membrane reduces internalization of D1R in response to dopamine, a mechanism that may normally

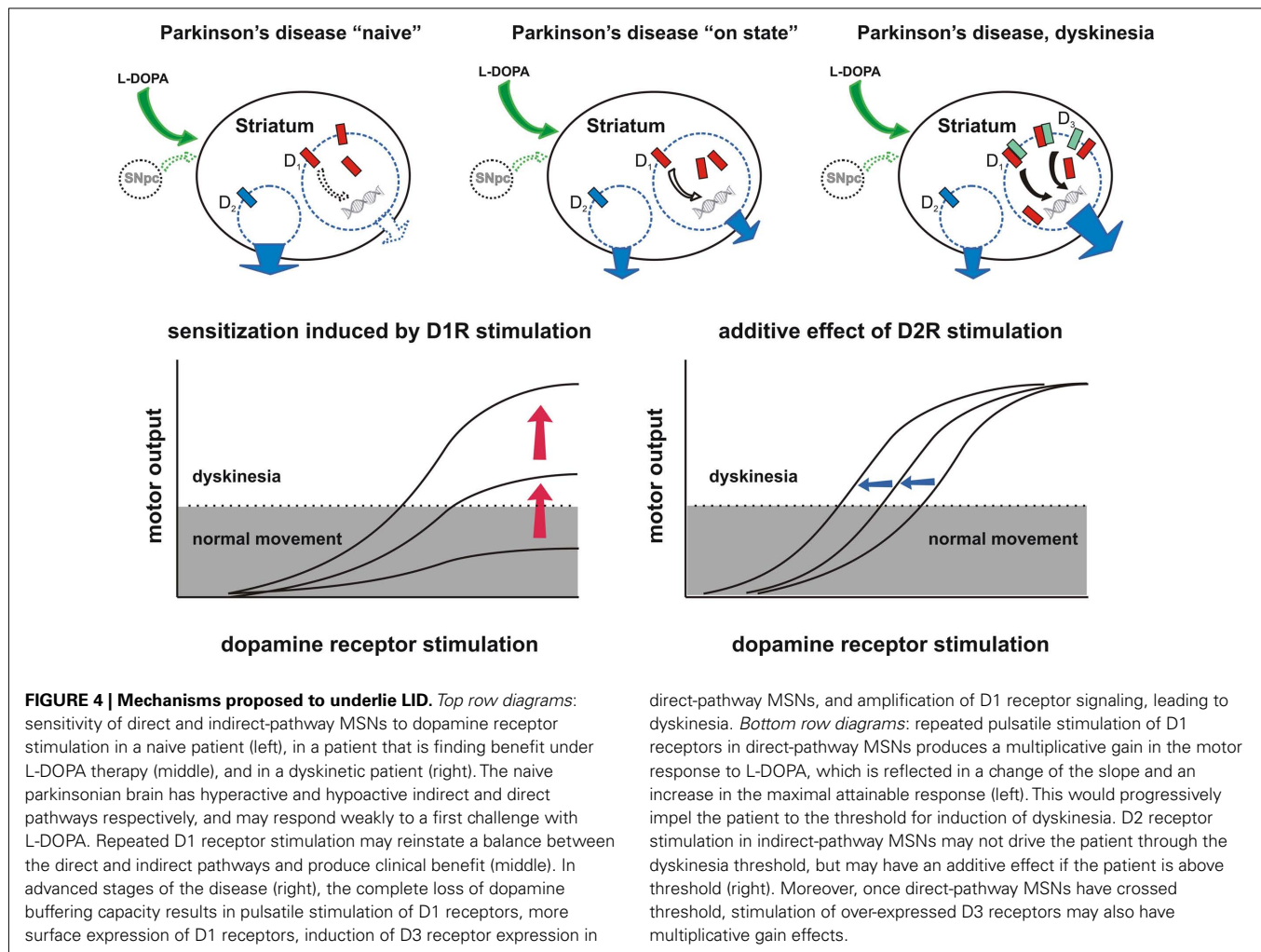
prevent excessive D1R activation (Berthet et al., 2009). It is worth considering whether the effects of D3R preferring drugs in LID result from their ability to modulate D1R-D3R membrane interactions rather than from their antagonist/partial agonist action at the ligand binding site.

LID IS ASSOCIATED WITH ALTERED SYNAPTIC PLASTICITY IN THE STRIATUM

Although a causal connection has been established between enhanced D1R signaling via ERK and LID, the functional changes linking these molecular events to behavior remain poorly understood. Once established, LID is very difficult to reverse and very easy to reestablish even after a long L-DOPA-free period (Nutt, 2000). Because of this, it has been suggested that long term changes in brain circuits occur during chronic L-DOPA therapy (Calabresi et al., 2000a) and dopamine denervation (Darmopil et al., 2009). ERK's established association with functional and structural forms of synaptic plasticity is consistent with this (Thomas and Huganir, 2004).

The critical role played by dopamine in corticostriatal long term potentiation (LTP) and LTD has been noticed 20 years ago (Calabresi et al., 1992). Early work suggested that D1R stimulation is necessary to induce LTP, whereas D2R stimulation is required for LTD (Calabresi et al., 1992, 2000b; Centonze et al., 1999, 2003; Tang et al., 2001). The mechanisms involved in LTP and LTD in direct and indirect-pathway MSNs have not been studied until very recently, with the availability of BAC transgenic mice expressing fluorescent proteins in specific neuronal populations (Wang et al., 2006; Kreitzer and Malenka, 2007; Shen et al., 2008). LTD can be readily induced in indirect pathway-MSNs via D2R-regulated endocannabinoid release. Moreover, this LTD is disrupted in animal models of Parkinson's disease and can be restored by D2-like agonists and CB1 receptor stimulation (Kreitzer and Malenka, 2007; Shen et al., 2008). What happens in the direct pathway neurons is less clear. One group could not induce LTD in direct pathway MSNs (Kreitzer and Malenka, 2007). However, Wang et al. (2006) and Shen et al. (2008) have been able to induce LTD in D1R-MSNs with different stimulation protocols. In rodent models of Parkinson's disease, LTD is favored over LTP in D1R-MSNs, but protocols aimed at inducing LTD result in LTP in the presence of D1-like agonists (Shen et al., 2008). The issue remains controversial and has been addressed in recent excellent reviews (Kreitzer and Malenka, 2008; Surmeier et al., 2009; Lovinger, 2010).

Concerning LID, there is evidence showing its association with altered functional plasticity in corticostriatal synapses. A pioneering slice study by Picconi et al. (2003) showed that excitatory synapses in MSNs show normal LTP in L-DOPA-treated 6-OHDA rats, and that depotentiation is selectively impaired in rats that have developed LID. As corticostriatal depotentiation is prevented by D1-like agonists, it seems likely that enhanced D1 signaling impedes depotentiation in dyskinetic rats. A deficit in depotentiation could lead to progressive enhancement of corticostriatal synaptic efficacy in the direct pathway, resulting in uncontrolled positive feedback to motor cortical areas (Figure 4). Recent *in vivo* studies support the idea that depotentiation of direct pathway MSNs is altered in 6-OHDA rats under chronic L-DOPA therapy (Belujon et al., 2010). Unfortunately, no study



has yet taken advantage of engineered mice expressing fluorescent proteins in D1R- or D2R-MSNs to establish how L-DOPA impacts on plasticity in each striatal output pathway.

The ultimate mechanisms that block depotentiation have not been clarified; in fact, depotentiation itself is poorly understood. It is well known that the D1R subtype has a facilitatory effect on NMDA receptors in MSNs (Cepeda and Levine, 1998). The interaction with the NMDA receptor occurs through different mechanisms, including PKA/DARPP-32-mediated signaling and direct protein–protein interactions in the postsynaptic membrane, which result in enhanced NMDA receptor currents and NMDA receptor redistribution across dendritic spine compartments (Cepeda and Levine, 2006). In animals with LID, NMDA receptors showing altered subunit composition form complexes with D1R and these complexes are trafficked abnormally between the postsynaptic membrane and intracellular compartments or extrasynaptic membrane sites (Fiorentini et al., 2006; Gardoni et al., 2006). However, it is not clear how such receptor reorganization is related to alterations in synaptic plasticity or to other functional changes associated with LID.

An aspect of NMDA receptor involvement in LID that has not received much attention is that it also activates the ERK1/2

cascade (Thomas and Huganir, 2004). However, NMDA receptor antagonists do not modify ERK phosphorylation induced by D1-like agonists in the dopamine-depleted striatum (Gerfen et al., 2002; Rylander et al., 2009). In contrast, mGluR5 antagonists attenuate LID and reduce ERK phosphorylation (Mela et al., 2007; Rylander et al., 2009), suggesting that mGluR5 plays a more important control over ERK1/2 than the NMDA receptor in the parkinsonian condition. Thus, in LID, D1 receptor activation may result in exaggerated ERK1/2 phosphorylation through multiple mechanisms, including reduced activity of PP-1, enhanced activity of Ras-GRF1, enhanced NMDA receptor, and mGluR5 activation.

In addition to alterations in functional plasticity, indirect evidence suggests that LID is associated with structural changes in neurons. Activity-regulated cytoskeletal-associated protein (arc) is markedly up-regulated in direct pathway MSNs in rats with 6-OHDA lesion treated chronically with L-DOPA, though it is not clear that this change is selectively associated with the development of LID (Sgambato-Faure et al., 2005). Although changes in arc expression and function are frequently involved in synaptic remodeling, further work is necessary to assess such changes in LID.

LID IS ASSOCIATED WITH WIDESPREAD FUNCTIONAL AND STRUCTURAL CHANGES IN THE BASAL GANGLIA

There is evidence that angiogenesis and changes in the blood brain barrier take place in parallel with LID in rats with nigrostriatal lesion. This is manifest in enhanced proliferation of endothelial cells, higher total microvessel length, and loss of blood brain barrier markers across most basal ganglia structures in the denervated hemisphere (Westin et al., 2006). The effect of L-DOPA on vessels and behavior was mimicked by administration of D1-like agonists and blocked by D1-like antagonists, with drugs acting on D2 family receptors having little or opposite effects. Moreover, pharmacological inhibition of the ERK1/2 signaling cascade reduced both LID and the microvascular changes induced by L-DOPA (Lindgren et al., 2009). However, these changes in the blood brain barrier were not corroborated by functional methods in a primate model of LID (Astradsson et al., 2009), so more work is needed to understand the functional significance of these findings. Changes in the microvasculature may be secondary to the higher metabolic demands imposed by chronic D1R receptor stimulation in rats with nigrostriatal lesion (Trugman and Wooten, 1986, 1987) or result from more direct actions of dopamine agonists on blood vessels (Hirano et al., 2008).

Widespread changes in metabolism and blood flow have been detected in LID. Rat pharmacological MRI studies revealed a correlation between the severity of dyskinesia induced by D1 receptor stimulation and the magnitude of blood oxygenation level-dependent signal (BOLD) in the striatum and motor cortex (Delfino et al., 2007). Moreover, in 6-OHDA rats, the BOLD response to D1 receptor stimulation showed sensitization with repeated drug administration in both striatum and motor cortex (Delfino et al., 2007). Similarly, nuclear magnetic resonance spectroscopy (NMR) studies in reserpinized animals showed increased glutamatergic activity after L-DOPA treatment, associated with increases in striatal c-fos expression and motor activity. These responses are blocked in D1R knock out animals (Rodrigues et al., 2007), suggesting the involvement of D1 receptor. Overall, these results are consistent with clinical findings. In patients with PD, the severity of involuntary arm and hand movements correlates with increases in blood flow measured with PET in the contralateral motor cortex and basal ganglia (Brooks et al., 2000; Hirano et al., 2008). In addition, L-DOPA-induced over-activation of the motor cortex is not observed in drug-naïve hemiparkinsonian patients (Buhmann et al., 2003). An interesting observation in pharmacological MRI studies is that a D3R-preferring agonist induces regional cerebral blood volume changes similar to those induced by D1-like agonists, but only if animals have developed LID. In the absence of LID, the D3-preferring agonist has no effect (Sánchez-Pernaute et al., 2007), further suggesting that D3R stimulation acts by potentiating D1R signaling in LID.

Importantly, it seems that metabolic changes are not restricted to motor areas. A 2-deoxyglucose accumulation study conducted in MPTP-lesioned non-human primates revealed increased metabolic activity in limbic and cognitive territories of the striatum only in L-DOPA-treated animals that had developed dyskinesia (Guigoni et al., 2005).

Less is known about the electrophysiological changes associated with the widespread metabolic effects occurring in LID. A

classic study by Fillion et al. (1991) revealed a parallel between the development of dyskinesia after repeated administration of apomorphine to MPTP-lesioned monkeys and changes in neuronal activity in the globus pallidus. Apomorphine, a mixed D1/D2 agonist with a short plasma half-life that is known to be very dyskinesia-inducing in patients, did not induce dyskinesia and had small effects on the activity of pallidal neurons in monkeys before MPTP lesioning. After induction of parkinsonism, however, repeated administration of apomorphine led to growing motor side effects and growing changes in pallidal activity. More specifically, dyskinesia was associated with a marked inhibition and reduction of burst firing in the internal segment of the globus pallidus (Fillion et al., 1991). This finding has been corroborated (Papa et al., 1999) and extended by showing that dyskinesia induced by D1-like agonists in the MPTP primate model has similar electrophysiological correlates (Boraud et al., 2001). Moreover, a limited number of recordings performed in patients undergoing functional neurosurgery also suggest that excessive inhibition of output neurons is involved in dyskinesia (Merello et al., 1999; Lozano et al., 2000). Overall, the data are consistent with the proposal that the gain of direct pathway striatal neurons increases in LID (**Figure 4**).

The studies by Fillion et al. (1991) and Boraud et al. (2001) show that D2-like agonists induce dyskinesia and changes in pallidal activity similar to those produced by apomorphine. In both studies the same monkeys were repeatedly challenged with a mixed D1/D2 agonist and a selective D2-like agonist, thus the D2 agonist effects were taking place in a primed brain. Moreover, both apomorphine and D2-like receptor agonists induced increases in activity in the external pallidal segment, which could have contributed to reducing neuronal firing in the internal pallidum. Thus, D2/D3 agonist-induced dyskinesia probably shares circuit mechanisms with LID.

Although the data are consistent with the view that increased gain of direct pathway striatal neurons produces excessive inhibition of basal ganglia output, there is still no direct evidence for this. Indeed, a recent study in drug-naïve 6-OHDA rats shows that D1-like receptor stimulation produces a strong induction of immediate early genes in the striatum without restoring the response of direct pathway MSNs to cortical stimulation to control levels (Ballion et al., 2009). The possibility remains that the hypothetical increase in the gain of corticostriatal connections requires repeated exposure to L-DOPA or D1-like agonists. However, in severely denervated 6-OHDA rats, a first challenge with L-DOPA can induce dyskinesia (see Onofrio et al., 1998, for similar findings in patients). Further work correlating the activity of direct- and indirect-pathway striatal MSNs to the degree of dopamine denervation and dyskinesia scores is necessary to determine the circuit mechanisms involved in LID.

ARE THE SIGNALING CHANGES OCCURRING IN LID UNIQUE TO THE PARKINSONIAN BRAIN?

L-DOPA does not induce dyskinesia unless dopaminergic neurons in the substantia nigra and striatal dopaminergic markers fall by more than 90% (see for example Winkler et al., 2002). More recent work correlating the spatial pattern of striatal denervation with the molecular changes associated to LID showed that FosB/ Δ FosB and prodynorphin induction is restricted to striatal areas where

the density of dopaminergic fibers drops below 5% of control levels (Pavon et al., 2006; Darmopil et al., 2009). Thus, denervation supersensitivity plays a pivotal role in the development of LID by allowing the activation of non-canonical signaling cascades by D1R stimulation, suggesting this is a mechanism unique to LID. However, there is evidence that similar signaling mechanisms are activated in dopamine-intact animals during sensitization to psychostimulants and drugs of abuse. For instance, there is evidence that a D1R-activated signaling cascade involving DARPP-32 and ERK1/2 phosphorylation, induction of Δ FosB, and prodynorphin expression, modifications of histone H3, a switch in gene expression control from CREB-dependent to Δ FosB-dependent, and impaired LTD of corticostriatal synapses, is causally involved in psychostimulant sensitization and drug addiction (Moratalla et al., 1996a; Kelz et al., 1999; Valjent et al., 2000; McClung and Nestler, 2003; Brami-Cherrier et al., 2005; Stipanovich et al., 2008; Fasano et al., 2009; Kasanetz et al., 2010).

Although a point-by-point comparison between drug addiction-related and LID-related mechanisms is beyond the scope of this review, it seems clear that a large part of the signaling cascade involved in LID can be activated in an intact brain by cocaine and psychostimulants, although with much less intensity and in considerably fewer striatal neurons (Moratalla et al., 1996b; Pavon et al., 2006). This raises the question why L-DOPA cannot activate it when the brain dopamine systems are intact. The most plausible explanation is that pulsatile stimulation of D1 receptors is sufficient to activate the non-canonical components of the signaling cascade, but that the buffering capacity of even a small number of dopaminergic terminals precludes L-DOPA from producing the large variations in dopamine concentration in the extracellular space that may be necessary to drive the cascade. Indeed, L-DOPA induces slow and moderate changes in dopamine concentration in the extracellular space when presynaptic mechanisms are intact but produces faster and more marked changes in rodent models of Parkinson's disease (Carta et al., 2006; Lindgren et al., 2010). Moreover, changes in dopamine concentration are more marked in patients with more severe parkinsonism (de la Fuente-Fernández et al., 2004). This is consistent with a recent report showing that L-DOPA does not induce dyskinesia in rats in which dopamine synthesis was knocked down but presynaptic dopaminergic terminals are preserved (Ulusoy et al., 2010). Importantly, these rats show dyskinesia in response to apomorphine, a short plasma half-life D1/D2 agonist whose effect over the dopamine receptors is not buffered by presynaptic terminals, indicating that the postsynaptic mechanisms involved in LID are functional. Moreover, the rats still do not show dyskinesia during L-DOPA administration after receiving apomorphine challenges that have been able to induce it (Ulusoy et al., 2010). In this sense, striatal TH+ neurons that appear after severe denervation and L-DOPA treatment are not able to provide enough buffering capacity to reduce dyskinesias (Darmopil et al., 2008). Overall, the data indicate that a good buffering capacity of dopamine concentration in the extracellular space may preclude sensitization to L-DOPA and attenuate the activation of already sensitized postsynaptic dyskinesia machinery.

This is not to say that supersensitivity of D1R signaling does not contribute to LID. Supersensitivity may explain why

the molecular changes associated to LID are several-fold more widespread and intense than those induced by psychostimulants. However, an almost complete loss of presynaptic dopaminergic terminals (implying a loss of buffering capacity) is a prerequisite for D1R signaling supersensitivity (Pavon et al., 2006; Darmopil et al., 2009). Thus, D1R supersensitivity and the loss of buffering capacity conspire together in LID.

DOES LID SHARE MECHANISMS WITH LONG TERM MEMORY?

Recent findings linking D1 receptor stimulation to long term memory storage further suggest that the signaling cascade involved in LID is not unique to the parkinsonian brain, or even to the striatum. Pharmacological studies show that blockade of D1-like receptors at the time of encoding reduces the persistence of hippocampal-dependent memories (O'Carroll et al., 2006; Bethus et al., 2010). Also, intrahippocampal administration of D1-like antagonists several hours after encoding blocks retention (Bernabeu et al., 1997). An important recent study shows that a long-lasting fear memory disappears if hippocampal D1 receptors are blocked 12 h after encoding, whereas a D1-like agonist applied at the same post-training time turns a labile memory into a persistent one (Rossato et al., 2009). Thus, sequential "waves" of dopamine at different time points are critical for long term memory storage in the hippocampus.

These pharmacological observations are backed up by studies in D1R knockout mice showing that this receptor subtype is essential for spatial memory tasks (El-Ghundi et al., 1999). Moreover, D1 knockouts show spatial learning and associative learning deficits concurrent with a reduction in LTP (Granado et al., 2008; Ortiz et al., 2010). Furthermore, mice in which hippocampal D1 receptors were knocked down with a small interfering RNA show deficits in spatial learning, fear learning, classical conditioning, and LTP, ruling out effects of developmental adaptations in the D1 knockouts (Ortiz et al., 2010). Importantly, it has been shown that D1R are important for both early- and late-LTP, indicating that the D1R subtype is involved in different kinds of memory, including new protein synthesis-independent and -dependent memory (Granado et al., 2008; Ortiz et al., 2010). Further studies *in vivo* showed that intact D1R are required for the acquisition of associative learning and for the endogenous, non-stimulated, increase in synaptic strength produced in the hippocampus during learning (Ortiz et al., 2010), supporting the important role of D1R in learning and memory.

Elucidation of the signaling cascade mediating the role of D1R in memory formation and retention is challenging, given the likely contribution of several other neurotransmitters and modulators that can share signaling mechanisms with dopamine. However, there is evidence that ERK1/2 phosphorylation (Bekinschtein et al., 2008) and histone H3 acetylation (Levenson et al., 2004), as well as Erg1 and Arc (Granado et al., 2008; Ortiz et al., 2010) are involved in the retention of hippocampal memories. Thus, at least some components of the signaling cascade implicated in LID are also active in the hippocampus at times when D1 receptor stimulation contributes to memory persistence.

An intriguing possibility is that phasic D1R stimulation is critical for inducing components of the signaling cascade that produce

physiological increases in synaptic strength. Even in the striatum, where tonic dopamine levels are relatively high, it is believed that the low affinity of D1R is not activated except during physiological surges of dopamine induced by burst firing of the dopaminergic neurons (Gonon, 1997). Thus, physiological surges of dopamine in the extracellular space could be involved in D1R promotion of memory storage in different brain areas. Longer-lasting and more marked non-physiological D1R stimulation induced by cocaine and psychostimulants in the dopamine-intact brain, or by L-DOPA in the brain that has lost the buffering capacity of striatal dopaminergic terminals, may induce abnormal plasticity and behavioral alterations.

IMPLICATIONS FOR PARKINSON'S DISEASE THERAPY

Why does pulsatile activation of the D1 receptor signaling cascade induce dyskinesia in Parkinson's disease instead of other behavioral abnormalities like addictive behaviors or impulse control disorders? The distinctive behavior resulting from pulsatile dopaminergic stimulation in the parkinsonian brain may reflect a preferential involvement of striatal motor areas, within which the extreme but heterogeneous loss of dopamine buffering capacity may produce a patchy pattern of molecular and functional post-synaptic changes (Darmopil et al., 2009). This non-physiological patchy pattern of plasticity would distort the flow of cortical information through the direct basal ganglia pathway, giving rise to abnormal movements instead of behavioral stereotypies. In this context, over-activation of dopamine signaling cascades in areas

that are not so extensively denervated in Parkinson's disease, like the ventral striatum and frontal cortex, may lead to behavioral abnormalities that are not unique to the parkinsonian brain, like addiction to L-DOPA, pathological gambling, and other impulse control disorders (Dagher and Robbins, 2009). This hypothesis can be interrogated by studying the behavioral consequences of intermittent stimulation of dopamine receptors after manipulating dopamine buffering capacity in specific brain areas.

This view opens questions about future directions in Parkinson's disease therapy. If the postsynaptic signaling involved in LID is not unique but part of physiological rewarding and memory storage mechanisms, targeting the non-canonical components of the signaling cascade may have undesirable consequences on behavior. In this context, increasing L-DOPA buffering capacity in the motor district of the striatum emerges as a more etiologically- and physiologically-rooted approach to the treatment of Parkinson's disease, of which the concept of "continuous dopaminergic stimulation" (Olanow et al., 2006) can be seen as an antecedent.

ACKNOWLEDGMENTS

The authors would like to thank Dr. S. Darmopil and Miss I. Ruiz De Diego for their help with the figures and art work. Funded by grants from the Spanish Ministerios de Ciencia e Innovación y de Sanidad y Política Social, ISCIII, ref number, BFU2010-20664, PNSD, RedRTA (RD06/0001/1011), and CIBERNED to RM. MGM is supported by FONCYT PICT 2008 2205, CONICET PIP 77, and UBACYT M562.

REFERENCES

- Andersson, M., Hilbertson, A., and Cenci, M. A. (1999). Striatal fosB expression is causally linked with L-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. *Neurobiol. Dis.* 6, 461–474.
- Andersson, M., Konradi, C., and Cenci, M. A. (2001). cAMP response element-binding protein is required for dopamine-dependent gene expression in the intact but not the dopamine-denervated striatum. *J. Neurosci.* 21, 9930–9943.
- Astradsson, A., Jenkins, B. G., Choi, J. K., Hallett, P. J., Levesque, M. A., McDowell, J. S., Brownell, A. L., Spealman, R. D., and Isacson, O. (2009). The blood-brain barrier is intact after levodopa-induced dyskinesias in parkinsonian primates – evidence from in vivo neuroimaging studies. *Neurobiol. Dis.* 35, 348–351.
- Aubert, I., Guigoni, C., Håkansson, K., Li, Q., Dovero, S., Barthe, N., Bioulac, B. H., Gross, C. E., Fisone, G., Bloch, B., and Bezard, E. (2005). Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. *Ann. Neurol.* 57, 17–26.
- Ballion, B., Frenois, F., Zold, C. L., Chetrit, J., Murer, M. G., and Gonon, F. (2009). D2 receptor stimulation, but not D1, restores striatal equilibrium in a rat model of Parkinsonism. *Neurobiol. Dis.* 2009 35, 376–384.
- Bateup, H. S., Santini, E., Shen, W., Birnbaum, S., Valjent, E., Surmeier, D. J., Fisone, G., Nestler, E. J., and Greengard, P. (2010). Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14845–14850.
- Bekinschtein, P., Cammarota, M., Kathe, C., Slipczuk, L., Rossato, J. I., Goldin, A., Izquierdo, I., and Medina, J. H. (2008). BDNF is essential to promote persistence of long-term memory storage. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2711–2716.
- Belujon, P., Lodge, D. J., and Grace, A. A. (2010). Aberrant striatal plasticity is specifically associated with dyskinesia following levodopa treatment. *Mov. Disord.* 25, 1568–1576.
- Bernabeu, R., Bevilacqua, L., Ardenghi, P., Bromberg, E., Schmitz, P., Bianchin, M., Izquierdo, I., and Medina, J. H. (1997). Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. *Proc. Natl. Acad. Sci. U.S.A.* 94, 7041–7046.
- Berthet, A., Porras, G., Doudnikoff, E., Stark, H., Cador, M., Bezard, E., and Bloch, B. (2009). Pharmacological analysis demonstrates dramatic alteration of D1 dopamine receptor neuronal distribution in the rat analog of L-DOPA-induced dyskinesia. *J. Neurosci.* 29, 4829–4835.
- Berton, O., Guigoni, C., Li, Q., Bioulac, B. H., Aubert, I., Gross, C. E., Dileone, R. J., Nestler, E. J., and Bezard, E. (2009). Striatal overexpression of DeltaJunD resets L-DOPA-induced dyskinesia in a primate model of Parkinson disease. *Biol. Psychiatry* 66, 554–561.
- Bethus, I., Tse, D., and Morris, R. G. (2010). Dopamine and memory: modulation of the persistence of memory for novel hippocampal NMDA receptor-dependent paired associates. *J. Neurosci.* 30, 1610–1618.
- Bézard, E., Ferry, S., Mach, U., Stark, H., Leriche, L., Boraud, T., Gross, C., and Sokoloff, P. (2003). Attenuation of levodopa-induced dyskinesia by normalizing dopamine D3 receptor function. *Nat. Med.* 9, 762–767.
- Blanchet, P. J., Gomez-Mancilla, B., Di Paolo, T., and Bédard, P. J. (1995). Is striatal dopaminergic receptor imbalance responsible for levodopa-induced dyskinesia? *Fundam. Clin. Pharmacol.* 9, 434–442.
- Boraud, T., Bezard, E., Bioulac, B., and Gross, C. E. (2001). Dopamine agonist-induced dyskinesias are correlated to both firing pattern and frequency alterations of pallidal neurones in the MPTP-treated monkey. *Brain* 124, 546–557.
- Bordet, R., Ridray, S., Carboni, S., Diaz, J., Sokoloff, P., and Schwartz, J. C. (1997). Induction of dopamine D3 receptor expression as a mechanism of behavioral sensitization to levodopa. *Proc. Natl. Acad. Sci. U.S.A.* 94, 3363–3367.
- Bordet, R., Ridray, S., Schwartz, J. C., and Sokoloff, P. (2000). Involvement of the direct striatonigral pathway in levodopa-induced sensitization in 6-hydroxydopamine-lesioned rats. *Eur. J. Neurosci.* 12, 2117–2123.
- Brami-Cherrier, K., Valjent, E., Hervé, D., Darragh, J., Corvol, J. C., Pages, C., Arthur, S. J., Girault, J. A., and Caboche, J. (2005). Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. *J. Neurosci.* 25, 11444–11454.
- Brooks, D. J., Piccini, P., Turjanski, N., and Samuel, M. (2000). Neuroimaging of dyskinesia. *Ann. Neurol.* 47(Suppl. 1), S154–S158.
- Buhmann, C., Glauche, V., Stürenburg, H. J., Oechsner, M., Weiller, C., and Büchel, C. (2003). Pharmacologically modulated fMRI – cortical responsiveness to levodopa in drug-naïve hemiparkinsonian patients. *Brain* 126, 451–461.

- Calabresi, P., Giamini, P., Centonze, D., and Bernardi, G. (2000a). Levodopa-induced dyskinesia: a pathological form of striatal synaptic plasticity? *Ann. Neurol.* 47(Suppl. 1), S60–S68.
- Calabresi, P., Gubellini, P., Centonze, D., Picconi, B., Bernardi, G., Chergui, K., Svenningsson, P., Fienberg, A. A., and Greengard, P. (2000b). Dopamine and cAMP-regulated phosphoprotein 32 kDa controls both striatal long-term depression and long-term potentiation, opposing forms of synaptic plasticity. *J. Neurosci.* 20, 8443–8451.
- Calabresi, P., Maj, R., Pisani, A., Mercuri, N. B., and Bernardi, G. (1992). Long-term synaptic depression in the striatum: physiological and pharmacological characterization. *J. Neurosci.* 12, 4224–4233.
- Carta, M., Lindgren, H. S., Lundblad, M., Stancampiano, R., Fadda, F., and Cenci, M. A. (2006). Role of striatal L-DOPA in the production of dyskinesia in 6-hydroxydopamine lesioned rats. *J. Neurochem.* 96, 1718–1727.
- Cenci, M. A., and Konradi, C. (2010). Maladaptive striatal plasticity in L-DOPA-induced dyskinesia. *Prog. Brain Res.* 183, 209–233.
- Centonze, D., Grande, C., Saulle, E., Martin, A. B., Gubellini, P., Pavón, N., Pisani, A., Bernardi, G., Moratalla, R., and Calabresi, P. (2003). Distinct roles of D1 and D5 dopamine receptors in motor activity and striatal synaptic plasticity. *J. Neurosci.* 23, 8506–8512.
- Centonze, D., Gubellini, P., Picconi, B., Calabresi, P., Giamini, P., and Bernardi, G. (1999). Unilateral dopamine denervation blocks corticostriatal LTP. *J. Neurophysiol.* 82, 3575–3579.
- Cepeda, C., and Levine, M. S. (1998). Dopamine and N-methyl-D-aspartate receptor interactions in the neostriatum. *Dev. Neurosci.* 20, 1–18.
- Cepeda, C., and Levine, M. S. (2006). Where do you think you are going? The NMDA-D1 receptor trap. *Sci. STKE* 2006, pe20.
- Corvol, J. C., Muriel, M. P., Valjent, E., Feger, J., Hanoun, N., Girault, J. A., Hirsch, E. C., and Hervé, D. (2004). Persistent increase in olfactory type G-protein alpha subunit levels may underlie D1 receptor functional hypersensitivity in Parkinson's disease. *J. Neurosci.* 24, 7007–7014.
- Cotzias, G. C., Papavasiliou, P. S., and Gellene, R. (1969). Modification of parkinsonism: chronic treatment with L-dopa. *N. Engl. J. Med.* 280, 337–345.
- Dagher, A., and Robbins, T. W. (2009). Personality, addiction, dopamine: insights from Parkinson's disease. *Neuron* 61, 502–510.
- Damier, P., Tremblay, L., Féger, J., and Hirsch, E. C. (2000). Development of dyskinesias induced by treatment for Parkinson's disease: potential role of first exposure to L-DOPA (or phenomenon of priming). *Rev. Neurol. (Paris)* 156, 224–235.
- Darmopil, S., Martín, A. B., De Diego, I. R., Ares, S., and Moratalla, R. (2009). Genetic inactivation of dopamine D1 but not D2 receptors inhibits L-DOPA-induced dyskinesia and histone activation. *Biol. Psychiatry* 66, 603–613.
- Darmopil, S., Muñetón-Gómez, V. C., de Ceballos, M. L., Bernson, M., and Moratalla, R. (2008). Tyrosine hydroxylase cells appearing in the mouse striatum after dopamine denervation are likely to be projection neurons regulated by L-DOPA. *Eur. J. Neurosci.* 27, 580–592.
- de la Fuente-Fernández, R., Sossi, V., Huang, Z., Furtado, S., Lu, J. Q., Calne, D. B., Ruth, T. J., and Stoessl, A. J. (2004). Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias. *Brain* 127, 2747–2754.
- Delfino, M., Kalisch, R., Czisch, M., Larremendy, C., Ricatti, J., Taravini, I. R., Trenkwalder, C., Murer, M. G., Auer, D. P., and Gershanik, O. S. (2007). Mapping the effects of three dopamine agonists with different dyskinesia potential and receptor selectivity using pharmacological functional magnetic resonance imaging. *Neuropsychopharmacology* 32, 1911–1921.
- Delfino, M. A., Stefano, A. V., Ferrario, J. E., Taravini, I. R., Murer, M. G., and Gershanik, O. S. (2004). Behavioral sensitization to different dopamine agonists in a parkinsonian rodent model of drug-induced dyskinesias. *Behav. Brain Res.* 152, 297–306.
- Doucet, J. P., Nakabeppu, Y., Bedard, P. J., Hope, B. T., Nestler, E. J., Jasmin, B. J., Chen, J. S., Iadarola, M. J., St-Jean, M., Wigle, N., Blanchet, P., Grondin, R., and Robertson, G. S. (1996). Chronic alterations in dopaminergic neurotransmission produce a persistent elevation of deltaFosB-like protein(s) in both the rodent and primate striatum. *Eur. J. Neurosci.* 8, 365–381.
- El-Ghundi, M., Fletcher, P. J., Drago, J., Sibley, D. R., O'Down, B. F., and George, S. R. (1999). Spatial learning deficit in dopamine D1 receptor knockout mice. *Eur. J. Pharmacol.* 383, 95–106.
- Fahn, S. (1996). Is levodopa toxic? *Neurology* 47(Suppl. 3), S184–S195.
- Fasano, S., Bezard, E., D'Antoni, A., Francardo, V., Indrigo, M., Qin, L., Doveró, S., Cerovic, M., Cenci, M. A., and Brambilla, R. (2010). Inhibition of Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) signaling in the striatum reverses motor symptoms associated with L-dopa-induced dyskinesia. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21824–21829.
- Fasano, S., D'Antoni, A., Orban, P. C., Valjent, E., Putignano, E., Vara, H., Pizzorusso, T., Giustetto, M., Yoon, B., Soloway, P., Maldonado, R., Caboche, J., and Brambilla, R. (2009). Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) controls activation of extracellular signal-regulated kinase (ERK) signaling in the striatum and long-term behavioral responses to cocaine. *Biol. Psychiatry* 66, 758–768.
- Filion, M., Tremblay, L., and Bédard, P. J. (1991). Effects of dopamine agonists on the spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. *Brain Res.* 547, 152–161.
- Fiorentini, C., Busi, C., Gorruso, E., Gotti, C., Spano, P., and Missale, C. (2008). Reciprocal regulation of dopamine D1 and D3 receptor function and trafficking by heterodimerization. *Mol. Pharmacol.* 74, 59–69.
- Fiorentini, C., Rizzetti, M. C., Busi, C., Bontempi, S., Collo, G., Spano, P., and Missale, C. (2006). Loss of synaptic D1 dopamine/N-methyl-D-aspartate glutamate receptor complexes in L-DOPA-induced dyskinesia in the rat. *Mol. Pharmacol.* 69, 805–812.
- Gardoni, F., Picconi, B., Ghiglieri, V., Polli, F., Bagetta, V., Bernardi, G., Cattabeni, F., Di Luca, M., and Calabresi, P. (2006). A critical interaction between NR2B and MAGUK in L-DOPA induced dyskinesia. *J. Neurosci.* 26, 2914–2922.
- Gerfen, C. R., Miyachi, S., Paletzki, R., and Brown, P. (2002). D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. *J. Neurosci.* 22, 5042–5054.
- Gold, S. J., Hoang, C. V., Potts, B. W., Porras, G., Pioli, E., Kim, K. W., Nadjar, A., Qin, C., LaHoste, G. J., Li, Q., Bioulac, B. H., Waugh, J. L., Gurevich, E., Neve, R. L., and Bezard, E. (2007). RGS9-2 negatively modulates L-3,4-dihydroxyphenylalanine-induced dyskinesia in experimental Parkinson's disease. *J. Neurosci.* 27, 14338–14348.
- Gonon, F. (1997). Prolonged and extrasynaptic excitatory action of dopamine mediated by D1 receptors in the rat striatum in vivo. *J. Neurosci.* 17, 5972–5978.
- Goulet, M., and Madras, B. K. (2000). D(1) dopamine receptor agonists are more effective in alleviating advanced than mild parkinsonism in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated monkeys. *J. Pharmacol. Exp. Ther.* 292, 714–724.
- Granado, N., Ortiz, O., Suárez, L. M., Martín, E. D., Ceña, V., Solís, J. M., and Moratalla, R. (2008). D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-Induced arc and zif268 expression in the hippocampus. *Cereb. Cortex* 18, 1–12.
- Grande, C., Zhu, H., Martin, A. B., Lee, M., Ortiz, O., Hiroi, N., and Moratalla, R. (2004). Chronic treatment with atypical neuroleptics induces striosomal FosB/DeltaFosB expression in rats. *Biol. Psychiatry* 55, 457–463.
- Grondin, R., Bédard, P. J., Britton, D. R., and Shiosaki, K. (1997). Potential therapeutic use of the selective dopamine D1 receptor agonist, A-86929: an acute study in parkinsonian levodopa-primed monkeys. *Neurology* 49, 421–426.
- Grondin, R., Doan, V. D., Grégoire, L., and Bédard, P. J. (1999). D1 receptor blockade improves L-dopa-induced dyskinesia but worsens parkinsonism in MPTP monkeys. *Neurology* 52, 771–776.
- Guigoni, C., Doudnikoff, E., Li, Q., Bloch, B., and Bezard, E. (2007). Altered D(1) dopamine receptor trafficking in parkinsonian and dyskinetic non-human primates. *Neurobiol. Dis.* 26, 452–463.
- Guigoni, C., Li, Q., Aubert, I., Dovero, S., Bioulac, B. H., Bloch, B., Crossman, A. R., Gross, C. E., and Bezard, E. (2005). Involvement of sensorimotor, limbic, and associative basal ganglia domains in L-3,4-dihydroxyphenylalanine-induced dyskinesia. *J. Neurosci.* 25, 2102–2107.
- Hirano, S., Asanuma, K., Ma, Y., Tang, C., Feigin, A., Dhawan, V., Carbon, M., and Eidelberg, D. (2008). Dissociation of metabolic and neurovascular responses to levodopa in the treatment of Parkinson's disease. *J. Neurosci.* 28, 4201–4209.
- Hiroi, N., and Graybiel, A. M. (1996). Atypical and typical neuroleptic treatments induce distinct programs of transcription factor expression in the striatum. *J. Comp. Neurol.* 374, 70–83.

- Hiroi, N., Martín, A. B., Grande, C., Alberti, I., Rivera, A., and Moratalla, R. (2002). Molecular dissection of dopamine receptor signaling. *J. Chem. Neuroanat.* 23, 237–242.
- Holloway, R. G., Shoulson, I., Fahn, S., Kiebertz, K., Lang, A., Marek, K., McDermott, M., Seibyl, J., Weiner, W., Musch, B., Kamp, C., Welsh, M., Shinaman, A., Pahwa, R., Barclay, L., Hubble, J., LeWitt, P., Miyasaka, J., Suchowersky, O., Stacy, M., Russell, D. S., Ford, B., Hammerstad, J., Riley, D., Standaert, D., Wooten, F., Factor, S., Jankovic, J., Atassi, F., Kurlan, R., Panisset, M., Rajput, A., Rodnitzky, R., Shults, C., Petsinger, G., Waters, C., Pfeiffer, R., Biglan, K., Borcher, L., Montgomery, A., Sutherland, L., Weeks, C., DeAngelis, M., Sime, E., Wood, S., Pantella, C., Harrigan, M., Fussell, B., Dillon, A., Alexander-Brown, B., Rainey, P., Tennis, M., Rost-Ruffner, E., Brown, D., Evans, S., Berry, D., Hall, J., Shirley, T., Dobson, J., Fontaine, D., Pfeiffer, B., Brocht, A., Bennett, S., Daigneault, S., Hodgeman, K., O'Connell, C., Ross, T., Richard, K., and Watts, A.; Parkinson Study Group. (2004). Pramipexole vs levodopa as initial treatment for Parkinson disease: a 4-year randomized 33 controlled trial. *Arch. Neurol.* 61, 1044–1053.
- Hsu, A., Togasaki, D. M., Bezard, E., Sokoloff, P., Langston, J. W., Di Monte, D. A., and Quirk, M. (2004). Effect of the D3 dopamine receptor partial agonist BP897 [N-[4-(2-methoxyphenyl) piperazinyl] butyl]-2-naphthamide] on L-3,4-dihydroxyphenylalanine-induced dyskinesias and parkinsonism in squirrel monkeys. *J. Pharmacol. Exp. Ther.* 311, 770–777.
- Hurley, M. J., Jolkonen, J., Stubbs, C. M., Jenner, P., and Marsden, C. D. (1996). Dopamine D3 receptors in the basal ganglia of the common marmoset and following MPTP and L-DOPA treatment. *Brain Res.* 709, 259–264.
- Jenner, P. (2008). Molecular mechanisms of L-DOPA-induced dyskinesia. *Nat. Rev. Neurosci.* 9, 665–677.
- Kasanez, F., Deroche-Gamonet, V., Berson, N., Balado, E., Lafourcade, M., Manzoni, O., and Piazza, P. V. (2010). Transition to addiction is associated with a persistent impairment in synaptic plasticity. *Science* 328, 1709–1712.
- Kelz, M. B., Chen, J., Carlezon, W. A. Jr., Whisler, K., Gilden, L., Beckmann, A. M., Steffen, C., Zhang, Y. J., Marotti, L., Self, D. W., Tkatch, T., Baranauskas, G., Surmeier, D. J., Neve, R. L., Duman, R. S., Picciotto, M. R., and Nestler, E. J. (1999). Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature* 401, 272–276.
- Kreitzer, A. C., and Malenka, R. C. (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature* 445, 643–647.
- Kreitzer, A. C., and Malenka, R. C. (2008). Striatal plasticity and basal ganglia circuit function. *Neuron* 60, 543–554.
- Kumar, R., Riddle, L., Griffin, S. A., Grundt, P., Newman, A. H., and Luedtke, R. R. (2009). Evaluation of the D3 dopamine receptor selective antagonist PG01037 on L-DOPA-dependent abnormal involuntary movements in rats. *Neuropharmacology* 56, 944–955.
- Larramendy, C., Taravini, I. R., Saborido, M. D., Ferrario, J. E., Murer, M. G., and Gershanik, O. S. (2008). Cabergoline and pramipexole fail to modify already established dyskinesias in an animal model of parkinsonism. *Behav. Brain Res.* 194, 44–51.
- Levenson, J. M., O'Riordan, K. J., Brown, K. D., Trinh, M. A., Molfese, D. L., and Sweatt, J. D. (2004). Regulation of histone acetylation during memory formation in the hippocampus. *J. Biol. Chem.* 279, 40545–40559.
- Lindgren, H. S., Andersson, D. R., Lagerkvist, S., Nissbrandt, H., and Cenci, M. A. (2010). L-DOPA-induced dopamine efflux in the striatum and the substantia nigra in a rat model of Parkinson's disease: temporal and quantitative relationship to the expression of dyskinesia. *J. Neurochem.* 112, 1465–1476.
- Lindgren, H. S., Ohlin, K. E., and Cenci, M. A. (2009). Differential involvement of D1 and D2 dopamine receptors in L-DOPA-induced angiogenic activity in a rat model of Parkinson's disease. *Neuropsychopharmacology* 34, 2477–2488.
- Lovinger, D. M. (2010). Neurotransmitter roles in synaptic modulation, plasticity and learning in the dorsal striatum. *Neuropharmacology* 58, 951–961.
- Lozano, A. M., Lang, A. E., Levy, R., Hutchison, W., and Dostrovsky, J. (2000). Neuronal recordings in Parkinson's disease patients with dyskinesias induced by apomorphine. *Ann. Neurol.* 47(Suppl. 1), S141–S146.
- Lundblad, M., Usiello, A., Carta, M., Håkansson, K., Fisone, G., and Cenci, M. A. (2005). Pharmacological validation of a mouse model of L-DOPA-induced dyskinesia. *Exp. Neurol.* 194, 66–75.
- Marcellino, D., Ferré, S., Casadó, V., Cortés, A., Le Foll, B., Mazzola, C., Drago, F., Saur, O., Stark, H., Soriano, A., Barnes, C., Goldberg, S. R., Lluís, C., Fuxe, K., and Franco, R. (2008). Identification of dopamine D1-D3 receptor heteromers. Indications for a role of synergistic D1-D3 receptor interactions in the striatum. *J. Biol. Chem.* 283, 26016–26025.
- Mazzucchi, C., Vantaggiato, C., Ciamei, A., Fasano, S., Pakhotin, P., Krezel, W., Welzl, H., Wolfer, D. P., Pagès, G., Valverde, O., Marowsky, A., Porrazzo, A., Orban, P. C., Maldonado, R., Ehrenguber, M. U., Cestari, V., Lipp, H. P., Chapman, P. F., Pouyssegur, J., and Brambilla, R. (2002). Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. *Neuron* 34, 807–820.
- McClung, C. A., and Nestler, E. J. (2003). Regulation of gene expression and cocaine reward by CREB and DeltaFosB. *Nat. Neurosci.* 6, 1208–1215.
- McClung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., and Nestler, E. J. (2004). DeltaFosB: a molecular switch for long-term adaptation in the brain. *Brain Res. Mol. Brain Res.* 132, 146–154.
- Mela, F., Marti, M., Dekundy, A., Danyasz, W., Morari, M., and Cenci, M. A. (2007). Antagonism of metabotropic glutamate receptor type 5 attenuates L-DOPA-induced dyskinesia and its molecular and neurochemical correlates in a rat model of Parkinson's disease. *J. Neurochem.* 101, 483–497.
- Mela, F., Millan, M. J., Brocco, M., and Morari, M. (2010). The selective D(3) receptor antagonist, S33084, improves parkinsonian-like motor dysfunction but does not affect L-DOPA-induced dyskinesia in 6-hydroxydopamine hemilesioned rats. *Neuropharmacology* 58, 528–536.
- Merello, M., Balej, J., Delfino, M., Cammarota, A., Betti, O., and Leiguarda, R. (1999). Apomorphine induces changes in GPe spontaneous outflow in patients with Parkinson's disease. *Mov. Disord.* 14, 45–49.
- Moratalla, R., Xu, M., Tonegawa, S., and Graybiel, A. M. (1996a). Cellular responses to psychomotor stimulant and neuroleptic drugs are abnormal in mice lacking the D1 dopamine receptor. *Proc. Natl. Acad. Sci. U.S.A.* 93, 14928–14933.
- Moratalla, R., Elibol, B., Vallejo, M., and Graybiel, A. M. (1996b). Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. *Neuron* 17, 147–156.
- Murer, M. G., Raisman-Vozari, R., and Gershanik, O. (1999). Levodopa in Parkinson's disease: neurotoxicity issue laid to rest? *Drug Saf.* 21, 339–352.
- Muriel, M. P., Bernard, V., Levey, A. I., Laribi, O., Abrous, D. N., Agid, Y., Bloch, B., and Hirsch, E. C. (1999). Levodopa induces a cytoplasmic localization of D1 dopamine receptors in striatal neurons in Parkinson's disease. *Ann. Neurol.* 46, 103–111.
- Nutt, J. G. (2000). Clinical pharmacology of levodopa-induced dyskinesia. *Ann. Neurol.* 47(Suppl. 1), S160–S164.
- Nutt, J. G. (2008). Pharmacokinetics and pharmacodynamics of levodopa. *Mov. Disord.* 23(Suppl. 3), S580–S584.
- O'Carroll, C. M., Martin, S. J., Sandin, J., Frenguelli, B., and Morris, R. G. (2006). Dopaminergic modulation of the persistence of one-trial hippocampus dependent memory. *Learn. Mem.* 13, 760–769.
- Olanow, C. W., Agid, Y., Mizuno, Y., Albanese, A., Bonuccelli, U., Damier, P., De Yebenes, J., Gershanik, O., Guttman, M., Grandas, F., Hallett, M., Hornykiewicz, O., Jenner, P., Katzenschlager, R., Langston, W. J., LeWitt, P., Melamed, E., Mena, M. A., Michel, P. P., Mytilineou, C., Obeso, J. A., Poewe, W., Quinn, N., Raisman-Vozari, R., Rajput, A. H., Rascol, O., Sampaio, C., and Stocchi, F. (2004). Levodopa in the treatment of Parkinson's disease: current controversies. *Mov. Disord.* 19, 997–1005.
- Olanow, C. W., Obeso, J. A., and Stocchi, F. (2006). Drug insight: Continuous dopaminergic stimulation in the treatment of Parkinson's disease. *Nat. Clin. Pract. Neurol.* 2, 382–392.
- Onofrio, C., Paci, A., and Thomas, C. (1998). Sudden appearance of invalidating dyskinesia-dystonia and off fluctuations after the introduction of levodopa in two dopaminomimetic drug naive patients with stage IV Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 65, 605–606.
- Ortiz, O., Delgado-García, J. M., Espadas, I., Bahí, A., Trullas, R., Dreyer, J. L., Gruart, A., and Moratalla, R. (2010). Associative learning and CA3-CA1 synaptic plasticity are impaired in D1R null, *Drd1a*^{-/-} mice and in hippocampal siRNA silenced *Drd1a* mice. *J. Neurosci.* 30, 12288–12300.
- Papa, S. M., Desimone, R., Fiorani, M., and Oldfield, E. H. (1999). Internal globus pallidus discharge is nearly suppressed during levodopa-induced dyskinesias. *Ann. Neurol.* 46, 732–738.

- Papavasiliou, P. S., Cotzias, G. C., Dübey, S. E., Steck, A. J., Fehling, C., and Bell, M. A. (1972). Levodopa in Parkinsonism: potentiation of central effects with a peripheral inhibitor. *N. Engl. J. Med.* 286, 8–14.
- Pavon, N., Martin, A. B., Mendiola, A., and Moratalla, R. (2006). ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. *Biol. Psychiatry* 59, 64–74.
- Picconi, B., Centonze, D., Håkansson, K., Bernardi, G., Greengard, P., Fisone, G., Cenci, M. A., and Calabresi, P. (2003). Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. *Nat. Neurosci.* 6, 501–506.
- Quik, M., Police, S., He, L., Di Monte, D. A., and Langston, J. W. (2000). Expression of D(3) receptor messenger RNA and binding sites in monkey striatum and substantia nigra after nigrostriatal degeneration: effect of levodopa treatment. *Neuroscience* 98, 263–273.
- Rascol, O. (1999). Dopamine agonists: what is the place of the newer compounds in the treatment of Parkinson's disease? *J. Neural Transm.* 55, 33–45.
- Rascol, O., Brooks, D. J., Korczyn, A. D., De Deyn, P. P., Clarke, C. E., Lang, A. E., and Abdalla, M. (056) Study Group. (2006). Development of dyskinesias in a 5-year trial of ropinirole and L-dopa. *Mov. Disord.* 21, 1844–1850.
- Rascol, O., Nutt, J. G., Blin, O., Goetz, C. G., Trugman, J. M., Soubrouillard, C., Carter, J. H., Currie, L. J., Fabre, N., Thalamos, C., Giordina, W. W., and Wright, S. (2001). Induction by dopamine D1 receptor agonist ABT-431 of dyskinesia similar to levodopa in patients with Parkinson disease. *Arch. Neurol.* 58, 249–254.
- Rodriguez, T. B., Granado, N., Ortiz, O., Cerdán, S., and Moratalla, R. (2007). Metabolic interactions between glutamatergic and dopaminergic neurotransmitter systems are mediated through D(1) dopamine receptors. *J. Neurosci. Res.* 85, 3284–3293.
- Rossato, J. I., Bevilacqua, L. R., Izquierdo, I., Medina, J. H., and Cammarota, M. (2009). Dopamine controls persistence of long-term memory storage. *Science* 325, 1017–1020.
- Rylander, D., Recchia, A., Mela, F., Dekundy, A., Danysz, W., and Cenci, M. A. (2009). Pharmacological modulation of glutamate transmission in a rat model of L-DOPA-induced dyskinesia: effects on motor behavior and striatal nuclear signaling. *J. Pharmacol. Exp. Ther.* 330, 227–235.
- Sánchez-Pernaute, R., Jenkins, B. G., Choi, J. K., Iris Chen, Y. C., and Isacson, O. (2007). In vivo evidence of D3 dopamine receptor sensitization in parkinsonian primates and rodents with L-DOPA-induced dyskinesias. *Neurobiol. Dis.* 27, 220–227.
- Santini, E., Alcacer, C., Cacciatore, S., Heiman, M., Hervé, D., Greengard, P., Girault, J. A., Valjent, E., and Fisone, G. (2009a). L-DOPA activates ERK signaling and phosphorylates histone H3 in the striatonigral medium spiny neurons of hemiparkinsonian mice. *J. Neurochem.* 108, 621–633.
- Santini, E., Heiman, M., Greengard, P., Valjent, E., and Fisone, G. (2009b). Inhibition of mTOR signaling in Parkinson's disease prevents L-DOPA-induced dyskinesia. *Sci. Signal.* 2, ra36.
- Santini, E., Sgambato-Faure, V., Li, Q., Savasta, M., Dovero, S., Fisone, G., and Bezard, E. (2010). Distinct changes in cAMP and extracellular signal-regulated protein kinase signalling in L-DOPA-induced dyskinesia. *PLoS ONE* 5, e12322. doi: 10.1371/journal.pone.0012322
- Santini, E., Valjent, E., Usiello, A., Carta, M., Borgkvist, A., and Girault, J. A. (2007). Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *J. Neurosci.* 27, 6995–7005.
- Schuster, S., Nadjar, A., Guo, J. T., Li, Q., Ittrich, C., Hengerer, B., and Bezard, E. (2008). The 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor lovastatin reduces severity of L-DOPA-induced abnormal involuntary movements in experimental Parkinson's disease. *J. Neurosci.* 28, 4311–4316.
- Sgambato-Faure, V., Buggia, V., Gilbert, F., Lévesque, D., Benabid, A. L., and Berger, F. (2005). Coordinated and spatial upregulation of arc in striatonigral neurons correlates with L-dopa-induced behavioral sensitization in dyskinetic rats. *J. Neuropathol. Exp. Neurol.* 64, 936–947.
- Shen, W., Flajolet, M., Greengard, P., and Surmeier, D. J. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. *Science* 321, 848–851.
- Stipanovich, A., Valjent, E., Matamalas, M., Nishi, A., Ahn, J. H., Maroteaux, M., Bertran-Gonzalez, J., Bami-Cherrier, K., Enslen, H., Corbillé, A. G., Filhol, O., Nairn, A. C., Greengard, P., Hervé, D., and Girault, J. A. (2008). A phosphatase cascade by which rewarding stimuli control nucleosomal response. *Nature* 453, 879–884.
- Surmeier, D. J., Plotkin, J., and Shen, W. (2009). Dopamine and synaptic plasticity in dorsal striatal circuits controlling action selection. *Curr. Opin. Neurobiol.* 19, 621–628.
- Tang, K., Low, M. J., Grandy, D. K., and Lovinger, D. M. (2001). Dopamine-dependent synaptic plasticity in striatum during in vivo development. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1255–1260.
- Taylor, J. L., Bishop, C., and Walker, P. D. (2005). Dopamine D1 and D2 receptor contributions to L-DOPA-induced dyskinesia in the dopamine-depleted rat. *Pharmacol. Biochem. Behav.* 81, 887–893.
- Thomas, G. M., and Haganir, R. L. (2004). MAPK cascade signalling and synaptic plasticity. *Nat. Rev. Neurosci.* 5, 173–183.
- Trugman, J. M., and Wooten, G. F. (1986). The effects of L-DOPA on regional cerebral glucose utilization in rats with unilateral lesions of the substantia nigra. *Brain Res.* 379, 264–274.
- Trugman, J. M., and Wooten, G. F. (1987). Selective D1 and D2 dopamine agonists differentially alter basal ganglia glucose utilization in rats with unilateral 6-hydroxydopamine substantia nigra lesions. *J. Neurosci.* 7, 2927–2935.
- Ulusoy, A., Sahin, G., and Kirik, D. (2010). Presynaptic dopaminergic compartment determines the susceptibility to L-DOPA-induced dyskinesia in rats. *Proc. Natl. Acad. Sci. U.S.A.* 107, 13159–13164.
- Valjent, E., Corvol, J. C., Pages, C., Besson, M. J., Maldonado, R., and Caboche, J. (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J. Neurosci.* 20, 8701–8709.
- Visanji, N. P., Fox, S. H., Johnston, T., Reyes, G., Millan, M. J., and Brotchie, J. M. (2009). Dopamine D3 receptor stimulation underlies the development of L-DOPA-induced dyskinesia in animal models of Parkinson's disease. *Neurobiol. Dis.* 35, 184–192.
- Voon, V., Fernagut, P. O., Wickens, J., Baunez, C., Rodriguez, M., Pavon, N., Juncos, J. L., Obeso, J. A., and Bezard, E. (2009). Chronic dopaminergic stimulation in Parkinson's disease: from dyskinesias to impulse control disorders. *Lancet Neurol.* 8, 1140–1149.
- Wang, Z., Kai, L., Day, M., Ronesi, J., Yin, H. H., Ding, J., Tkatch, T., and Lovinger, D. M., Surmeier, D. J. (2006). Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. *Neuron* 50, 443–452.
- Westin, J. E., Lindgren, H. S., Gardi, J., Nyengaard, J. R., Brundin, P., Mohapel, P., and Cenci, M. A. (2006). Endothelial proliferation and increased blood-brain barrier permeability in the basal ganglia in a rat model of 3,4-dihydroxyphenyl-L-alanine-induced dyskinesia. *J. Neurosci.* 26, 9448–9461.
- Westin, J. E., Vercammen, L., Strome, E. M., Konradi, C., and Cenci, M. A. (2007). Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D1 receptors. *Biol. Psychiatry* 62, 800–810.
- Winkler, C., Kirik, D., Björklund, A., and Cenci, M. A. (2002). L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of Parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. *Neurobiol. Dis.* 10, 165–186.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 11 May 2011; paper pending published: 06 June 2011; accepted: 25 July 2011; published online: 11 August 2011.
Citation: Murer MG and Moratalla R (2011) Striatal signaling in L-DOPA-induced dyskinesia: common mechanisms with drug abuse and long term memory involving D1 dopamine receptor stimulation. *Front. Neuroanat.* 5:51. doi: 10.3389/fnana.2011.00051
Copyright © 2011 Murer and Moratalla. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Deciphering the actions of antiparkinsonian and antipsychotic drugs on cAMP/DARPP-32 signaling

Alessandra Bonito-Oliva, Michael Feyder and Gilberto Fisone*

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Edited by:

Emmanuel Valjent, Université Montpellier 1 and 2, France

Reviewed by:

Chip Gerfen, National Institutes of Health, USA

Michel Cyr, Neuroscience Research Group, Canada

*Correspondence:

Gilberto Fisone, Department of Neuroscience, Karolinska Institutet, Retzius väg 8, 171 77 Stockholm, Sweden.
e-mail: gilberto.fisone@ki.se

The basal ganglia are affected by several neuropsychiatric and neurodegenerative diseases, many of which are treated with drugs acting on the dopamine system. For instance, the loss of dopaminergic input to the striatum, which is the main pathological feature of Parkinson's disease, is counteracted by administering the dopamine precursor, L-DOPA. Furthermore, psychotic disorders, including schizophrenia, are treated with drugs that act as antagonists at the D2-type of dopamine receptor (D2R). The use of L-DOPA and typical antipsychotic drugs, such as haloperidol, is limited by the emergence of motor side-effects, particularly after prolonged use. Striatal medium spiny neurons (MSNs) represent an ideal tool to investigate the molecular changes implicated in these conditions. MSNs receive a large glutamatergic innervation from cortex, thalamus, and limbic structures, and are controlled by dopaminergic projections originating in the midbrain. There are two large populations of striatal MSNs, which differ based on their connectivity to the output nuclei of the basal ganglia and on their ability to express dopamine D1 receptors (D1Rs) or D2Rs. Administration of L-DOPA promotes cAMP signaling and activates the dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) in the D1R-expressing MSNs, which form the striatonigral, or direct pathway. Conversely, haloperidol activates the cAMP/DARPP-32 cascade in D2R-expressing MSNs, which form the striatopallidal, or indirect pathway. This review describes the effects produced on downstream effector proteins by stimulation of cAMP/DARPP-32 signaling in these two groups of MSNs. Particular emphasis is given to the regulation of the GluR1 subunit of the α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate glutamate receptor, the extracellular signal-regulated protein kinases 1 and 2, focusing on functional role and potential pathological relevance.

Keywords: DARPP-32, dopamine D1 receptor, dopamine D2 receptor, ERK, L-DOPA, haloperidol, medium spiny neurons

Dysfunctions of the dopamine system are implicated in many neurodegenerative and neuropsychiatric disorders, including Parkinson's disease (PD) and schizophrenia. The cardinal symptoms of PD (tremor, rigidity, and hypokinesia) are caused by the progressive degeneration of the midbrain dopaminergic neurons of the substantia nigra pars compacta (Hornykiewicz, 1963; Braak et al., 2003). These cells project to the dorso-lateral part of the striatum, which is the major component of the basal ganglia, a group of subcortical nuclei involved in the control of motor function. The dopamine precursor L-DOPA is widely used in the treatment of PD (Cotzias et al., 1967; Birkmayer and Hornykiewicz, 1998); however, prolonged use of this drug is complicated by the appearance of dystonic and choreic movements, or dyskinesia, which are manifested in parallel with the therapeutic, anti-kinetic action of L-DOPA (Obeso et al., 2000).

In contrast to PD, schizophrenia and psychotic disorders are treated with drugs which reduce dopaminergic transmission. In particular, conventional antipsychotic drugs, such as haloperidol, act as selective and potent antagonists at D2-type of dopamine receptors (Creese et al., 1976; Seeman et al., 1976). Even the use of these substances, however, is limited by the emergence

of motor complications, generally referred to as extrapyramidal side-effects (EPS), which include tardive dyskinesia, dystonia, and parkinsonism (Miyamoto et al., 2005).

The development of motor disorders in response to administration of L-DOPA and haloperidol depends in large part on the ability of these drugs to affect transmission in the striatum, which is enriched in D1- and D2-type of dopamine receptors. The study of the effects produced in this brain region by antiparkinsonian and antipsychotic drugs is important not only to elucidate the mechanisms underlying L-DOPA-induced dyskinesia (LID) and EPS, but also to examine the impact on motor dysfunctions of aberrant signaling processes occurring in well-defined neuronal populations.

DISTINCT ROLES OF STRIATAL MEDIUM SPINY NEURONS IN MOTOR FUNCTION

In the striatum, fibers originating from midbrain dopaminergic neurons innervate two populations of principal GABAergic neurons, collectively denominated medium spiny neurons (MSNs). More than 20 years ago, these two groups of neurons were proposed to form separate projection pathways, exerting opposing

effects on basal ganglia output structures (i.e., substantia nigra pars reticulata and internal segment of the globus pallidus). It was suggested that the MSNs directly connected to these structures would disinhibit thalamo-cortical neurons and promote motor activity, whereas the MSNs connected to these nuclei indirectly (via external segment of the globus pallidus and subthalamic nucleus) would increase the inhibition of efferent targets within the thalamus and depress motor function (Albin et al., 1989; Alexander and Crutcher, 1990; DeLong, 1990).

Another important concept emerging during those years was that dopamine acted by stimulating the MSNs of the direct, or striatonigral pathway, and inhibiting those of the indirect, or striatopallidal pathway (Albin et al., 1989; Gerfen et al., 1990). These opposing effects were suggested to depend on the expression of dopamine D1 receptors (D1Rs) in the MSNs of the direct pathway and of dopamine D2 receptors (D2Rs) in the MSNs of the indirect pathway (Gerfen et al., 1990). The hypothesis of a contrasting action of dopamine on direct and indirect MSNs was supported by the observation that D1Rs activate, whereas D2Rs inhibit, adenylyl cyclase and cAMP synthesis (Kebabian and Calne, 1979; cf. below), thereby leading to opposite regulations of downstream effector targets and neuronal excitability.

The idea that D1Rs and D2Rs are selectively expressed in distinct groups of MSNs has gained increasing acceptance and, recently, it has been exploited to revisit and further analyze the functional properties of the MSNs of the direct and indirect pathway.

The development of techniques to engineer bacterial artificial chromosomes (BACs) has allowed the generation of mice expressing Cre recombinase, epitope-tagged proteins, and fluorescent probes in the D1R- or in the D2R-expressing MSNs of the direct and indirect pathway (Gong et al., 2003, 2007; Valjent et al., 2009). Using Cre-dependent viral delivery of channelrhodopsin-2, a light activated proton pump (Boyden et al., 2005; Kravitz et al., 2010) demonstrated that optogenetic activation of the D1R-expressing MSNs of the direct pathway promotes locomotion, whereas activation of the D2R-expressing MSNs of the indirect pathway depresses motor function.

Another study examined the effects on motor behaviors of cell-specific inactivation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), an important mediator of cAMP signaling and striatal excitability (Greengard, 2001). It was found that deletion of DARPP-32 in D1R-expressing MSNs reduced basal locomotor activity in the open-field. In contrast, inactivation of DARPP-32 in D2R-expressing MSNs increased locomotion (Bateup et al., 2010). A similar enhancement of motor function was observed following ablation of striatopallidal MSNs (Durieux et al., 2009).

DOPAMINE SIGNALING IN STRIATAL MSNs

As previously mentioned, D1Rs and D2Rs mediate opposite regulations of cAMP signaling. In the striatum, activation of D1Rs in the direct MSNs leads to G_{olf} -mediated stimulation of adenylyl cyclase, the enzyme responsible for the synthesis of cAMP (Zhuang et al., 2000; Herve et al., 2001). The ability of D1Rs to induce G-protein-dependent activation of cAMP signaling promotes transmission at α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate

(AMPA; cf. below) and *N*-methyl-D-aspartate (NMDA) glutamate receptors (Surmeier et al., 1995; Blank et al., 1997; Snyder et al., 1998; Liu et al., 2004). In addition, D1R-mediated activation of cAMP signaling increases L-type Ca^{2+} channel currents and decreases K^{+} channel currents (Kitai and Surmeier, 1993; Surmeier et al., 1995; Galarraga et al., 1997; Gao et al., 1997). These multiple regulations elevate the ability of sustained release of glutamate to produce depolarized up-states close to spike threshold (Wickens and Wilson, 1998), thereby promoting the excitability of striatonigral MSNs (Surmeier et al., 2007).

Activation of D2Rs in the indirect MSNs is coupled to $G_{i/o}$ -mediated inhibition of adenylyl cyclase, which decreases cAMP synthesis (Kebabian and Calne, 1979; Stoof and Kebabian, 1981). This negative modulation is especially evident on the adenylyl cyclase type 5 isoform, which is preferentially expressed in striatal MSNs (Glatt and Snyder, 1993; Mons and Cooper, 1994; Robinson and Caron, 1997). D2R-mediated activation of $G_{i/o}$ also leads to decreased cell excitability via G-protein-mediated activation of inwardly rectifying K^{+} channels (Wickman et al., 1994; Greif et al., 1995; Kuzhikandathil et al., 1998; Hopf et al., 2003). Moreover, D2Rs have been shown to regulate L-type Ca^{2+} currents in striatal MSNs through a G-protein- and Ca^{2+} -dependent mechanism which does not require suppression of cAMP signaling (Hernandez-Lopez et al., 2000). The coordinated modulation of ion channels exerted by D2R via activation of $G_{i/o}$ protein leads to reduced responsiveness of striatopallidal MSNs to glutamate, diminished transition of MSNs to depolarized up-states and reduced excitability (Surmeier et al., 2007).

In summary, the opposite regulation exerted by D1Rs and D2Rs on the responsiveness of MSNs to glutamate is in line with the functional model of basal ganglia described above, in which dopamine promotes motor activity via concomitant activation of direct MSNs and inhibition of indirect MSNs (Albin et al., 1989; Alexander and Crutcher, 1990; DeLong, 1990; Surmeier et al., 2007).

L-DOPA AND HALOPERIDOL IN THE STRIATUM: FOCUS ON cAMP SIGNALING IN MSNs

Studies performed in experimental models of PD indicate that administration of L-DOPA results in multiple changes in signal transduction, which occur specifically in the MSNs of the direct pathway. These changes depend on the loss of striatal dopamine, which results in the development of a remarkable sensitization at striatal D1Rs (Guigoni et al., 2005; Santini et al., 2008). Such sensitization potentiates the ability of L-DOPA to promote cAMP signaling, leading to stimulation of cAMP-dependent protein kinase (PKA) and phosphorylation of DARPP-32 (Picconi et al., 2003; Santini et al., 2007, 2010; Lebel et al., 2010). As discussed below, prolonged activation of this signaling cascade is implicated in the development of LID.

In contrast to L-DOPA, the actions of antipsychotic drugs (e.g., haloperidol) in the striatum are generally attributed to their ability to antagonize D2Rs on the MSNs of the indirect pathway. The primary effect of D2R blockade is the removal of the inhibition exerted by the $G_{i/o}$ protein on adenylyl cyclase, leading to increased cAMP signaling. This effect, in turn, depends on basal adenylyl cyclase activity, which is maintained through the action of the

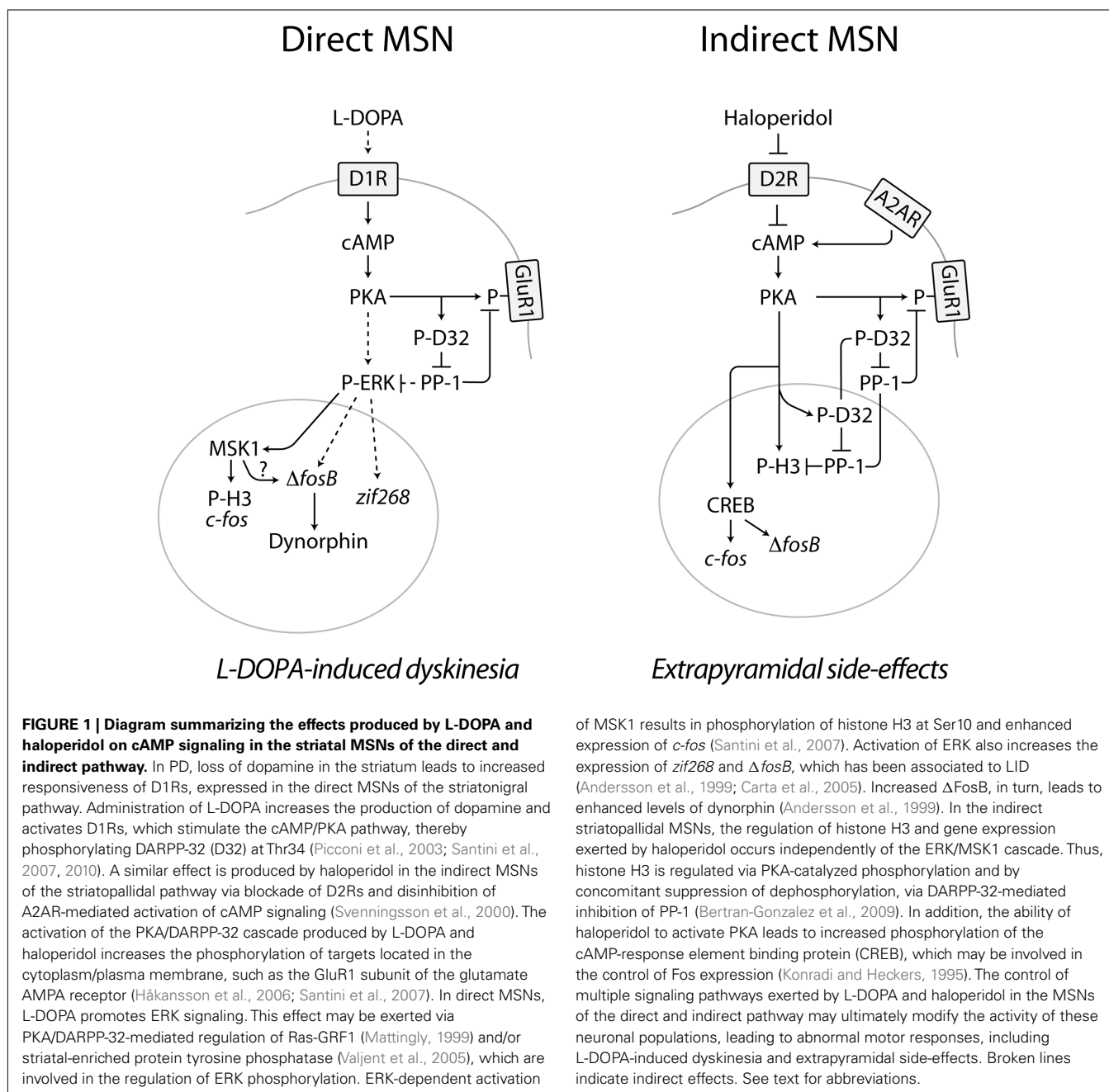
neuromodulator adenosine. The MSNs of the indirect pathway express high levels of adenosine A2A receptors (A2ARs; Schiffmann et al., 1991; Fink et al., 1992), which, similarly to D1Rs, are coupled to G_{olf} -mediated activation of adenylyl cyclase (Kull et al., 2000; Herve et al., 2001). Thus, in the MSNs of the indirect pathway, D2Rs and A2ARs exert opposite regulations on cAMP signaling. An important consequence of this functional arrangement is that blockade of A2AR-mediated transmission prevents the ability of D2R antagonists, including haloperidol, to increase cAMP signaling in indirect MSNs (Svenningsson et al., 2000; Håkansson et al., 2006; Bertran-Gonzalez et al., 2009; cf. **Figure 1**).

In summary, both L-DOPA and haloperidol promote cAMP-dependent signaling in striatal MSNs. The effect of L-DOPA is

exerted in the MSNs of the direct pathway, via D1R-mediated activation of adenylyl cyclase. In contrast, the effect of haloperidol is exerted on the MSNs of the indirect pathway through blockade of D2Rs and disinhibition of adenylyl cyclase (**Figure 1**).

DARPP-32: A MEDIATOR OF THE ACTIONS OF ANTIPARKINSONIAN AND ANTIPSYCHOTIC DRUGS

The ability of L-DOPA and haloperidol to promote cAMP signaling is reflected by the large increase in the phosphorylation of DARPP-32 produced by administration of these drugs. DARPP-32 is highly expressed in the MSNs of the direct and indirect pathway and plays an important role in dopamine transmission (Fienberg et al., 1998; Greengard, 2001). Activation of D1Rs in direct



MSNs increases PKA-dependent phosphorylation of DARPP-32 on Thr34 (Nishi et al., 1997; Svenningsson et al., 1998). A similar effect is produced by activation of A2ARs in indirect MSNs (Svenningsson et al., 1998). Phosphorylation of Thr34 converts DARPP-32 into an inhibitor of protein phosphatase-1 (PP-1; Hemmings et al., 1984), which amplifies the effects of the cAMP/PKA cascade by reducing dephosphorylation of downstream target proteins (Fienberg et al., 1998; Greengard, 2001; cf. **Figure 1**).

In the dopamine depleted striatum, administration of L-DOPA increases the phosphorylation of DARPP-32 at Thr34 (Picconi et al., 2003; Santini et al., 2007, 2010; Lebel et al., 2010). In rodent and non-human primate models of PD, this effect is associated to the manifestation of abnormal involuntary movements, which are regarded as a behavioral correlate of dyskinesia (Cenci et al., 2002). In line with these observations, it has been shown that LID is attenuated in DARPP-32 knock out mice (Santini et al., 2007). Interestingly, a large reduction of LID has also been observed in mice in which DARPP-32 is specifically inactivated in the MSNs of the direct pathway, whereas deletion of DARPP-32 in indirect MSNs does not affect the development of dyskinesia (Bateup et al., 2010). These findings indicate the importance of enhanced D1R/PKA/DARPP-32 signaling in LID and identify the MSNs of the direct pathway as a key neuronal substrate implicated in this condition.

Administration of D2R antagonists, including haloperidol, promotes PKA-dependent phosphorylation of DARPP-32 (Svenningsson et al., 2000). This effect is produced via disinhibition of adenylyl cyclase, whose basal activation is maintained by A2ARs (cf. above). In support of this idea, it has been shown that both pharmacological and genetic inactivation of A2ARs prevent the increase in DARPP-32 phosphorylation induced by eticlopride, a potent D2R antagonist (Svenningsson et al., 2000). The effect of haloperidol has been examined in BAC transgenic mice over-expressing DARPP-32 with different epitope tags in D1R- or D2R-expressing MSNs (Bateup et al., 2008). In these animals, tag-specific immunoprecipitation of DARPP-32 indicates that the increase in Thr34 phosphorylation induced by haloperidol occurs specifically in the D2R-expressing MSNs of the indirect pathway (Bateup et al., 2008).

Other studies examined the role of DARPP-32 in the changes of motor activity produced by haloperidol. Blockade of D2Rs results in a state of rigid immobility, or catalepsy, which represents a surrogate marker of EPS. This form of motor depression is thought to reflect the ability of haloperidol and other D2R antagonists to activate indirect MSNs, through removal of the inhibition exerted on these neurons by D2Rs (cf. above; Albin et al., 1989; Alexander and Crutcher, 1990; DeLong, 1990; Gerfen et al., 1990). Activation of the MSNs of the indirect pathway would lead, in turn, to inhibition of thalamo-cortical neurons (Albin et al., 1989; Alexander and Crutcher, 1990; DeLong, 1990; Gerfen et al., 1990) and reduction of motor cortex activity (Parr-Brownlie and Hyland, 2005). Deletion of DARPP-32 in indirect MSNs decreases haloperidol-induced catalepsy, whereas DARPP-32 inactivation in direct MSNs only slightly affects this behavioral response (Bateup et al., 2010). These findings indicate the importance of the PKA/DARPP-32 signaling pathway in haloperidol-induced motor depression and point to the MSNs of

the indirect pathway as a critical neuronal population involved in EPS.

REGULATION OF GluR1 PHOSPHORYLATION

Activation of the cAMP/PKA/DARPP-32 cascade increases the phosphorylation of the GluR1 subunit of the AMPA glutamate receptor (Banke et al., 2000; Snyder et al., 2000). Phosphorylation of GluR1 at the PKA site, Ser845, promotes glutamate transmission by increasing AMPA channel currents (Roche et al., 1996; Banke et al., 2000) and enhancing cell surface expression of the receptor (Mangiacavalli and Wolf, 2004). In the dopamine depleted striatum, L-DOPA enhances Ser845 phosphorylation and this effect has been associated to LID in both mice and monkeys (Santini et al., 2007, 2010). Indeed, GluR1 hyperphosphorylation may be causally linked to dyskinesia, which is currently treated with drugs that reduce glutamatergic transmission (Goetz et al., 2005).

GluR1 phosphorylation at Ser845 is also increased by administration of haloperidol (Håkansson et al., 2006). This effect depends on disinhibition of PKA in indirect MSNs, since it is abolished by pre-treatment with an A2AR antagonist (cf. above; Håkansson et al., 2006). In addition, haloperidol fails to phosphorylate GluR1 in DARPP-32 knock out mice, or in mice in which the PKA phosphorylation site of DARPP-32 (i.e., Thr34) is replaced with an alanine (T34A mutant mice; Håkansson et al., 2006). Thus, in indirect MSNs, haloperidol increases the phosphorylation of the AMPA glutamate receptor via activation of the cAMP/PKA/DARPP-32/PP-1 cascade. In view of the permissive role played by PKA-dependent phosphorylation of GluR1 on glutamatergic transmission (Roche et al., 1996; Banke et al., 2000; Mangiacavalli and Wolf, 2004), this effect of haloperidol is likely to promote the excitability of indirect MSNs, thereby leading to motor depression and, possibly, to the generation of EPS.

The activation of cAMP signaling produced by L-DOPA and haloperidol in direct and indirect MSNs is involved in the behavioral responses associated to these drugs. However, the increases in DARPP-32 and GluR1 phosphorylation discussed above are transient, whereas LID and EPS are most likely caused by long-term adaptive responses involving dysregulated gene expression.

DOWNSTREAM OF THE cAMP/DARPP-32 CASCADE I. EFFECTS OF L-DOPA IN DIRECT MSNs

Seminal studies carried out by Gerfen et al. (1990) in 6-OHDA-lesioned rats showed that, in the striatum, dopamine deafferentation decreased the transcription of mRNA coding for substance P, a neuropeptide specifically expressed in the direct MSNs. Conversely, the 6-OHDA lesion increased the levels of mRNA coding for the neuropeptide enkephalin, which is expressed in indirect MSNs (Gerfen et al., 1990). In addition to these changes, which reflect the opposite regulation exerted by dopamine on direct and indirect MSNs (cf. above), it was found that repeated administration of a D1R agonist increased mRNA for dynorphin, specifically in direct MSNs (Gerfen et al., 1990). Later studies in the same experimental model showed that a similar increase in dynorphin expression occurs also in response to chronic administration of L-DOPA and that this effect correlates with the severity of abnormal involuntary movements (i.e., dyskinesia) produced by this drug (Andersson et al., 1999). These observations prompted the

investigation of the mechanisms mediating the modifications in the expression of dynorphin and other genes potentially involved in LID.

The extracellular signal-regulated protein kinases 1 and 2 (ERK) have emerged as key signaling components involved in the control of gene expression and synaptic plasticity (Thomas and Huganir, 2004). In the striatum, ERK signaling has been implicated in the actions of addictive drugs and in corticostriatal long-term potentiation (Valjent et al., 2000, 2005; Xie et al., 2009). Stimulation of the D1R/PKA/DARPP-32 cascade leads to the phosphorylation and activation of ERK (Valjent et al., 2005; Santini et al., 2007; but see also Gerfen et al., 2008), which controls a variety of downstream effector proteins in the nucleus and cytoplasm (Thomas and Huganir, 2004). In the dopamine depleted striatum, activation of D1Rs or administration of L-DOPA increase the phosphorylation of ERK and its nuclear target, mitogen- and stress-activated kinase 1 (MSK1; Gerfen et al., 2002; Pavon et al., 2006; Santini et al., 2007, 2009; Westin et al., 2007). This, in turn, leads to phosphorylation of histone H3 at Ser10 (Santini et al., 2007, 2009; Darmopil et al., 2009), which is thought to promote transcriptional activation (Nowak and Corces, 2004).

Evidence obtained in rodent and monkey models show that activation of ERK signaling is implicated in LID (Santini et al., 2007; Schuster et al., 2008; Fasano et al., 2010). Studies in BAC transgenic mice expressing enhanced green fluorescent protein (EGFP) under the control of the promoter for either the D1R (*drd1a*-EGFP mice) or the D2R (*drd2*-EGFP mice; Santini et al., 2009) indicate that activation of the ERK/MSK1/H3 cascade occurs selectively in the D1R-containing MSNs of the direct pathway (Santini et al., 2009). Therefore, it is likely that, in these neurons, abnormal activation of PKA/DARPP-32 and ERK signaling in response to L-DOPA promotes transcriptional responses ultimately responsible for the development of dyskinesia.

Chronic administration of cocaine, a drug which promotes dopaminergic transmission, leads to the accumulation of stable, alternatively spliced isoforms of the transcription factor FosB, generally denominated as Δ FosB (Hope et al., 1994; Nestler et al., 2001). Enhanced Δ FosB levels have been also observed in the striata of rodent and non-human primate models of PD following chronic administration of L-DOPA (Andersson et al., 1999; Pavon et al., 2006; Berton et al., 2009; Fasano et al., 2010), as well as in postmortem samples from parkinsonian patients treated with L-DOPA (Tekumalla et al., 2001).

Interestingly, the ability of L-DOPA to increase Δ FosB expression correlates with the emergence of dyskinesia (Doucet et al., 1996; Andersson et al., 1999; Pavon et al., 2006; Berton et al., 2009; Fasano et al., 2010). This effect is produced in response to activation of D1Rs (Doucet et al., 1996; Darmopil et al., 2009) and is restricted to the MSNs of the direct pathway (Andersson et al., 1999; Darmopil et al., 2009), where activation of ERK is also occurring (Darmopil et al., 2009; Santini et al., 2009). Thus, in mice and monkeys, the increase in Δ FosB associated to LID is abolished by inactivation of Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1), a brain specific component of the Ras-ERK signaling cascade (Fasano et al., 2010).

Several lines of evidence indicate that overexpression of Δ FosB is implicated in LID. Experiments performed in 6-OHDA-lesioned rats showed that downregulation of Δ FosB, achieved by striatal injection of a *fosB* antisense oligonucleotide, decreases L-DOPA-induced abnormal involuntary movements (Andersson et al., 1999). A similar effect has been recently observed in the macaque following viral-induced overexpression of Δ JunD, a dominant negative of Δ FosB (Berton et al., 2009; Cao et al., 2010). Furthermore, in the rat, LID is reduced or exacerbated by overexpression of Δ JunD and Δ FosB, respectively (Cao et al., 2010).

Taken together, the studies described above indicate that LID is produced by sensitized D1R transmission, which leads to cAMP-dependent activation of ERK/MSK1 signaling. This, in turn, results in enhanced phosphorylation of histone H3 at Ser10 and increased expression of the transcription factor Δ FosB (Figure 1). One important task for future studies will be the identification of specific sets of genes regulated by this intracellular pathway. In this regard it is interesting to note that the increase in dynorphin associated to LID has been shown to depend on the ability of chronic L-DOPA to induce Δ FosB (Andersson et al., 1999).

DOWNSTREAM OF THE cAMP/DARPP-32 CASCADE II. EFFECTS OF HALOPERIDOL IN INDIRECT MSNs

As discussed above, the changes in gene expression associated to LID are primarily linked to the ability of L-DOPA to act on sensitized D1Rs, leading to sequential activation of PKA/DARPP-32 and ERK signaling (Pavon et al., 2006; Santini et al., 2007, 2008; Westin et al., 2007; Lebel et al., 2010). Administration of haloperidol and other D2R antagonists is also known to induce the expression of several immediate early genes acting as transcription factors, including *c-fos*, *fosB*, and *zif268* (Dragunow et al., 1990; Miller, 1990; Robertson and Fibiger, 1992; MacGibbon et al., 1994, 1995). This effect is particularly evident in the dorsal striatum and has been correlated to the propensity of antipsychotic drugs to induce EPS (Robertson and Fibiger, 1992; MacGibbon et al., 1994).

The increase in *c-fos* expression produced by haloperidol occurs selectively in the enkephalin-positive MSNs of the indirect pathway (Robertson et al., 1992). Recent work in *drd1a*- and *drd2*-EGFP mice has confirmed this initial observation, showing that haloperidol increases c-Fos and Zif268 specifically in D2R-expressing MSNs (Bertran-Gonzalez et al., 2008).

The control exerted by haloperidol on gene expression depends on the ability of this drug to promote cAMP signaling by antagonizing D2R-mediated transmission (cf. above). In support of this idea, it has been shown that genetic inactivation of the regulatory II β subunit of PKA, which is highly expressed in the striatum (Cadd and McKnight, 1989), abolishes the effect of haloperidol on *c-fos* expression (Adams et al., 1997). In line with this observation, intra-striatal injection of antisense oligonucleotides against the cAMP-response element binding protein, a transcription factor activated by PKA, prevents haloperidol-mediated induction of c-Fos protein (Konradi and Heckers, 1995). Other studies showed that the increase in enkephalin mRNA produced by acute and chronic administration of haloperidol is reduced in the absence of A2ARs, which are necessary to maintain normal cAMP signaling in the striatal MSNs of the indirect pathway (cf. above; Chen et al., 2001). Interestingly, the ability of haloperidol to induce catalepsy

is also attenuated in both PKA- and A2AR-deficient mice (Adams et al., 1997; Chen et al., 2001), suggesting that reduced cAMP signaling in indirect MSNs may attenuate EPS.

The ability of haloperidol to promote cAMP/DARPP-32 signaling is accompanied by increased ERK phosphorylation in a small number of MSNs belonging to the indirect pathway (Bertran-Gonzalez et al., 2008, 2009). This modest effect contrasts with the relatively large increase in ERK phosphorylation detected in striatal tissue by western blotting (Pozzi et al., 2003). In fact, a considerable proportion of the increase in ERK phosphorylation produced by haloperidol is confined to the terminals of midbrain dopaminergic neurons. In support of this possibility it has been shown that, in the striatum, haloperidol increases ERK-dependent phosphorylation of tyrosine hydroxylase, whose localization is restricted to dopaminergic fibers (Håkansson et al., 2004). This effect may be secondary to the block exerted by haloperidol on inhibitory dopamine D2 autoreceptors located on cell bodies and terminals of nigrostriatal dopaminergic neurons (Håkansson et al., 2004; Fisone et al., 2007).

Administration of haloperidol results in a large increase in phosphorylation of histone H3 at Ser10, which occurs in the D2R-expressing MSNs of the indirect pathway (Bertran-Gonzalez et al., 2008, 2009). This effect differs from the more restricted and modest increase in ERK phosphorylation (cf. above; Bertran-Gonzalez et al., 2008, 2009), suggesting that ERK signaling is not involved in the phosphorylation of histone H3 produced by blockade of D2Rs. This idea is confirmed by the observation that neither pharmacological inhibition of ERK, nor genetic inactivation of MSK1, affects haloperidol-induced phosphorylation of histone H3 (Bertran-Gonzalez et al., 2009).

Several lines of evidence indicate that the ability of D2R antagonists, including antipsychotic drugs, to promote histone H3 phosphorylation at Ser10 depends exclusively on activation of the cAMP/DARPP-32 cascade. For instance, the ability of haloperidol to increase the levels of phospho-Ser10-Lys14-acetylated histone H3 is prevented by blockade of PKA (Li et al., 2004). A similar effect has been observed following genetic attenuation of the G_{olf} protein, which couples A2ARs to activation of adenylyl cyclase (Kull et al., 2000; Herve et al., 2001), or pharmacological blockade of A2ARs (Bertran-Gonzalez et al., 2009). Finally, the ability of haloperidol to regulate histone H3 also requires PKA-dependent activation and nuclear translocation of DARPP-32 (Stipanovich et al., 2008; Bertran-Gonzalez et al., 2009). Thus, in striatopallidal MSNs, the PKA/DARPP-32 signaling pathway regulates histone H3 phosphorylation independently of the ERK/MSK1 cascade, which is generally thought to be critically involved in the generation of the nucleosomal response (Thomson et al., 1999; **Figure 1**).

CONCLUSION

The advent of sophisticated methodological approaches based on the use of BAC transgenic mice (Heintz, 2001) and optogenetics (Deisseroth, 2011) has allowed a refined analysis of the circuitries and mechanisms involved in a multitude of physiopathological conditions. In this regard, the striatal MSNs of the direct and indirect pathway have represented a particularly interesting subject of study. These neurons are the primary target of medications used

in the treatment of PD and schizophrenia and are distinguishable based on the organization of their connectivity within the basal ganglia and of their ability to express distinct sets of neurotransmitter receptors (Albin et al., 1989; Gerfen et al., 1990). This latter feature has made it possible to generate transgenic mice in which the physiological properties of direct and indirect MSNs, as well as their ability to respond to a variety of psychoactive substances, can be examined by cell-specific genetic manipulations (Gong et al., 2003, 2007; Valjent et al., 2009).

This review focused on the changes in signal transduction produced in striatal MSNs by L-DOPA and haloperidol, with particular emphasis on the potential involvement of these changes in the movement disorders caused by prolonged use of these drugs. The emerging picture indicates that LID and EPS are caused by dysregulation of cAMP signaling in direct and indirect MSNs, respectively. In line with this idea, increased activity of PKA and DARPP-32 in these two groups of neurons has been implicated in L-DOPA- and haloperidol-induced motor disorders (Santini et al., 2007; Bateup et al., 2010). The activation of the PKA/DARPP-32 signaling cascade produced by administration of L-DOPA and typical antipsychotic drugs leads to chromatin modifications (e.g., histone H3 phosphorylation at Ser10) and to the expression of several immediate early genes acting as transcription factors (e.g., *fos-c*, *fosB/ΔfosB*, and *zif268*; **Figure 1**). Future studies will be necessary to examine the relative contribution of these various responses to the development and manifestation of LID and EPS. It will also be essential to identify molecular targets regulated by this complex transcriptional machinery and evaluate their possible involvement in aberrant motor behaviors.

An intriguing point emerging from recent studies concerns the existence of distinct mechanisms by which L-DOPA and haloperidol promote nucleosomal response and gene expression in striatal MSNs. In the case of L-DOPA, these effects require D1R/PKA/DARPP-32-mediated activation of ERK/MSK1 signaling. Thus, pharmacological or genetic inactivation of ERK prevents the increase in gene expression and the phosphorylation of histone H3 induced by L-DOPA and associated to LID (Santini et al., 2007; Fasano et al., 2010). In contrast, the ability of haloperidol to modify histone H3 and gene expression does not appear to involve activation of ERK signaling. This is indicated by the modest effect on ERK phosphorylation produced by haloperidol in striatal MSNs and, most importantly, by the observation that genetic inactivation of MSK1 does not affect the ability of haloperidol to phosphorylate histone H3 (Bertran-Gonzalez et al., 2009).

Taken together, the above observations indicate that cAMP-dependent regulation of long-term responses in direct and indirect MSNs engages, at least in part, different signaling pathways (**Figure 1**). This distinction may be exploited to design therapeutic approaches tailored to counteract motor disturbances produced by dysfunctional signaling in one or the other neuronal population. For instance, drugs interfering with ERK signaling may be particularly suited for the treatment of LID and less efficacious to counteract EPS, since haloperidol produces only a modest activation of the ERK/MSK1 cascade in indirect MSNs. Indeed, it has been shown that administration of ERK inhibitors, or genetic

inactivation of Ras-GRF1, reduce LID in animal models of PD (Santini et al., 2007; Fasano et al., 2010). Further work on the characterization of signal transduction in direct and indirect MSNs will open new vistas on the functioning of the striatum in physiological and pathological conditions and provide information for the treatment of diseases caused by altered transmission in this brain region.

REFERENCES

- Adams, M. R., Brandon, E. P., Chartoff, E. H., Idzerda, R. L., Dorsa, D. M., and McKnight, G. S. (1997). Loss of haloperidol induced gene expression and catalepsy in protein kinase A-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 94, 12157–12161.
- Albin, R. L., Young, A. B., and Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends Neurosci.* 12, 366–375.
- Alexander, G. E., and Crutcher, M. D. (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci.* 13, 266–271.
- Andersson, M., Hilbertson, A., and Cenci, M. A. (1999). Striatal fosB expression is causally linked with L-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. *Neurobiol. Dis.* 6, 461–474.
- Banke, T. G., Bowie, D., Lee, H., Huganir, R. L., Schousboe, A., and Traynelis, S. F. (2000). Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. *J. Neurosci.* 20, 89–102.
- Bateup, H. S., Santini, E., Shen, W., Birnbaum, S., Valjent, E., Surmeier, D. J., Fisone, G., Nestler, E. J., and Greengard, P. (2010). Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14845–14850.
- Bateup, H. S., Svenningsson, P., Kuroiwa, M., Gong, S., Nishi, A., Heintz, N., and Greengard, P. (2008). Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. *Nat. Neurosci.* 11, 932–939.
- Berton, O., Guigoni, C., Li, Q., Bioulac, B. H., Aubert, I., Gross, C. E., Dileone, R. J., Nestler, E. J., and Bezaud, E. (2009). Striatal overexpression of DeltaJunD resets L-DOPA-induced dyskinesia in a primate model of Parkinson disease. *Biol. Psychiatry* 66, 554–561.
- Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamalas, M., Herve, D., Valjent, E., and Girault, J. A. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J. Neurosci.* 28, 5671–5685.
- Bertran-Gonzalez, J., Hakansson, K., Borgkvist, A., Irinopoulou, T., Brami-Cherrier, K., Usiello, A., Greengard, P., Herve, D., Girault, J. A., Valjent, E., and Fisone, G. (2009). Histone H3 phosphorylation is under the opposite tonic control of dopamine D2 and adenosine A2A receptors in striatopallidal neurons. *Neuropsychopharmacology* 34, 1710–1720.
- Birkmayer, W., and Hornykiewicz, O. (1998). The effect of L-3,4-dihydroxyphenylalanine (=DOPA) on akinesia in parkinsonism. *Parkinsonism Relat. Disord.* 4, 59–60.
- Blank, T., Nijholt, I., Teichert, U., Kugler, H., Behrsing, H., Fienberg, A., Greengard, P., and Spiess, J. (1997). The phosphoprotein DARPP-32 mediates cAMP-dependent potentiation of striatal N-methyl-D-aspartate responses. *Proc. Natl. Acad. Sci. U.S.A.* 94, 14859–14864.
- Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8, 1263–1268.
- Braak, H., Del Tredici, K., Rub, U., De Vos, R. A., Jansen Steur, E. N., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24, 197–211.
- Cadd, G., and McKnight, G. S. (1989). Distinct patterns of cAMP-dependent protein kinase gene expression in mouse brain. *Neuron* 3, 71–79.
- Cao, X., Yasuda, T., Uthayathas, S., Watts, R. L., Mouradian, M. M., Mochizuki, H., and Papa, S. M. (2010). Striatal overexpression of DeltaFosB reproduces chronic levodopa-induced involuntary movements. *J. Neurosci.* 30, 7335–7343.
- Carta, A. R., Tronci, E., Pinna, A., and Morelli, M. (2005). Different responsiveness of striatonigral and striatopallidal neurons to L-DOPA after a subchronic intermittent L-DOPA treatment. *Eur. J. Neurosci.* 21, 1196–1204.
- Cenci, A. M., Whishaw, I. Q., and Schallert, T. (2002). Animal models of neurological deficits: how relevant is the rat? *Nat. Rev. Neurosci.* 3, 574–579.
- Chen, J.-F., Moratalla, R., Impagnatiello, E., Grandy, D. K., Cuellar, B., Rubinstein, M., Beilstein, M. A., Hackett, E., Fink, S. J., Low, M. J., Ongini, E., and Schwarzschild, M. A. (2001). The role of the D2 dopamine receptor (D2R) in A2A adenosine receptor (A2A R)-mediated behavioral and cellular responses as revealed by A2A and D2 receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1970–1975.
- Cotzias, G. C., Van Woert, M. H., and Schiffer, L. M. (1967). Aromatic amino acids and modification of parkinsonism. *N. Engl. J. Med.* 276, 374–379.
- Creese, I., Burt, D. R., and Snyder, S. H. (1976). Dopamine receptor binding predicts clinical and pharmacological potencies of anti-schizophrenic drugs. *Science* 192, 481–483.
- Darmopil, S., Martin, A. B., De Diego, I. R., Ares, S., and Moratalla, R. (2009). Genetic inactivation of dopamine D1 but not D2 receptors inhibits L-DOPA-induced dyskinesia and histone activation. *Biol. Psychiatry* 66, 603–613.
- Deisseroth, K. (2011). Optogenetics. *Nat. Methods* 8, 26–29.
- DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends Neurosci.* 13, 281–285.
- Doucet, J. P., Nakabeppu, Y., Bedard, P. J., Hope, B. T., Nestler, E. J., Jasmin, B. J., Chen, J. S., Iadarola, M. J., St-Jean, M., Wigle, N., Blanchet, P., Grondin, R., and Robertson, G. S. (1996). Chronic alterations in dopaminergic neurotransmission produce a persistent elevation of deltaFosB-like protein(s) in both the rodent and primate striatum. *Eur. J. Neurosci.* 8, 365–381.
- Dragunow, M., Robertson, G. S., Faull, R. L., Robertson, H. A., and Jansen, K. (1990). D2 dopamine receptor antagonists induce fos and related proteins in rat striatal neurons. *Neuroscience* 37, 287–294.
- Durieux, P. F., Bearzatto, B., Guiducci, S., Buch, T., Waisman, A., Zoli, M., Schiffmann, S. N., and De Kerchove d'Exaerde, A. (2009). D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat. Neurosci.* 12, 393–395.
- Fasano, S., Bezard, E., D'Antoni, A., Francardo, V., Indrigo, M., Qin, L., Dovero, S., Ceric, M., Cenci, M. A., and Brambilla, R. (2010). Inhibition of Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) signaling in the striatum reverts motor symptoms associated with L-dopa-induced dyskinesia. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21824–21829.
- Fienberg, A. A., Hiroi, N., Mermelstein, P. G., Song, W., Snyder, G. L., Nishi, A., Cheramy, A., O'Callaghan, J. P., Miller, D. B., Cole, D. G., Corbett, R., Haile, C. N., Cooper, D. C., Onn, S. P., Grace, A. A., Ouimet, C. C., White, F. J., Hyman, S. E., Surmeier, D. J., Girault, J., Nestler, E. J., and Greengard, P. (1998). DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. *Science* 281, 838–842.
- Fink, J. S., Weaver, D. R., Rivkees, S. A., Peterfreund, R. A., Pollack, A. E., Adler, E. M., and Reppert, S. M. (1992). Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptor in rat striatum. *Mol. Brain Res.* 14, 186–195.
- Fisone, G., Hakansson, K., Borgkvist, A., and Santini, E. (2007). Signaling in the basal ganglia: postsynaptic and presynaptic mechanisms. *Physiol. Behav.* 92, 8–14.
- Galarraga, E., Hernández-López, S., Reyes, A., Barral, J., and Bargas, J. (1997). Dopamine facilitates striatal EPSPs through an L-type Ca²⁺ conductance. *Neuroreport* 8, 2183–2186.
- Gao, T., Yatani, A., Dell'Acqua, M. L., Sako, H., Green, S. A., Dascal, N., Scott, J. D., and Hosey, M. M. (1997). cAMP-dependent regulation of cardiac L-type Ca²⁺ channels requires membrane targeting of PKA and

ACKNOWLEDGMENTS

Gilberto Fisone was supported by Swedish Research Council grant 13482 and by the Swedish Brain Foundation. Alessandra Bonito-Oliva was supported by a fellowship from the Foundation Blanceflor Boncompagni-Ludovisi, née Bildt. Michael Feyder was supported by the National Institutes of Health – Karolinska Institutet Graduate Training Partnership Program.

- phosphorylation of channel subunits. *Neuron* 19, 185–196.
- Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J. Jr., and Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250, 1429–1432.
- Gerfen, C. R., Miyachi, S., Paletzki, R., and Brown, P. (2002). D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. *J. Neurosci.* 22, 5042–5054.
- Gerfen, C. R., Paletzki, R., and Worley, P. (2008). Differences between dorsal and ventral striatum in Drd1a dopamine receptor coupling of dopamine- and cAMP-regulated phosphoprotein-32 to activation of extracellular signal-regulated kinase. *J. Neurosci.* 28, 7113–7120.
- Glatt, C. E., and Snyder, S. H. (1993). Cloning and expression of an adenylyl cyclase localized to the corpus striatum. *Nature* 361, 536–538.
- Goetz, C. G., Poewe, W., Rascol, O., and Sampaio, C. (2005). Evidence-based medical review update: pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. *Mov. Disord.* 20, 523–539.
- Gong, S., Doughty, M., Harbaugh, C. R., Cummins, A., Hatten, M. E., Heintz, N., and Gerfen, C. R. (2007). Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J. Neurosci.* 27, 9817–9823.
- Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E., and Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425, 917–925.
- Greengard, P. (2001). The neurobiology of slow synaptic transmission. *Science* 294, 1024–1030.
- Greif, G. J., Lin, Y. J., Liu, J. C., and Freedman, J. E. (1995). Dopamine-modulated potassium channels on rat striatal neurons: specific activation and cellular expression. *J. Neurosci.* 15, 4533–4544.
- Guigoni, C., Aubert, I., Li, Q., Gurevich, V. V., Benovic, J. L., Ferry, S., Mach, U., Stark, H., Leriche, L., Hakansson, K., Bioulac, B. H., Gross, C. E., Sokoloff, P., Fisone, G., Gurevich, E. V., Bloch, B., and Bezard, E. (2005). Pathogenesis of levodopa-induced dyskinesia: focus on D1 and D3 dopamine receptors. *Parkinsonism Relat. Disord.* 11(Suppl. 1), S25–S29.
- Håkansson, K., Galdi, S., Hendrick, J., Snyder, G., Greengard, P., and Fisone, G. (2006). Regulation of phosphorylation of the GluR1 AMPA receptor by dopamine D2 receptors. *J. Neurochem.* 96, 482–488.
- Håkansson, K., Pozzi, L., Usiello, A., Haycock, J., Borrelli, E., and Fisone, G. (2004). Regulation of striatal tyrosine hydroxylase phosphorylation by acute and chronic haloperidol. *Eur. J. Neurosci.* 20, 1108–1112.
- Heintz, N. (2001). BAC to the future: the use of bac transgenic mice for neuroscience research. *Nat. Rev. Neurosci.* 2, 861–870.
- Hemmings, H. C. Jr., Greengard, P., Tung, H. Y., and Cohen, P. (1984). DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. *Nature* 310, 503–505.
- Hernandez-Lopez, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H., and Surmeier, D. J. (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca²⁺ currents and excitability via a novel PLC[β]/IP3-calcineurin-signaling cascade. *J. Neurosci.* 20, 8987–8995.
- Herve, D., Le Moine, C., Corvol, J. C., Belluscio, L., Ledent, C., Fienberg, A. A., Jaber, M., Studler, J. M., and Girault, J. A. (2001). α 1(olf) levels are regulated by receptor usage and control dopamine and adenosine action in the striatum. *J. Neurosci.* 21, 4390–4399.
- Hope, B. T., Nye, H. E., Kelz, M. B., Self, D. W., Iadarola, M. J., Nakabeppu, Y., Duman, R. S., and Nestler, E. J. (1994). Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* 13, 1235–1244.
- Hopf, F. W., Cascini, M. G., Gordon, A. S., Diamond, I., and Bonci, A. (2003). Cooperative activation of dopamine D1 and D2 receptors increases spike firing of nucleus accumbens neurons via G-protein betagamma subunits. *J. Neurosci.* 23, 5079–5087.
- Hornykiewicz, O. (1963). Die topische Lokalisation und das Verhalten von Noradrenalin und Dopamin (3-Hydroxytyramin) in der Substantia nigra des normalen und Parkinsonkranken Menschen. *Wien. Klin. Wochenschr.* 56, 426–427.
- Kebabian, J. W., and Calne, D. B. (1979). Multiple receptors for dopamine. *Nature* 277, 93–96.
- Kitai, S. T., and Surmeier, D. J. (1993). Cholinergic and dopaminergic modulation of potassium conductances in neostriatal neurons. *Adv. Neurol.* 60, 40–52.
- Konradi, C., and Heckers, S. (1995). Haloperidol-induced Fos expression in striatum is dependent upon transcription factor cyclic AMP response element binding protein. *Neuroscience* 65, 1051–1061.
- Kravitz, A. V., Freeze, B. S., Parker, P. R., Kay, K., Thwin, M. T., Deisseroth, K., and Kreitzer, A. C. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature* 466, 622–626.
- Kull, B., Svenningsson, P., and Fredholm, B. B. (2000). Adenosine A(2A) receptors are colocalized with and activate g(olf) in rat striatum. *Mol. Pharmacol.* 58, 771–777.
- Kuzhikandathil, E. V., Yu, W., and Oxford, G. S. (1998). Human dopamine D3 and D2L receptors couple to inward rectifier potassium channels in mammalian cell lines. *Mol. Cell. Neurosci.* 12, 390–402.
- Lebel, M., Chagniel, L., Bureau, G., and Cyr, M. (2010). Striatal inhibition of PKA prevents levodopa-induced behavioural and molecular changes in the hemiparkinsonian rat. *Neurobiol. Dis.* 38, 59–67.
- Li, J., Guo, Y., Schroeder, F. A., Youngs, R. M., Schmidt, T. W., Ferris, C., Konradi, C., and Akbarian, S. (2004). Dopamine D2-like antagonists induce chromatin remodeling in striatal neurons through cyclic AMP-protein kinase A and NMDA receptor signaling. *J. Neurochem.* 90, 1117–1131.
- Liu, J. C., Defazio, R. A., Espinosa-Jeffrey, A., Cepeda, C., De Vellis, J., and Levine, M. S. (2004). Calcium modulates dopamine potentiation of N-methyl-D-aspartate responses: electrophysiological and imaging evidence. *J. Neurosci. Res.* 76, 315–322.
- MacGibbon, G. A., Lawlor, P. A., Bravo, R., and Dragunow, M. (1994). Clozapine and haloperidol produce a differential pattern of immediate early gene expression in rat caudate-putamen, nucleus accumbens, lateral septum and islands of Calleja. *Brain Res. Mol. Brain Res.* 23, 21–32.
- MacGibbon, G. A., Lawlor, P. A., Hughes, P., Young, D., and Dragunow, M. (1995). Differential expression of inducible transcription factors in basal ganglia neurons. *Brain Res. Mol. Brain Res.* 34, 294–302.
- Mangiavacchi, S., and Wolf, M. E. (2004). D1 dopamine receptor stimulation increases the rate of AMPA receptor insertion onto the surface of cultured nucleus accumbens neurons through a pathway dependent on protein kinase A. *J. Neurochem.* 88, 1261–1271.
- Mattingly, R. R. (1999). Phosphorylation of serine 916 of Ras-GRF1 contributes to the activation of exchange factor activity by muscarinic receptors. *J. Biol. Chem.* 274, 37379–37384.
- Miller, J. C. (1990). Induction of c-fos mRNA expression in rat striatum by neuroleptic drugs. *J. Neurochem.* 54, 1453–1455.
- Miyamoto, S., Duncan, G. E., Marx, C. E., and Lieberman, J. A. (2005). Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol. Psychiatry* 10, 79–104.
- Mons, N., and Cooper, D. M. (1994). Selective expression of one Ca(2+)-inhibitable adenylyl cyclase in dopaminergically innervated rat brain regions. *Brain Res. Mol. Brain Res.* 22, 236–244.
- Nestler, E. J., Barrot, M., and Self, D. W. (2001). DeltaFosB: a sustained molecular switch for addiction. *Proc. Natl. Acad. Sci. U.S.A.* 98, 11042–11046.
- Nishi, A., Snyder, G. L., and Greengard, P. (1997). Bidirectional regulation of DARPP-32 phosphorylation by dopamine. *J. Neurosci.* 17, 8147–8155.
- Nowak, S. J., and Corces, V. G. (2004). Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. *Trends Genet.* 20, 214–220.
- Obeso, J. A., Olanow, C. W., and Nutt, J. G. (2000). Levodopa motor complications in Parkinson's disease. *Trends Neurosci.* 23, S2–S7.
- Parr-Brownlie, L. C., and Hyland, B. I. (2005). Bradykinesia induced by dopamine D2 receptor blockade is associated with reduced motor cortex activity. *J. Neurosci.* 25, 5700–5709.
- Pavon, N., Martin, A. B., Mendiola, A., and Moratalla, R. (2006). ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. *Biol. Psychiatry* 59, 64–74.

- Picconi, B., Centonze, D., Hakanson, K., Bernardi, G., Greengard, P., Fisone, G., Cenci, M. A., and Calabresi, P. (2003). Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. *Nat. Neurosci.* 6, 501–506.
- Pozzi, L., Hakanson, K., Usiello, A., Borgkvist, A., Lindsog, M., Greengard, P., and Fisone, G. (2003). Opposite regulation by typical and atypical anti-psychotics of ERK1/2, CREB and Elk-1 phosphorylation in mouse dorsal striatum. *J. Neurochem.* 86, 451–459.
- Robertson, G. S., and Fibiger, H. C. (1992). Neuroleptics increase c-fos expression in the forebrain: contrasting effects of haloperidol and clozapine. *Neuroscience* 46, 315–328.
- Robertson, G. S., Vincent, S. R., and Fibiger, H. C. (1992). D1 and D2 dopamine receptors differentially regulate c-fos expression in striatonigral and striatopallidal neurons. *Neuroscience* 49, 285–296.
- Robinson, S. W., and Caron, M. G. (1997). Selective inhibition of adenylyl cyclase type V by the dopamine D3 receptor. *Mol. Pharmacol.* 52, 508–514.
- Roche, K. W., O'Brien, R. J., Mammen, A. L., Bernhardt, J., and Haganir, R. L. (1996). Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* 16, 1179–1188.
- Santini, E., Alcaer, C., Cacciato, S., Heiman, M., Herve, D., Greengard, P., Girault, J. A., Valjent, E., and Fisone, G. (2009). L-DOPA activates ERK signaling and phosphorylates histone H3 in the striatonigral medium spiny neurons of hemiparkinsonian mice. *J. Neurochem.* 108, 621–633.
- Santini, E., Sgambato-Faure, V., Li, Q., Savasta, M., Dovero, S., Fisone, G., and Bezdard, E. (2010). Distinct changes in cAMP and extracellular signal-regulated protein kinase signalling in L-DOPA-induced dyskinesia. *PLoS ONE* 5, e12322. doi: 10.1371/journal.pone.0012322
- Santini, E., Valjent, E., and Fisone, G. (2008). Parkinson's disease: levodopa-induced dyskinesia and signal transduction. *FEBS J.* 275, 1392–1399.
- Santini, E., Valjent, E., Usiello, A., Carta, M., Borgkvist, A., Girault, J. A., Herve, D., Greengard, P., and Fisone, G. (2007). Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *J. Neurosci.* 27, 6995–7005.
- Schiffmann, S. N., Jacobs, O., and Vanderhaeghe, J. J. (1991). Striatum restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an in situ hybridization histochemistry study. *J. Neurochem.* 57, 1062–1067.
- Schuster, S., Nadjar, A., Guo, J. T., Li, Q., Itrich, C., Hengerer, B., and Bezdard, E. (2008). The 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor lovastatin reduces severity of L-DOPA-induced abnormal involuntary movements in experimental Parkinson's disease. *J. Neurosci.* 28, 4311–4316.
- Seeman, P., Lee, T., Chau-Wong, M., and Wong, K. (1976). Anti-psychotic drug doses and neuroleptic/dopamine receptors. *Nature* 261, 717–719.
- Snyder, G. L., Allen, P. B., Fienberg, A. A., Valle, C. G., Haganir, R. L., Nairn, A. C., and Greengard, P. (2000). Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. *J. Neurosci.* 20, 4480–4488.
- Snyder, G. L., Fienberg, A. A., Haganir, R. L., and Greengard, P. (1998). A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. *J. Neurosci.* 18, 10297–10303.
- Stipanovich, A., Valjent, E., Matamalas, M., Nishi, A., Ahn, J. H., Maroteaux, M., Bertran-Gonzalez, J., Brami-Cherrier, K., Enslen, H., Corbille, A. G., Filhol, O., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2008). A phosphatase cascade by which rewarding stimuli control nucleosomal response. *Nature* 453, 879–884.
- Stoof, J. C., and Kebabian, J. W. (1981). Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature* 294, 366–368.
- Surmeier, D. J., Bargas, J., Hemmings, H. C. Jr., Nairn, A. C., and Greengard, P. (1995). Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* 14, 385–397.
- Surmeier, D. J., Ding, J., Day, M., Wang, Z., and Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci.* 30, 228–235.
- Svenningsson, P., Lindsog, M., Ledent, C., Parmentier, M., Greengard, P., Fredholm, B. B., and Fisone, G. (2000). Regulation of the phosphorylation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa in vivo by dopamine D1, dopamine D2, and adenosine A2A receptors. *Proc. Natl. Acad. Sci. U.S.A.* 97, 1856–1860.
- Svenningsson, P., Lindsog, M., Rognoni, F., Fredholm, B. B., Greengard, P., and Fisone, G. (1998). Activation of adenosine A2A and dopamine D1 receptors stimulates cyclic AMP-dependent phosphorylation of DARPP-32 in distinct populations of striatal projection neurons. *Neuroscience* 84, 223–228.
- Tekumalla, P. K., Calon, F., Rahman, Z., Birdi, S., Rajput, A. H., Hornykiewicz, O., Di Paolo, T., Bedard, P. J., and Nestler, E. J. (2001). Elevated levels of DeltaFosB and RGS9 in striatum in Parkinson's disease. *Biol. Psychiatry* 50, 813–816.
- Thomas, G. M., and Haganir, R. L. (2004). MAPK cascade signalling and synaptic plasticity. *Nat. Rev. Neurosci.* 5, 173–183.
- Thomson, S., Clayton, A. L., Hazzalin, C. A., Rose, S., Barratt, M. J., and Mahadevan, L. C. (1999). The nucleosomal response associated with immediate-early gene induction is mediated via alternative MAP kinase cascades: MSK1 as a potential histone H3/HMG-14 kinase. *EMBO J.* 18, 4779–4793.
- Valjent, E., Bertran-Gonzalez, J., Herve, D., Fisone, G., and Girault, J. A. (2009). Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci.* 32, 538–547.
- Valjent, E., Corvol, J. C., Pages, C., Besson, M. J., Maldonado, R., and Caboche, J. (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J. Neurosci.* 20, 8701–8709.
- Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., Stipanovich, A., Caboche, J., Lombroso, P. J., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 102, 491–496.
- Westin, J. E., Vercammen, L., Strome, E. M., Konradi, C., and Cenci, M. A. (2007). Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D1 receptors. *Biol. Psychiatry* 62, 800–810.
- Wickens, J. R., and Wilson, C. J. (1998). Regulation of action-potential firing in spiny neurons of the rat neostriatum in vivo. *J. Neurophysiol.* 79, 2358–2364.
- Wickman, K. D., Iniguez-Lluhl, J. A., Davenport, P. A., Taussig, R., Krapivinsky, G. B., Linder, M. E., Gilman, A. G., and Clapham, D. E. (1994). Recombinant G-protein beta gamma-subunits activate the muscarinic-gated atrial potassium channel. *Nature* 368, 255–257.
- Xie, G. Q., Wang, S. J., Li, J., Cui, S. Z., Zhou, R., Chen, L., and Yuan, X. R. (2009). Ethanol attenuates the HFS-induced, ERK-mediated LTP in a dose-dependent manner in rat striatum. *Alcohol. Clin. Exp. Res.* 33, 121–128.
- Zhuang, X., Belluscio, L., and Hen, R. (2000). G(olf)alpha mediates dopamine D1 receptor signaling. *J. Neurosci.* 20, RC91.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 11 May 2011; accepted: 27 June 2011; published online: 12 July 2011.

Citation: Bonito-Oliva A, Feyder M and Fisone G (2011) Deciphering the actions of antiparkinsonian and antipsychotic drugs on cAMP/DARPP-32 signaling. *Front. Neuroanat.* 5:38. doi: 10.3389/fnana.2011.00038

Copyright © 2011 Bonito-Oliva, Feyder and Fisone. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Huntington's disease and striatal signaling

Emmanuel Roze^{1,2}, Emma Cahill¹, Elodie Martin¹, Cecilia Bonnet³, Peter Vanhoutte¹, Sandrine Betuing¹ and Jocelyne Caboche^{1*}

¹ UMRS 952, INSERM, UMR 7224, CNRS Université Pierre et Marie Curie – Paris-6, Paris, France

² Pôle des Maladies du Système Nerveux, Département de Neurologie, Hôpital Pitié-Salpêtrière, Assistance publique-Hôpitaux de Paris, Paris, France

³ Department of Neurology, First Faculty of Medicine, Charles University in Prague and General University Hospital, Prague, Czech Republic

Edited by:

Emmanuel Valjent, Université Montpellier 1&2, France

Reviewed by:

Michelle E. Ehrlich, Mount Sinai School of Medicine, USA

Sandrine Humbert, Institut Curie-INSERM U1005-CNRS UMR3306, France

*Correspondence:

Jocelyne Caboche, Laboratoire de Physiopathologie des Maladies du Système Nerveux Central, Université Pierre et Marie Curie, 9 Quai Saint Bernard, 75005 Paris, France.
e-mail: jocelyne.caboche@snv.jussieu.fr

Huntington's Disease (HD) is the most frequent neurodegenerative disease caused by an expansion of polyglutamines (CAG). The main clinical manifestations of HD are chorea, cognitive impairment, and psychiatric disorders. The transmission of HD is autosomal dominant with a complete penetrance. HD has a single genetic cause, a well-defined neuropathology, and informative pre-manifest genetic testing of the disease is available. Striatal atrophy begins as early as 15 years before disease onset and continues throughout the period of manifest illness. Therefore, patients could theoretically benefit from therapy at early stages of the disease. One important characteristic of HD is the striatal vulnerability to neurodegeneration, despite similar expression of the protein in other brain areas. Aggregation of the mutated Huntingtin (HTT), impaired axonal transport, excitotoxicity, transcriptional dysregulation as well as mitochondrial dysfunction, and energy deficits, are all part of the cellular events that underlie neuronal dysfunction and striatal death. Among these non-exclusive mechanisms, an alteration of striatal signaling is thought to orchestrate the downstream events involved in the cascade of striatal dysfunction.

Keywords: polyglutamine, Huntingtin, excitotoxicity, mitochondrial dysfunctions, transcriptional deregulation

CLINICAL ASPECTS

Huntington's disease (HD) is a fatal disorder with a general prevalence of about 10 per 100,000 births, with some regions of the world having a higher prevalence of up to 700 per 100,000 (Harper, 1992; Paradisi et al., 2008). The typical age of onset is usually between 35 and 50 years but is very variable ranging from 1 to 85 years or more. The disease begins before 30 years of age in about 15% of patients and is then referred to as juvenile HD. The duration of the disease from onset to death is about 15–20 years. Clinical features of HD can be divided into three groups: movement disorders, cognitive impairment, and psychiatric manifestations. Chorea is the most characteristic movement disorder of classical HD and is characterized by brief, involuntary, abnormal movements, which appear unpredictably in all the parts of the body (Quinn and Schrag, 1998). As the disease progresses, the choreic movements generally tend to diminish and be replaced by akineto-rigid parkinsonism that can be associated with dystonic postures (Penney et al., 1990; Kremer et al., 1992). At an advanced stage of the disease, a high proportion of HD patients have falls (Busse et al., 2009), and a loss of independent ambulation, which may precipitate admission to nursing homes (Wheelock et al., 2003). Other movement disorders can be occasionally observed during the course of the disease, including tics (Kerbeshian et al., 1991; Jankovic et al., 1995) and myoclonia (Vogel et al., 1991; Carella et al., 1993; Thompson et al., 1994). Cognitive impairment plays a major role in the functional decline and loss of autonomy of the patients. It can precede motor symptoms or occur during the course of the disease, and usually leads, in turn, to dementia. Cognitive alteration has a sub-cortical profile with predominant impairment of executive/attention functions (Caine et al., 1978;

Bamford et al., 1995; Ho et al., 2003; Peinemann et al., 2005). Instrumental functions (language, praxia, and gnosis) and memory are generally better preserved in HD than in other types of dementia (Caine et al., 1977; Hodges et al., 1990; Pilon et al., 1993, 1994). Neurobehavioral symptoms are very frequent. They can be the initial manifestations of the disease or occur at any time during the course of the disease (Shiwach and Norbury, 1994; Kirkwood et al., 2002). Irritability, agitation, apathy, anxiety, social withdrawal, impulsiveness, alcohol abuse, obsessive-compulsive disorder (Cummings and Cunningham, 1992; Patzold and Brune, 2002), hostility, and sexual disorders are common (Pflanz et al., 1991; Fedoroff et al., 1994; Paulsen et al., 2001). Mood disorders are very frequent along HD, including depression (Caine and Shoulson, 1983; Folstein et al., 1983; Di Maio et al., 1993; Cummings, 1995; Jensen et al., 1998) and manic episodes (Mendez, 2000). Various types of psychotic disorders can also be observed (Cummings, 1995; Rosenblatt and Leroi, 2000). HD patients have a risk of suicide that is 10 times higher than in the general population. Sleep disorders and weight loss are also frequently encountered in HD patients (Morton et al., 2005; Arnulf et al., 2008; Aziz et al., 2008; Videnovic et al., 2009).

Contrary to patients with typical HD, those with the juvenile form (or the Westphal variant) do not display chorea. These forms are characterized by a combination of progressive akineto-rigid parkinsonism, dystonia, ataxia, dementia, and psychiatric disorders (Siesling et al., 1997; Gonzalez-Alegre and Afifi, 2006). Seizures can also occur. Patients with childhood-onset HD can develop non-specific encephalopathy resulting in seizures, myoclonus, and rapid cognitive deterioration (Siesling et al., 1997; Gambardella et al., 2001; Gonzalez-Alegre and Afifi, 2006).

GENETICS

The mutation responsible for HD is located at the 5' terminal part of the *HTT* gene on chromosome 4p16.3 (The Huntington Collaborative Research Group, 1993). The mutation consists of an unstable expansion of the CAG repeat sequence, located in exon 1, at the NH₂-terminal part of the protein. The mutated protein causes neuronal dysfunction and death, particularly in the striatum and cortex, although it is ubiquitously expressed. The penetrance of the mutation is almost complete. The *HTT* gene is normal when it contains less than 27 CAG repeats. Between 27 and 35 CAG repeats do not cause HD but may expand in successive generations. Intermediate alleles (between 36 and 39 repeats) repetitions are usually associated with late onset disease and may express a variable penetrance as the patient may die before disease onset. Individuals with 39 CAG repeats or greater will develop symptoms of HD (Kenney et al., 2007; Reynolds, 2008; Semaka et al., 2008). About 10% of HD patients have no family history of HD (Goldberg et al., 1993; Davis et al., 1994), with some of these patients receiving the mutant allele from an asymptomatic father with an intermediate allele. Such alleles do not cause HD but show instability on replication and tend to expand in successive generation with greater instability in spermatogenesis than in oogenesis (Zuhlke et al., 1993; Ranen et al., 1995). This instability of increased number of CAG repeats over successive generations explains the phenomenon of genetic anticipation, which is defined by the tendency of an earlier disease onset in successive generations (Goldberg et al., 1993; Myers et al., 1993; Alford et al., 1996). The age of onset cannot be predicted from the CAG repeat length in clinical practice. However, the number of repeats inversely correlates with the age of onset (Andrew et al., 1993; Duyao et al., 1993; Snell et al., 1993; Wexler et al., 2004; Andresen et al., 2007).

NEUROPATHOLOGY

Brain weight may be reduced by as much as 25–30% in advanced HD cases. Gross pathology of HD is limited to the brain, with atrophy predominating in the caudate–putamen and to a lesser extent, the cerebral cortex. The neuropathological signature of HD is the prominent striatal neuron loss and the presence of intranuclear inclusion bodies, which mainly consist of the accumulation of abnormal expansion of polyglutamines [Exp-Huntingtin (HTT)]. A grading system for the striatal neuropathology was established using macroscopic and microscopic criteria (Vonsattel's grade; Vonsattel et al., 1985). It defines five grades ranging from 0 to 4 with increasing severity. The grade correlates closely with the extent of clinical disability. The most vulnerable neuronal population is the medium spiny neurons (MSNs) of the striatum. According to the Vonsattel's grade, the striato-pallidal MSNs, which express enkephalin and dopaminergic D₂ receptors, degenerate first (grade 2). Then striato-nigral MSNs which express substance P and dopaminergic D₁ receptors degenerate (grade 3). The degeneration of MSNs occurs according to a dorso-ventral and medio-lateral gradient and is associated with a reduced expression of substance P, leu-enkephalin, calcineurin, calbindin, histamine H₂-receptors, dopamine receptors, cannabinoid receptors, and Adenosine A₂ receptors (Goto et al., 1989; Martinez-Mir et al., 1991; Richfield et al., 1991; Richfield and Herkenham, 1994). The striatal interneurons, aspiny striatal cholinergic, and

somatostatin containing neurons, are relatively spared (Lange et al., 1976; Dawbarn et al., 1985; Ferrante et al., 1985, 1987, 1991). Another characteristic neuropathological change is a modification of the dendritic arborization of spiny neurons, with an axonal retraction before cell death (Graveland et al., 1985; Kiechle et al., 2002).

MOLECULAR MECHANISMS OF THE DISEASE

Whether neuronal degeneration in HD is due to the loss of normal HTT properties or a gain of toxic functions, or both, is not fully elucidated. In addition, age-related “normal” alterations in cellular functioning may accelerate HD pathogenesis (Diguett et al., 2009). Importantly, HTT is required for normal embryonic development as the loss of the protein leads to lethality of mouse embryos around day 8.5 (Duyao et al., 1995; Zeitlin et al., 1995) and the selective knockdown of the protein in neurons and testis produces apoptosis in these tissues (Dragatsis et al., 2000). The wild-type HTT is a ubiquitous protein, expressed in most cells of the organism and within virtually all cellular compartments (DiFiglia et al., 1997; Gutekunst et al., 1999; Kegel et al., 2002, 2005; Hoffner et al., 2005; Caviston et al., 2007; Rockabrand et al., 2007; Strehlow et al., 2007). Cell-based assays have focused on striatal specific cell-autonomous effects of Exp-HTT on HD neuropathology, since striatal but not cortical neurons in culture, show spontaneous degeneration in presence of Exp-HTT (Saudou et al., 1998; Arrasate et al., 2004; Garcia et al., 2004). Evidence in favor of cell-autonomous effects also include that a lentiviral mediated delivery of Exp-HTT to rat striatum results in a progressive pathology characterized by the appearance of ubiquitinated HTT aggregates, loss of dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) staining, and cell death (De Almeida et al., 2002). Nevertheless, in a genetic model with striatal specific expression of Exp-HTT, cell-autonomous nuclear accumulation of Exp-HTT aggregates in striatal neurons were observed, but no significant locomotor deficits nor striatal neuropathology, were found (Gu et al., 2007). This work suggested a “two-hit” hypothesis in which both cell-autonomous toxicity and pathological cell–cell interactions are critical to HD pathogenesis.

Huntingtin has multiple interacting partners, some of them exhibiting enhanced binding with Exp-HTT, while a handful prefer associating with the wild-type HTT (Harjes and Wanker, 2003; Li and Li, 2004). Among the binding partners of HTT are a dozen transcription factors which appear to affect the transcriptional profile in HD brain tissue or cells (Luthi-Carter et al., 2000, 2002; Sipione et al., 2002; Sugars and Rubinshtein, 2003; Zhai et al., 2005; Kuhn et al., 2007). HTT is also considered as a scaffold protein, orchestrating the intracellular trafficking, signaling pathways, and transcriptional activity that are required for neuronal homeostasis; these functions being strongly hindered by the Exp-HTT expression (see below). Exp-HTT is cleaved by various intracellular proteases, including caspases, and this proteolytic processing plays a key role in the pathophysiology of HD since the cleaved N-terminal fragment is much more toxic than the full-length mutant protein. These cleaved versions of Exp-HTT can also undergo aggregate formation (Scherzinger et al., 1997; Huang et al., 1998; Perutz et al., 2002), which is thought to interfere with normal cellular functioning. Aggregates of insoluble proteins are found in the

brain of HD patients and in various HD models. They are enriched in striatal neurons and first appear in the neuropil of striatal MSNs (Li et al., 2000, 2001; Lee et al., 2004). By sequestering the wild-type HTT (Martindale et al., 1998) or associated proteins involved in transcription (Kazantsev et al., 1999; Steffan et al., 2000; Nucifora et al., 2001) or transport (Gunawardena et al., 2003; Trushina et al., 2004), these aggregates are thought to alter the fate of neuronal cells. Aggregate-mediated toxicity could be attributed to defects in RNA synthesis, cell survival, microtubule-dependent trafficking, or the ubiquitin–proteasome system (DiFiglia et al., 1997; Harjes and Wanker, 2003; Li and Li, 2004; Bennett et al., 2007). Nevertheless, the toxicity of aggregates still remains an issue of debate. Several studies have indicated that Exp-HTT aggregates are connected with neurodegeneration or cytotoxicity (Davies et al., 1997; DiFiglia et al., 1997; Ordway et al., 1997), whereas other studies have suggested that aggregate formation is either not intimately correlated with cytotoxicity (Saudou et al., 1998; Arrasate et al., 2004; Slow et al., 2005; Sawada et al., 2007; Mitra et al., 2009) or plays a protective role in cells (Arrasate et al., 2004; Mitra et al., 2009). Some authors argue that smaller oligomeric aggregates may be more toxic than larger ones (Gong et al., 2008). Furthermore, Exp-HTT can misfold into distinct β -sheet aggregates, called amyloids, which can be either toxic or non-toxic depending on their conformation (Nekooki-Machida et al., 2009). Aggregate toxicity can also depend on the cellular compartment in which they are localized, for example they could be more toxic in the neurites than in the nucleus (Li et al., 2000, 2001; Lee et al., 2004). Importantly, aggregates cannot be cleared by the ubiquitin–proteasome system (Bence et al., 2001; Jana et al., 2001; Verhoef et al., 2002; Venkatraman et al., 2004) and alter the normal efficacy of the clearance-machinery (Bennett et al., 2007; Hunter et al., 2007). The lower basal activity of the ubiquitin–proteasome system in neurons as compared to glial cells may account for the preferential accumulation of aggregates in neurons (Tydlacka et al., 2008). It is noteworthy that inhibiting the aggregation of Exp-HTT can alleviate the symptoms in various models of HD (Carmichael et al., 2000; Jana et al., 2000; Vacher et al., 2005; Ravikumar et al., 2006; Chopra et al., 2007; Herbst and Wanker, 2007; Perrin et al., 2007; Sarkar et al., 2007; Seo et al., 2007).

CHANGES IN AXONAL TRANSPORT AND SYNAPTIC DYSFUNCTION

Vesicular transport is altered in HD and is linked to a subsequent synaptic dysfunction (Gunawardena et al., 2003; Szebenyi et al., 2003; Gauthier et al., 2004; Trushina et al., 2004; Caviston and Holzbaur, 2009; Sinadinos et al., 2009). These defaults of trafficking are mainly due to an impaired interaction between Exp-HTT and motor proteins (Szebenyi et al., 2003; Lee et al., 2004), but can also be a consequence of neuritic aggregates that act as physical roadblocks (Gunawardena et al., 2003; Szebenyi et al., 2003; Trushina et al., 2004). According to the non-autonomous theory, the “neurotrophin disorder hypothesis” is the most widely accepted (Zuccato and Cattaneo, 2009). The release of brain derived neurotrophic factor (BDNF) from cortical afferences, which provides an important neurotrophic support to striatal neurons, is impaired in HD. Transcriptional dysregulation of BDNF in cortical neurons was first described (Zuccato et al., 2001; see Transcriptional

Dysregulation chapter below). Then, altered axonal transport deficit of BDNF from cortical neurons, was shown to participate in the default of BDNF release in the striatum (Gauthier et al., 2004). Wild-type HTT interacts with the molecular motor complex that transports organelles along the microtubules in axons (Gauthier et al., 2004; Caviston and Holzbaur, 2009). This interaction is altered in HD, due to Exp-HTT expression, which decreases axonal transport and the release of BDNF from cortical neurons to their terminals within the striatum (**Figure 1**). This is thought to participate in the striatal vulnerability in HD. One approach

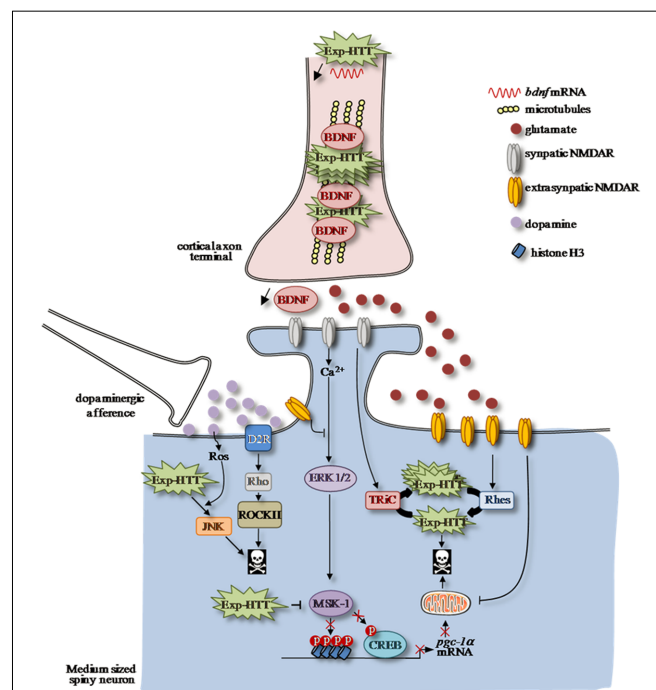


FIGURE 1 | Altered striatal signaling pathways in HD. Afferent corticostriatal and nigro-striatal projections modulate striatal signaling, which is impaired in Huntington's Disease (HD). Neurotrophic factor BDNF release from cortical afferences is decreased in cortico-striatal synapses as a consequence of Exp-HTT-mediated down-regulation of *bdnf* transcription and axonal transport in cortical neurons. A shift from synaptic to extrasynaptic NMDAR-dependent signaling participates to striatal neurons to death. In a physiological condition, calcium influx through synaptic NMDAR promotes activation of the MAPkinase/ERK signaling pathway along with its nuclear target, the MSK-1 protein, which phosphorylates the transcription factor CREB. MSK-1 activation promotes chromatin remodeling, which is crucial for CREB-mediated *pgc-1α* transcription, a key gene involved in mitochondria biogenesis. Synaptic NMDARs also promote formation of non-toxic Exp-HTT inclusion via TRIC. In HD, localization and activity of extrasynaptic NMDAR are enhanced by Exp-HTT, which promotes neuronal cell injury and death. The toxic effect of extrasynaptic NMDAR is partly due to (i) the upregulation of Rhes expression that disaggregates the non-toxic Exp-HTT inclusions (ii) to an impairment of mitochondrial functions and (iii) to a decrease of the ERK/MSK-1/CREB signaling module to the *pgc-1α* promoter. Dopamine release from nigro-striatal inputs promotes oxidative stress via the production of reactive oxygen species (ROS), which potentiates activation of the pro-apoptotic JNK pathway induced by Exp-HTT. In addition, Dopamine potentiates Exp-HTT-mediated striatal neurons death through activation of the Rho/ROCK signaling pathway downstream D2R.

to compensate for the defective BDNF transport is to mimic the phosphorylation of Exp-HTT on Ser421, in order to restore the interaction between Exp-HTT and dynactin, and their association with microtubules (Colin et al., 2008). Interestingly, increasing tubulin acetylation using a specific histone deacetylase (HDAC6) inhibitor restores the recruitment of motor proteins, including kinesin-1 and dynein to microtubules, and increases BDNF axonal transport in cortical neurons (Dompierre et al., 2007).

Huntingtin is also involved in the trafficking and secretion of vesicles from the Golgi apparatus (Strehlow et al., 2007). In particular, it promotes, acting in concert with transglutaminase 2 (TGase 2) and HJS1b, the budding of vesicles containing BDNF from the Golgi to the cytoplasm (Borrell-Pages et al., 2006a; Del Toro et al., 2006). Modulation of TGase 2 and HJS1b expression by cystamine or cysteamine increases the release of BDNF in mice and monkey models of HD (Borrell-Pages et al., 2006b). The trafficking of neural proteins from the Golgi is also regulated by HTT-interacting protein 14 (HIP14), which normally interacts with HTT to regulate the trafficking of neuronal proteins and their synaptic release through palmitoylation of cysteine string protein (CSP; Yanai et al., 2006; Ohyama et al., 2007). Finally, Exp-HTT increases c-Jun-Kinase3 (JNK3) activity, which phosphorylates kinesin on Ser176 and reduces its binding to microtubules (Morfini et al., 2009). Thus, inhibition of the neuron-specific JNK3 can also protect from defects in fast axonal transport induced by Exp-HTT.

EXCITOTOXICITY AND STRIATAL SIGNALING

In HD, increased glutamate levels within the striatum are thought to arise from reduction of glial glutamate uptake (Liévens et al., 2001; Behrens et al., 2002). This is because of selective down-regulation of the glutamate transporter GLT1, which is mainly expressed in astrocytes. In *Drosophila* glia, Exp-HTT antagonize epidermal growth factor receptor (EGFR) Ras-extracellular signal-regulated kinase (ERK) signaling pathway, resulting in down-regulation of the glutamate transporter levels (Liévens et al., 2006). In addition, the expression of glutamine synthetase, an enzyme that converts glutamate to glutamine in glia, was also found to be altered (Liévens et al., 2001; Behrens et al., 2002). These findings demonstrated that the HD mutation results in a progressively deranged glutamate handling in the brain, beginning before the onset of symptoms in mice. They also provided evidence for a contribution of excitotoxicity to the pathophysiology of HD.

NMDAR-MEDIATED EXCITOTOXICITY IN HD

Ineffective management of Ca²⁺ homeostasis by striatal neurons ultimately leads to cell death via numerous signaling pathways. This excitotoxicity mostly implicates the *N*-Methyl-D-Aspartate type glutamate receptors (NMDAR) due to their high calcium permeability and slow activation/deactivation kinetics (Rothman and Olney, 1995; Dingleline et al., 1999; Cull-Candy et al., 2001). The earliest evidence in support of a role for NMDAR-mediated excitotoxicity in HD came from rodent studies where the administration of NMDAR agonists mimicked some clinical and pathological features of the disease (Beal et al., 1986; Sanberg et al., 1989; Ferrante et al., 1993). Studies have since switched from these chemical HD models to genetic mouse models where the

expression of Exp-HTT protein has been linked to NMDAR-mediated excitotoxicity (Levine et al., 1999; Cepeda et al., 2001; Zeron et al., 2004). However, comparisons between HD models are complicated as the mRNA and protein expression levels of the NMDAR subunits vary between models and the disease state (Fan et al., 2007). Furthermore, age-related dependence to excitotoxicity has been recently highlighted in the transgenic YAC128 HD mice model, which display enhanced sensitivity to excitotoxicity in the early phase of the disease, prior to development of cognitive dysfunction and motor abnormalities, and resistance to excitotoxic stress as the disease progresses (Graham et al., 2009).

TARGETING NMDAR SUBUNITS

Pharmacological analyses have indicated that striatal NMDARs are most commonly formed from heterotetramers containing two obligatory NMDAR type 1 subunits (GluN1, previously known as NR1) plus a combination of two NMDAR type 2 subunits, GluN2A and GluN2B, as heterodimers or as heterotrimers (GluN1 plus GluN2A and GluN2B). Some literature points to NMDARs containing principally GluN2A as being pro-survival whereas GluN2B-containing NMDARs act as the excitotoxic mediators (Zeron et al., 2002; Liu et al., 2007). As the expression of GluN2B is significantly higher than GluN2A in the striatum than in other brain regions (Landwehrmeyer et al., 1995; Kuppenbender et al., 2000), a GluN2B-mediated excitotoxicity is consistent with the striatal vulnerability for neurodegeneration in HD. Interestingly, recent work has demonstrated a greater contribution by GluN2A containing NMDAR in the D1 expressing MSNs than D2 expressing MSNs which degenerate first in HD (Jocoy et al., 2011). In the YAC transgenic FVB/N mouse model of HD, NMDA-induced cell death is prevented by a specific GluN2B antagonist ifenprodil (Zeron et al., 2002). Likewise, double mutant mice expressing Exp-HTT and overexpressing GluN2B displayed an exacerbated striatal degeneration (Heng et al., 2009). The logical conclusion from these studies is that selective antagonists of GluN2B such as ifenprodil, RO-25,6981, and CP101,606 should offer some neuroprotection. However, the use of three different GluN2B antagonists had no beneficial effects in the R6/2 mouse model of HD (Tallaksen-Greene et al., 2010). In addition, both ifenprodil and RO-25,6981 increase cell death induced by oxidative stress (Papadia et al., 2008) and blockade of spontaneous GluN2B activity exacerbates staurosporine-induced cell death (Martel et al., 2009). Therefore, as the outcome depends on the considered model and the particular excitotoxic insult the argument is left open as to whether GluN2B merits specific targeting in HD excitotoxicity.

GLUTAMATE RECEPTOR LOCALIZATION AND EXCITOTOXICITY

Instead of a definite GluN2B subunit-specific implication for excitotoxic signaling, work by Hardingham and Bading fueled the hypothesis that activation of extrasynaptic NMDAR (those located at the cell body, dendritic shaft or on the neck of spines) promotes cell death whereas stimulation of synaptic NMDAR promotes cell survival (Hardingham and Bading, 2010). These hypotheses may not be in complete opposition as extrasynaptic NMDAR contain more GluN2B than synaptic ones (Tovar and Westbrook, 1999; Steigerwald et al., 2000; Jocoy et al., 2011). Stimulation of calcium entry via synaptic NMDAR is well tolerated and coupled to

activation of the MAPkinase/ERK signaling pathway along with its target the transcription factor cyclic AMP-response element binding (CREB), which regulates genes such as *bdnf* (Hardingham et al., 2001, 2002). On the other hand these pro-survival mediators are dominantly opposed by the activation of extrasynaptic NMDAR which leads to the loss of mitochondrial membrane potential, inhibition of ERK signaling, CREB shut off (Vanhoutte and Bading, 2003), and an induction of a distinct genomic program including the gene *clca1a*, which encodes a calcium-activated chloride channel sufficient to kill neurons (Zhang et al., 2007). In HD, the striatal specific signaling partners of synaptic versus extrasynaptic NMDAR have begun to be analyzed. Ras homolog enriched in striatum (Rhes) is localized in the striatum, where it specifically bind to Exp-HTT and elicits both a decrease in ubiquitination and an increase in sumoylation of Exp-HTT, which leads to its disaggregation and favors the formation of neurotoxic soluble microaggregates (Subramaniam et al., 2009). Okamoto et al. (2009) found that Rhes expression is reduced when extrasynaptic receptors are blocked, whereas antagonism of synaptic NMDAR leads to a decrease in Exp-HTT aggregates and cell death. This study distinguished between the two receptor pools by the use of the uncompetitive NMDAR antagonist memantine, which selectively antagonizes extrasynaptic NMDAR at low concentrations (Rammes et al., 2008). The formation of non-toxic Exp-HTT inclusions is dependent on synaptic NMDAR activation and the induction of the T-complex-1 (TCP-1) subunit of TCP-1 ring complex (TRiC), which associates with heat shock protein 70 (Hsp 70) to favor the formation of these inclusions. By contrast, activation of extrasynaptic NMDAR in the presence of Exp-HTT down-regulates the protective PPAR alpha-co-activator-1 α (PGC-1 α) cascade (see below) via an inhibition of ERK activation and CREB phosphorylation (Okamoto et al., 2009). Inhibition of extrasynaptic NMDAR after *in vivo* treatment with a low dose of memantine over a period of 8 months increased TCP-1 protein levels, inclusion formation, and improved the motor function of YAC128 HD mice. By using the same approach, Milnerwood et al. (2010) showed that a blockade of extrasynaptic NMDAR restores basal levels of CREB activation and significantly overcomes motor learning deficits of YAC128 mice. Even at pre-symptomatic stages, YAC128 mice express more NMDAR subunits at extrasynaptic sites than YAC18 control mice, and consequently have a heightened response to glutamate spillover dependent on an extrasynaptic GluN2B-containing NMDAR (Milnerwood et al., 2010).

In addition to lateral NMDAR receptor localization, altered NMDA receptor trafficking may also participate to neuronal excitotoxicity in the striatum. Accelerated NMDA receptor trafficking and increased expression at the cell surface were found in striatal neurons from the YAC72 HD mouse model (Fan et al., 2007). This phenomenon can be related to an altered interaction between Exp-HTT and postsynaptic density 95 (PSD-95), a scaffold protein necessary for NMDAR stability, which has been previously described (Roche et al., 2001; Sun et al., 2001). Recently, association of PSD-95 with GluN2B in striatal tissue has been shown to be enhanced by Exp-HTT (Fan et al., 2009). Treatment of cultured MSNs with a TAT coupled peptide that blocks binding of GluN2B with PSD-95, reduces NMDAR surface expression in both

YAC transgenic and WT MSN and rescues cells from NMDAR excitotoxicity.

The HIP1, which normally interacts with HTT, is involved in the intracellular trafficking of glutamate receptors of the AMPA subtypes. Exp-HTT, which interacts less efficiently with HIP1 than its normal counterpart, participates to increased membranal expression of AMPA receptors and hence to excitotoxic neuronal death in HD (Metzler et al., 2007). Altered calcium homeostasis in HD could be due to an abnormal interaction between Exp-HTT and the type 1 inositol 1,4,5-trisphosphate receptor (InsP3R1), which regulates the cytoplasmic calcium clearance by the endoplasmic reticulum (Tang et al., 2004, 2009). Disrupting the interaction between InsP3R1 and Exp-HTT normalizes calcium signaling, protects from glutamate-induced apoptosis in striatal neurons *in vitro*, and reduces neuronal pathology and motor deficits in a mouse model of HD *in vivo*.

STRIATAL VULNERABILITY: THE DOPAMINERGIC HYPOTHESIS

In addition to the glutamatergic stimulation, some evidence indicate that Dopamine (DA) stimulation may play a key role in excitotoxicity in HD (Reynolds et al., 1998; Charvin et al., 2005, 2008; Cyr et al., 2006; Stack et al., 2007; Tang et al., 2007; Benchoua et al., 2008). Knock-out mice for the DA transporter (DAT) show spontaneous striatal death accompanied by behavioral alterations that resemble HD specifically during aging (Cyr et al., 2003). In an elegant study, a double mutant mouse strain with both enhanced dopamine transmission and endogenous expression of a mutant HTT gene was generated. This strain was generated by crossing the DAT knock-out mouse with a knock-in mouse model of HD containing 92 CAG repeats (Cyr et al., 2006). These double mutant mice exhibited increased behavioral and neuropathological hallmarks of HD, including neuropil aggregates in MSN projection neurons. DA released from nigro-striatal inputs, is present in high concentrations within the striatum and enhances sensitivity to glutamatergic inputs. It may also produce oxidative stress via the production of reactive oxygen species (ROS), a cellular process that increases with aging (Jakel and Maragos, 2000). In particular, cultures of striatal neurons from R6/2 HD mice are sensitized to DA-induced oxidative stress, leading to neuronal autophagy (Petersen et al., 2001). ROS produced by low doses of DA potentiate activation of the pro-apoptotic JNK pathway induced by Exp-HTT (Garcia et al., 2004; Charvin et al., 2005), the pharmacological inhibition of which is neuroprotective in the R6/2 transgenic mouse model of HD (Apostol et al., 2008). DA may also render striatal neurons more vulnerable to Exp-HTT via dopamine receptor-mediated mechanisms (Charvin et al., 2005, 2008). Depending on the cell line model of HD, expressing either the full length or truncated versions of Exp-HTT, D1, or D2 receptors stimulation seems to be different. Full-length Exp-HTT is required for alteration of calcium signaling (Zhang et al., 2008). In striatal neurons from YAC128 transgenic or Q111 knock-in mice, both expressing full-length Exp-HTT, D1 receptor stimulation potentiates calcium influx via NMDA receptors, and hence excitotoxic processes, including mitochondrial depolarization, and caspase activation (Cepeda et al., 2001; Zeron et al., 2002, 2004; Starling et al., 2005; Tang et al., 2007). More recently, a

calcium-dependent activation of calpain was shown to be involved in striatal death after convergent activation of NMDA and D1 receptors in HD cell models (Paoletti et al., 2008). Increases in calcium influx lead to the cleavage of Cdk5 co-activator p35 into p25, which enables an aberrant toxic activation of Cdk5 (Paoletti et al., 2008). By contrast, when associated with p35 as a co-activator, Cdk5 is known to be protective via phosphorylation of Exp-HTT and the blockade of caspase-induced cleavage, resulting in attenuated aggregate formation and toxicity (Luo et al., 2005; Anne et al., 2007). Consistently, Cdk5/p35 suppresses the formation of aggregates induced by a short fragment of Exp-HTT (exon 1), via a new, unexpected role on microtubule stability and hence inclusion formation (Kaminosono et al., 2008). Together, these data highlight the complexity of Cdk5 activity and the importance of targeting selectively p25 to block Exp-HTT-induced inclusion formation and neuronal death.

A central role of DARPP-32 (Dopaminergic and cAMP-regulated phosphoprotein) has also been proposed (Metzler et al., 2010). DARPP-32 phosphorylation at Thr34 is induced by a D1 agonist (SKF 81297) and inhibits in turn the activity of PP1, a phosphatase that dephosphorylates the Ser421 residue of the HTT protein. Of interest, inhibition of PP1 can offer protection from NMDA-induced excitotoxicity in YAC128 mice, through increased phosphorylation of Ser421-HTT. The loss of DARPP-32 expression described in HD results in an increase of PP1 activity followed by a decrease of Ser421-Exp-HTT phosphorylation (Metzler et al., 2010). As its cleaved version, Exp-HTT is not sensitive to D1 agonists, probably because it is not sensitive to calcium overload and calcium-dependent proteolytic processes produced by D1 receptor stimulation. By contrast, D2 receptors stimulation potentiates Exp-HTT-induced aggregate formation, deficiency of mitochondrial complex II protein activity, and neuronal death (Charvin et al., 2005; Benchoua et al., 2008). *In vivo*, in a rat model of HD based on lentiviral-mediated expression of Exp-HTT exon 1 in the striatum (De Almeida et al., 2002), an early and chronic treatment with the D2 antagonist, haloperidol decanoate, protects striatal neurons from Exp-HTT-induced dysfunction, and aggregates formation (Charvin et al., 2008). Striatal signaling mediated by D2 receptor stimulation on Exp-HTT toxicity has been recently elucidated. Inhibition of the Rho/ROCK pathway using selective inhibitors or knockdown of ROCK-II expression reversed D2 agonist-mediated aggregate formation, neuritic retraction and neuronal death induced by Exp-HTT (Deyts et al., 2009).

MITOCHONDRIAL DYSFUNCTION AND ENERGY DEFICITS

Defects in energy metabolism in brain and muscles, has long been proposed to be involved in HD, from clinical (Djousse et al., 2002; Hamilton et al., 2004) biochemical (Arenas et al., 1998; Turner et al., 2007) and neuroimaging studies (Jenkins et al., 1998). In HD patients there is strong evidence for reduced glucose consumption in the brain, more specifically in the basal ganglia (Grafton et al., 1992; Kuwert et al., 1993) as well as increased lactate concentrations in the basal ganglia and occipital cortex (Jenkins et al., 1993), and lactate-to-pyruvate levels in the CSF (Jenkins et al., 1998). Various mechanisms that underlie the energy deficit in the HD brain have been proposed (Mochel et al., 2007; Mochel and Haller, 2011). They include impaired oxidative phosphorylation,

oxidative stress, impaired mitochondrial calcium handling, abnormal mitochondria trafficking, decreased glycolysis, and transcriptional deregulation of PGC-1 α . A deficiency of respiratory chain complex II, i.e., succinate dehydrogenase (SDH), in HD has been proposed since the observation that accidental ingestion of 3-nitropropionic acid (3-NP), an irreversible inhibitor of SDH, reproduces the clinical and neuropathological characteristics of HD in humans (Brouillet et al., 1999). In rodents, systemic 3-NP administration reproduces selective striatal degeneration, despite an altered SDH activity in multiple brain regions (Brouillet et al., 1998). Conversely, restoration of the complex II activity level is neuroprotective in a cellular model of HD (Benchoua et al., 2006). Activation of the pro-apoptotic JNK pathway is observed selectively in striatal neurons of 3-NP-administered rats *in vivo*, and overexpression of a dominant negative, non-phosphorylatable version of c-Jun *in vitro* inhibits striatal degeneration induced by 3-NP (Garcia et al., 2002). DA signaling regulates SDH enzymatic activity (Benchoua et al., 2008) and hence may account for the vulnerability of striatal neurons in HD. Furthermore, as detailed in the next section Exp-HTT-induced transcriptional dysregulation is now thought to contribute to altered bioenergetics.

TRANSCRIPTIONAL DYSREGULATION

Transcriptional dysregulation is an early event in the neuropathological process. Altered levels of dopaminergic receptor and neuropeptide mRNAs observed in patient's brain tissues (Augood et al., 1996, 1997) are also observed in pre-symptomatic HD's transgenic mice, suggesting that changes in transcription underlie neurodegeneration rather than reflecting non-specific degradation of all RNAs in affected neurons (Cha et al., 1998, 1999). Subsequently, multiple genes encoding neurotransmitter receptors, enzymes, and proteins involved in neuron structure, stress responses, and axonal transport were found to be dysregulated (Luthi-Carter et al., 2000, 2002; Sugars and Rubinstein, 2003; Cha, 2007; Runne et al., 2007), with overlaps of altered transcripts between various mouse models of HD and brain of HD patients (Kuhn et al., 2007). Interestingly, more than 80% of classically admitted striatal-enriched genes (genes with higher relative expression in the striatum compared with other brain regions) are decreased in a mouse model of HD as well as in human HD postmortem brain (Desplats et al., 2006). A down-regulation of novel striatal-enriched genes involved in vesicle transport and trafficking, tryptophan metabolism, and neuroinflammation were identified more recently in both HD mouse striatum and caudate from HD patients (Mazarei et al., 2010). Of interest, most of HD-induced dysregulation of the striatal transcriptome can be largely attributed to the intrinsic effects of mutant HTT, in the absence of expression in cortical neurons (Thomas et al., 2011).

Transcriptional dysregulation can be found in large genomic regions in a coordinated fashion and this dysregulation is associated with disease progression. Attempts were made to use transcriptional dysregulation as a biomarker in HD. Genome-wide expression profiling of the blood from HD's patients revealed significant differences in symptomatic patients (Borovecki et al., 2005), but not moderate-stage patients (Runne et al., 2007). Thus, these biomarkers need to be further validated before their widespread use in clinical trials.

MOLECULAR MECHANISMS OF TRANSCRIPTIONAL DYSREGULATION IN HD

Within the nucleus Exp-HTT, under its soluble or aggregated form, interacts with and inhibits the activity of proteins involved in the normal transcriptional machinery. These include TATA binding protein (TBP), transcription factor II F (TFIIF), and tyrosine-aminotransferase II (TATII) 130 (Shimohata et al., 2000; Suhr et al., 2001; Dunah et al., 2002; Li et al., 2002). Exp-HTT also sequesters transcription factors involved in cell viability, including CREB protein (CBP), p53, specificity protein 1 (Sp1), nuclear factor-kappa B (NF- κ B), nuclear receptor co-repressor (NCoR), and CA150 (Boutell et al., 1999; Li et al., 2000; Steffan et al., 2000; Nucifora et al., 2001; Dunah et al., 2002; Bae et al., 2005; Arango et al., 2006). Expression levels of BDNF, and its receptor TrkB, are decreased in the striatum of HD patient's, suggesting a deficit in cortical neurotrophic support of the striatum (Ferrer et al., 2000; Zuccato et al., 2001; Lynch et al., 2007; Strand et al., 2007). In animal models of HD, cortical BDNF expression is reduced (Zuccato et al., 2001). Moreover, downregulating BDNF in striatum in mice worsens the HD phenotype, whereas elevating BDNF expression in the forebrain alleviates the HD phenotype (Canals et al., 2004; Strand et al., 2007; Gharami et al., 2008; Xie et al., 2010). The molecular mechanisms by which Exp-HTT drives the down-regulation of BDNF expression in cortical neurons have been unraveled. Wild-type HTT sequesters R element-1 silencing transcription factor (REST), a transcriptional repressor of neuronal survival factors including BDNF, within the cytoplasm. The HTT mutation leads to REST release within the nucleus, where it exerts a potent inhibitory role on the transcription of BDNF and other neuronal genes (Zuccato et al., 2001, 2003, 2007). Furthermore, REST mRNA levels are increased in R6/2 mouse model of HD and NG108 neuronal-like model of HD. At the transcriptional level, Sp1 binds to the Sp factor binding sites contained in the promoter of REST and contributes to Exp-HTT-mediated REST upregulation (Ravache et al., 2010). BDNF expression is also a component of the neuroprotective transcriptional response mediated by NF- κ B in neurons (Lipsky et al., 2001). In addition, the loss of BDNF expression and low levels of NF- κ B activity in neurons could lead to impairments of cognitive functions (Kaltschmidt et al., 2005; Meffert and Baltimore, 2005), a common feature of neurodegenerative disorders such as HD.

A LINK BETWEEN TRANSCRIPTIONAL DYSREGULATION AND ENERGY DEFICITS IN HD

A link between Exp-HTT-induced transcriptional dysregulation and energy deficits has been recently described. Exp-HTT binds to the tumor suppression gene p53 more avidly than wild-type HTT and has been reported to increase p53 protein levels, nuclear localization and transcriptional activity in neuronal cultures and transgenic mice (Bae et al., 2005). Augmented p53 activity mediates mitochondrial membrane depolarization and decreases complex IV activity, and p53 inhibition or genetic deletion ameliorates these changes in a cell culture model. PGC-1 α is a transcriptional co-activator that regulates mitochondrial biogenesis and oxidative phosphorylation (Cui et al., 2006; Weydt et al., 2006). A role of PGC-1 α in HD pathogenesis was suspected from the observation of selective striatal lesions in PGC-1 α knock-mice (Lin et al., 2004).

A direct link between CREB phosphorylation and transcriptional regulation at the PGC-1 α promoter has been observed in neuronal cells (Cui et al., 2006). Impairment of the CREB/PGC-1 α signaling cascade by suppression of excitatory synaptic activity or by stimulation of extracellular NMDA receptors increases the vulnerability of HD neuronal cells (Okamoto et al., 2009). Mitogen and stress-activated protein kinase-1 (MSK-1), a nuclear protein kinase activated downstream of ERK, was shown to control the expression levels of PGC-1 α via increased binding of phosphorylated-CREB and Histone H3 at the promoter region of PGC-1 α . Overexpression of MSK-1 was protective against Exp-HTT-induced striatal dysfunctions *in vitro* (Roze et al., 2008) and *in vivo* (Martin et al., 2011; Figure 1).

ACTING ON CHROMATIN REMODELING TO IMPROVE TRANSCRIPTIONAL DYSREGULATION IN HD

Chromatin remodeling that underlies DNA decompaction was first described in dividing cells, but is also one of the prime events of transcription in post-mitotic mature neurons (Taniura et al., 2007). This "above the genome" molecular mechanism, also called an epigenetic mechanism, gates DNA access, and hence transcription. It is critically controlled by post-translational modifications of histones (H2A and H2B, H3 and H4), a group of highly basic proteins tightly linked to DNA. In particular, the methylation and acetylation state of histones is closely linked to the regions of transcriptional activity by regulating transcription factor access to promoter regions in the DNA. Histone acetylation at a promoter generally increases transcription and the enzymes that catalyze these reactions are histone acetyltransferases (HATs). By contrast, HDACs catalyze deacetylation. Exp-HTT interacts with CBP and blocks its intrinsic HAT activity (Steffan et al., 2001). Administration of HDACs inhibitors, including SAHA, sodium butyrate, and phenylbutyrate, has demonstrated their therapeutic role in several HD models (Steffan et al., 2000; Ferrante et al., 2003; Hockly et al., 2003; Gardian et al., 2005), as they improved behavioral performance and neuronal survival. Interestingly, administration of a new benzamide-type HDAC inhibitor with lower potential toxicity than previous HDAC inhibitors, HDACi 4b, also restores the transcription of critical striatal genes and improves motor and neuropathological phenotype of R6/2 HD mice (Thomas et al., 2008). All these HDAC inhibitors act broadly across various classes of HDACs (Lesort et al., 1999). Inhibitors targeting a specific class of HDACs may result in a better benefit to side effect ratio (Pallos et al., 2008). Finally, it must be emphasized that the levels of acetylated histones are not decreased globally in HD mice models, but rather selectively in the promoters of genes that are specifically down-regulated in HD (Sadri-Vakili et al., 2007).

Methylation of histones plays the opposite, inhibitory role on transcription. One of the proteins involved in methyltransferase activity at histone H3 (K9) is ERG-associated protein with SET domain (ESET). ESET expression is increased in HD patients and R6/2 HD mice (Ryu et al., 2006). Sp1 acts as a transcriptional activator of the ESET promoter at guanosine-cytosine (GC)-rich DNA binding sites (Yang et al., 2003). Inhibiting Sp1 binding to these sites using mitramycin (a clinically approved antitumor antibiotic) suppressed basal ESET promoter activity in a dose-dependent manner. The combined pharmacological treatment of

mithramycin and cystamine, down-regulates ESET gene expression and hypertrimethylation of histone H3. This treatment significantly ameliorates the behavioral and neuropathological phenotype of R6/2 HD mice and improves their survival. Owing to its HEAT repeat α -solenoid structure, HTT acts as a facilitator of the epigenetic silencer polycomb repressive complex 2 (PRC2; Seong et al., 2010). The polyglutamine region augments PRC2 stimulation and hence H3 trimethylation on specific promoters, including *Hoxb9*. In general, the DNA/RNA binding agents anthracyclines are thought to provide a significant therapeutic potential by correcting the pathological nucleosome changes and realigning transcription. Two such agents, chromomycin and mithramycin, were found to improve altered nucleosomal homeostasis, and by virtue of normalizing the shift in the balance between methylation and acetylation in HD mice, could alter a subset of down-regulated genes accompanied by a significant improvement of the behavioral and neuropathological phenotype observed in HD mice (Stack et al., 2007).

The transcriptional co-repressor transglutaminase 2 (TG2), which is up-regulated in HD, interacts physically with Histone H3 and could contribute to gene silencing via hyperpolyamination of histone tails (McConoughey et al., 2010). The inhibition of TG2 increases PGC-1 α expression, but also that of 40% of genes that are dysregulated (Karpuj et al., 1999; Lesort et al., 1999) in HD striatal neurons. Histone H2A ubiquitinylation is increased in R6/2 HD mice and the association of ubiquitinated H2A with the promoters of down-regulated genes is increased in an *in vitro* model of HD (Kim et al., 2008). This transcriptional repression is rescued by restoration of the ubiquitinated H2A level. In addition, histone H2B ubiquitinylation is decreased in R6/2 HD mice and association of ubiquitinated H2B with promoters positively correlates with transcriptional level in R6/2 mice. This transcriptional modulation by H2 ubiquitinylation is thought to occur through a subsequent interference with methylation of histone H3 (Kim et al., 2008).

Histone H3 phosphorylation is also critical to induce the nucleosomal response and gene transcription at some promoters. MSK-1 is critically involved in Histone H3 phosphorylation in the striatum (Brami-Cherrier et al., 2005, 2009). It is deficient in the striatum of R6/2 mice and postmortem caudate of HD patients (Roze et al., 2008). Restoring MSK-1 expression and subsequent striatal H3 phosphorylation in an *in vitro* model

system of HD protects against neuronal alteration induced by the Exp-HTT including neuritic retraction, aggregate formation, and neuronal death (Roze et al., 2008). *In vivo*, in a rat model of HD based on striatal lentiviral expression of Exp-HTT, overexpression of MSK-1 induced hyperphosphorylation of H3 and CREB, along with an overexpression of PGC-1 α (Martin et al., 2011). Similarly to PGC-1 α , MSK-1 protects from Exp-HTT-induced striatal dysfunctions, including DARPP-32 down-regulation and neuronal death. Furthermore MSK-1 knock-out mice are more susceptible to 3-NP-induced striatal lesion, and aging MSK-1 knock-out mice show spontaneous striatal degeneration (Martin et al., 2011).

CONCLUSION

The regulation of neuronal death has been intensely investigated and, due to its paramount implications for neurodegenerative disease, has sparked one of the most prolific research fields in the past decades. In particular, basic research has provided novel insights into the molecular machinery of neuronal signaling, and how it mediates neuronal dysfunction and death in progressive neurodegenerative diseases. HD involves a complex pathological cascade with multiple deleterious mechanisms, affecting predominantly the striatal neurons. This striatal vulnerability to HD-induced neuronal dysfunction reflects both the particular characteristics of striatal neurons (cell-autonomous alterations) and their location within the functional neuronal networks (non-cell-autonomous alterations). The various pathological events are not proceeding in succession but instead take place in parallel with continuous reciprocal interactions. This parallel and interactive nature should be taken into account for both the general understanding of the HD-related neurodegeneration and any therapeutic approach.

ACKNOWLEDGMENTS

This work was supported by Centre National pour la Recherche Scientifique, Université Pierre and Marie Curie, Ministère de la Recherche Scientifique. Jocelyne Caboche was funded by Fondation pour la Recherche sur le Cerveau and Hereditary Disease Foundation. Emma Cahill is supported by the Ecole des Neurosciences de Paris, Elodie Martin was a recipient from Ministère de la Recherche Scientifique, and Fondation Huntington France. Cecilia Bonnet is supported by the Czech Ministry of Education, Grantov Agency UK and IGA.

REFERENCES

- Alford, R. L., Ashizawa, T., Jankovic, J., Caskey, C. T., and Richards, C. S. (1996). Molecular detection of new mutations, resolution of ambiguous results and complex genetic counseling issues in Huntington disease. *Am. J. Med. Genet.* 66, 281–286.
- Andersen, J. M., Gayan, J., Cherny, S. S., Brocklebank, D., Alkorta-Aranburu, G., Addis, E. A., Cardon, L. R., Housman, D. E., and Wexler, N. S. (2007). Replication of twelve association studies for Huntington's disease residual age of onset in large Venezuelan kindreds. *J. Med. Genet.* 44, 44–50.
- Andrew, S. E., Goldberg, Y. P., Kremer, B., Telenius, H., Theilmann, J., Adam, S., Starr, E., Squitieri, F., Lin, B., Kalchman, M. A., Graham, R. K., and Hayden, M. R. (1993). The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. *Nat. Genet.* 4, 398–403.
- Anne, S. L., Saudou, F., and Humbert, S. (2007). Phosphorylation of huntingtin by cyclin-dependent kinase 5 is induced by DNA damage and regulates wild-type and mutant huntingtin toxicity in neurons. *J. Neurosci.* 27, 7318–7328.
- Apostol, B. L., Simmons, D. A., Zuccato, C., Illes, K., Pallos, J., Casale, M., Conforti, P., Ramos, C., Roarke, M., Kathuria, S., Cattaneo, E., Marsh, J. L., and Thompson, L. M. (2008). CEP-1347 reduces mutant huntingtin-associated neurotoxicity and restores BDNF levels in R6/2 mice. *Mol. Cell. Neurosci.* 39, 8–20.
- Arango, M., Holbert, S., Zala, D., Brouillet, E., Pearson, J., Regulier, E., Thakur, A. K., Aebischer, P., Wetzel, R., Deglon, N., and Neri, C. (2006). CA150 expression delays striatal cell death in overexpression and knock-in conditions for mutant huntingtin neurotoxicity. *J. Neurosci.* 26, 4649–4659.
- Arenas, J., Campos, Y., Ribacoba, R., Martin, M. A., Rubio, J. C., Ablanedo, P., and Cabello, A. (1998). Complex I defect in muscle from patients with Huntington's disease. *Ann. Neurol.* 43, 397–400.
- Arnulf, I., Nielsen, J., Lohmann, E., Schiefer, J., Wild, E., Jennum, P., Konofal, E., Walker, M., Oudiette, D., Tabrizi, S., and Durr, A. (2008). Rapid eye movement sleep

- disturbances in Huntington disease. *Arch. Neurol.* 65, 482–488.
- Arrasate, M., Mitra, S., Schweitzer, E. S., Segal, M. R., and Finkbeiner, S. (2004). Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 431, 805–810.
- Augood, S. J., Faull, R. L., and Emson, P. C. (1997). Dopamine D1 and D2 receptor gene expression in the striatum in Huntington's disease. *Ann. Neurol.* 42, 215–221.
- Augood, S. J., Faull, R. L., Love, D. R., and Emson, P. C. (1996). Reduction in enkephalin and substance P messenger RNA in the striatum of early grade Huntington's disease: a detailed cellular in situ hybridization study. *Neuroscience* 72, 1023–1036.
- Aziz, N. A., Van Der Burg, J. M., Landwehrmeyer, G. B., Brundin, P., Stijnen, T., and Roos, R. A. (2008). Weight loss in Huntington disease increases with higher CAG repeat number. *Neurology* 71, 1506–1513.
- Bae, B. I., Xu, H., Igarashi, S., Fujimuro, M., Agrawal, N., Taya, Y., Hayward, S. D., Moran, T. H., Montell, C., Ross, C. A., Snyder, S. H., and Sawa, A. (2005). p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease. *Neuron* 47, 29–41.
- Bamford, K. A., Caine, E. D., Kido, D. K., Cox, C., and Shoulson, I. (1995). A prospective evaluation of cognitive decline in early Huntington's disease: functional and radiographic correlates. *Neurology* 45, 1867–1873.
- Beal, M. F., Kowall, N. W., Ellison, D. W., Mazurek, M. F., Swartz, K. J., and Martin, J. B. (1986). Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature* 321, 168–171.
- Behrens, P. F., Franz, P., Woodman, B., Lindenberg, K. S., and Landwehrmeyer, G. B. (2002). Impaired glutamate transport and glutamate–glutamine cycling: downstream effects of the Huntington mutation. *Brain* 125, 1908–1922.
- Bence, N. F., Sampat, R. M., and Kopito, R. R. (2001). Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 292, 1552–1555.
- Benchoua, A., Trioulier, Y., Diguët, E., Malgorn, C., Gaillard, M. C., Dufour, N., Elalouf, J. M., Krajewski, S., Hantraye, P., Deglon, N., and Brouillet, E. (2008). Dopamine determines the vulnerability of striatal neurons to the N-terminal fragment of mutant huntingtin through the regulation of mitochondrial complex II. *Hum. Mol. Genet.* 17, 1446–1456.
- Benchoua, A., Trioulier, Y., Zala, D., Gaillard, M. C., Lefort, N., Dufour, N., Saudou, F., Elalouf, J. M., Hirsch, E., Hantraye, P., Deglon, N., and Brouillet, E. (2006). Involvement of mitochondrial complex II defects in neuronal death produced by N-terminus fragment of mutated huntingtin. *Mol. Biol. Cell* 17, 1652–1663.
- Bennett, E. J., Shaler, T. A., Woodman, B., Ryu, K. Y., Zaitseva, T. S., Becker, C. H., Bates, G. P., Schulman, H., and Kopito, R. R. (2007). Global changes to the ubiquitin system in Huntington's disease. *Nature* 448, 704–708.
- Borovecki, F., Lovrecic, L., Zhou, J., Jeong, H., Then, F., Rosas, H. D., Hersch, S. M., Hogarth, P., Bouzou, B., Jensen, R. V., and Krainc, D. (2005). Genome-wide expression profiling of human blood reveals biomarkers for Huntington's disease. *Proc. Natl. Acad. Sci. U.S.A.* 102, 11023–11028.
- Borrell-Pages, M., Canals, J. M., Cordeiro, F. P., Parker, J. A., Pineda, J. R., Grange, G., Bryson, E. A., Guillermier, M., Hirsch, E., Hantraye, P., Cheetham, M. E., Neri, C., Alberch, J., Brouillet, E., Saudou, F., and Humbert, S. (2006a). Cystamine and cysteamine increase brain levels of BDNF in Huntington disease via HSP1b and transglutaminase. *J. Clin. Invest.* 116, 1410–1424.
- Borrell-Pages, M., Zala, D., Humbert, S., and Saudou, F. (2006b). Huntington's disease: from huntingtin function and dysfunction to therapeutic strategies. *Cell. Mol. Life Sci.* 63, 2642–2660.
- Boutell, J. M., Thomas, P., Neal, J. W., Weston, V. J., Duce, J., Harper, P. S., and Jones, A. L. (1999). Aberrant interactions of transcriptional repressor proteins with the Huntington's disease gene product, huntingtin. *Hum. Mol. Genet.* 8, 1647–1655.
- Brami-Cherrier, K., Roze, E., Girault, J. A., Betuing, S., and Caboche, J. (2009). Role of the ERK/MSK1 signalling pathway in chromatin remodelling and brain responses to drugs of abuse. *J. Neurochem.* 108, 1323–1335.
- Brami-Cherrier, K., Valjent, E., Herve, D., Darragh, J., Corvol, J. C., Pages, C., Arthur, S. J., Girault, J. A., and Caboche, J. (2005). Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. *J. Neurosci.* 25, 11444–11454.
- Brouillet, E., Conde, F., Beal, M. F., and Hantraye, P. (1999). Replicating Huntington's disease phenotype in experimental animals. *Prog. Neurobiol.* 59, 427–468.
- Brouillet, E., Guyot, M. C., Mittoux, V., Altaïrac, S., Conde, F., Palfi, S., and Hantraye, P. (1998). Partial inhibition of brain succinate dehydrogenase by 3-nitropropionic acid is sufficient to initiate striatal degeneration in rat. *J. Neurochem.* 70, 794–805.
- Busse, M. E., Wiles, C. M., and Rosser, A. E. (2009). Mobility and falls in people with Huntington's disease. *J. Neurol. Neurosurg. Psychiatr.* 80, 88–90.
- Caine, E. D., Ebert, M. H., and Weingartner, H. (1977). An outline for the analysis of dementia. The memory disorder of Huntingtons disease. *Neurology* 27, 1087–1092.
- Caine, E. D., Hunt, R. D., Weingartner, H., and Ebert, M. H. (1978). Huntington's dementia. Clinical and neuropsychological features. *Arch. Gen. Psychiatry* 35, 377–384.
- Caine, E. D., and Shoulson, I. (1983). Psychiatric syndromes in Huntington's disease. *Am. J. Psychiatry* 140, 728–733.
- Canals, J. M., Pineda, J. R., Torres-Peraza, J. F., Bosch, M., Martin-Ibanez, R., Munoz, M. T., Mengod, G., Ernfor, P., and Alberch, J. (2004). Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with encephalineric neuronal degeneration in Huntington's disease. *J. Neurosci.* 24, 7727–7739.
- Carella, F., Scaïoli, V., Ciano, C., Binelli, S., Oliva, D., and Girotti, F. (1993). Adult onset myoclonic Huntington's disease. *Mov. Disord.* 8, 201–205.
- Carmichael, J., Chatellier, J., Woolfson, A., Milstein, C., Fersht, A. R., and Rubinsztein, D. C. (2000). Bacterial and yeast chaperones reduce both aggregate formation and cell death in mammalian cell models of Huntington's disease. *Proc. Natl. Acad. Sci. U.S.A.* 97, 9701–9705.
- Caviston, J. P., and Holzbaur, E. L. (2009). Huntingtin as an essential integrator of intracellular vesicular trafficking. *Trends Cell Biol.* 19, 147–155.
- Caviston, J. P., Ross, J. L., Antony, S. M., Tokito, M., and Holzbaur, E. L. (2007). Huntingtin facilitates dynein/dynactin-mediated vesicle transport. *Proc. Natl. Acad. Sci. U.S.A.* 104, 10045–10050.
- Cepeda, C., Ariano, M. A., Calvert, C. R., Flores-Hernandez, J., Chandler, S. H., Leavitt, B. R., Hayden, M. R., and Levine, M. S. (2001). NMDA receptor function in mouse models of Huntington disease. *J. Neurosci. Res.* 66, 525–539.
- Cha, J. H. (2007). Transcriptional signals in Huntington's disease. *Prog. Neurobiol.* 83, 228–248.
- Cha, J. H., Frey, A. S., Alsdorf, S. A., Kerner, J. A., Kosinski, C. M., Mangiarini, L., Penney, J. B. Jr., Davies, S. W., Bates, G. P., and Young, A. B. (1999). Altered neurotransmitter receptor expression in transgenic mouse models of Huntington's disease. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 354, 981–989.
- Cha, J. H., Kosinski, C. M., Kerner, J. A., Alsdorf, S. A., Mangiarini, L., Davies, S. W., Penney, J. B., Bates, G. P., and Young, A. B. (1998). Altered brain neurotransmitter receptors in transgenic mice expressing a portion of an abnormal human Huntington disease gene. *Proc. Natl. Acad. Sci. U.S.A.* 95, 6480–6485.
- Charvin, D., Roze, E., Perrin, V., Deyts, C., Betuing, S., Pages, C., Regulier, E., Luthi-Carter, R., Brouillet, E., Deglon, N., and Caboche, J. (2008). Haloperidol protects striatal neurons from dysfunction induced by mutated huntingtin in vivo. *Neurobiol. Dis.* 29, 22–29.
- Charvin, D., Vanhoutte, P., Pages, C., Borrelli, E., and Caboche, J. (2005). Unraveling a role for dopamine in Huntington's disease: the dual role of reactive oxygen species and D2 receptor stimulation. *Proc. Natl. Acad. Sci. U.S.A.* 102, 12218–12223.
- Chopra, V., Fox, J. H., Lieberman, G., Dorsey, K., Matson, W., Waldmeier, P., Housman, D. E., Kazantsev, A., Young, A. B., and Hersch, S. (2007). A small-molecule therapeutic lead for Huntington's disease: preclinical pharmacology and efficacy of C2-8 in the R6/2 transgenic mouse. *Proc. Natl. Acad. Sci. U.S.A.* 104, 16685–16689.
- Colin, E., Zala, D., Liot, G., Rangone, H., Borrell-Pages, M., Li, X. J., Saudou, F., and Humbert, S. (2008). Huntingtin phosphorylation acts as a molecular switch for anterograde/retrograde transport in neurons. *EMBO J.* 27, 2124–2134.
- Cui, L., Jeong, H., Borovecki, F., Parkhurst, C. N., Tanese, N., and Krainc, D. (2006). Transcriptional repression of PGC-1 α by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell* 127, 59–69.
- Cull-Candy, S., Brickley, S., and Farrant, M. (2001). NMDA receptor subunits: diversity, development and disease. *Curr. Opin. Neurobiol.* 11, 327–335.

- Cummings, J. L. (1995). Behavioral and psychiatric symptoms associated with Huntington's disease. *Adv. Neurol.* 65, 179–186.
- Cummings, J. L., and Cunningham, K. (1992). Obsessive-compulsive disorder in Huntington's disease. *Biol. Psychiatry* 31, 263–270.
- Cyr, M., Beaulieu, J. M., Laakso, A., Sotnikova, T. D., Yao, W. D., Bohn, L. M., Gainetdinov, R. R., and Caron, M. G. (2003). Sustained elevation of extracellular dopamine causes motor dysfunction and selective degeneration of striatal GABAergic neurons. *Proc. Natl. Acad. Sci. U.S.A.* 100, 11035–11040.
- Cyr, M., Sotnikova, T. D., Gainetdinov, R. R., and Caron, M. G. (2006). Dopamine enhances motor and neuropathological consequences of polyglutamine expanded huntingtin. *FASEB J.* 20, 2541–2543.
- Davies, S. W., Turmaine, M., Cozens, B. A., DiFiglia, M., Sharp, A. H., Ross, C. A., Scherzinger, E., Wanker, E. E., Mangiarini, L., and Bates, G. P. (1997). Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* 90, 537–548.
- Davis, M. B., Bateman, D., Quinn, N. P., Marsden, C. D., and Harding, A. E. (1994). Mutation analysis in patients with possible but apparently sporadic Huntington's disease. *Lancet* 344, 714–717.
- Dawbarn, D., De Quidt, M. E., and Emson, P. C. (1985). Survival of basal ganglia neuropeptide Y-somatostatin neurones in Huntington's disease. *Brain Res.* 340, 251–260.
- De Almeida, L. P., Ross, C. A., Zala, D., Aebischer, P., and Deglon, N. (2002). Lentiviral-mediated delivery of mutant huntingtin in the striatum of rats induces a selective neuropathology modulated by polyglutamine repeat size, huntingtin expression levels, and protein length. *J. Neurosci.* 22, 3473–3483.
- Del Toro, D., Canals, J. M., Gines, S., Kojima, M., Egea, G., and Alberch, J. (2006). Mutant huntingtin impairs the post-Golgi trafficking of brain-derived neurotrophic factor but not its Val66Met polymorphism. *J. Neurosci.* 26, 12748–12757.
- Desplats, P. A., Kass, K. E., Gilmartin, T., Stanwood, G. D., Woodward, E. L., Head, S. R., Sutcliffe, J. G., and Thomas, E. A. (2006). Selective deficits in the expression of striatal-enriched mRNAs in Huntington's disease. *J. Neurochem.* 96, 743–757.
- Deyts, C., Galan-Rodriguez, B., Martin, E., Bouveyron, N., Roze, E., Charvin, D., Caboche, J., and Betuing, S. (2009). Dopamine D2 receptor stimulation potentiates PolyQ-Huntingtin-induced mouse striatal neuron dysfunctions via Rho/ROCK-II activation. *PLoS ONE* 4, e8287. doi: 10.1371/journal.pone.0008287
- Di Maio, L., Squitieri, F., Napolitano, G., Campanella, G., Trofater, J. A., and Conneally, P. M. (1993). Onset symptoms in 510 patients with Huntington's disease. *J. Med. Genet.* 30, 289–292.
- DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P., and Aronin, N. (1997). Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* 277, 1990–1993.
- Diguët, E., Petit, F., Escartin, C., Cambron, K., Bizat, N., Dufour, N., Hantraye, P., Deglon, N., and Brouillet, E. (2009). Normal aging modulates the neurotoxicity of mutant huntingtin. *PLoS ONE* 4, e4637. doi: 10.1371/journal.pone.0004637
- Dingledine, R., Borges, K., Bowie, D., and Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacol. Rev.* 51, 7–61.
- Djousse, L., Knowlton, B., Cupples, L. A., Marder, K., Shoulson, I., and Myers, R. H. (2002). Weight loss in early stage of Huntington's disease. *Neurology* 59, 1325–1330.
- Dompierre, J. P., Godin, J. D., Charvin, B. C., Cordelieres, F. P., King, S. J., Humbert, S., and Saudou, F. (2007). Histone deacetylase 6 inhibition compensates for the transport deficit in Huntington's disease by increasing tubulin acetylation. *J. Neurosci.* 27, 3571–3583.
- Dragatsis, I., Dietrich, P., and Zeitlin, S. (2000). Expression of the Huntingtin-associated protein 1 gene in the developing and adult mouse. *Neurosci. Lett.* 282, 37–40.
- Dunah, A. W., Jeong, H., Griffin, A., Kim, Y. M., Standaert, D. G., Hersch, S. M., Mouradian, M. M., Young, A. B., Tanese, N., and Krainc, D. (2002). Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science* 296, 2238–2243.
- Duyao, M., Ambrose, C., Myers, R., Novellotto, A., Persichetti, F., Frontali, M., Folstein, S., Ross, C., Franz, M., Abbott, M., Gray, J., Conneally, P., Young, A., Penney, J., Hollingsworth, Z., Shoulson, I., Lazzarini, A., Falek, A., Koroshetz, W., Sax, D., Bird, E., Vonsattel, J., Bonilla, E., Alvir, J., Bickham Conde, J., Cha, J.-H., Dure, L., Gomez, F., Ramos, M., Sanchez-Ramos, J., Snodgrass, S., de Young, M., Wexler, N., Moscovitz, C., Penchaszadeh, G., MacFarlane, H., Anderson, M., Jenkins, B., Srinidhi, J., Barnes, G., Gusella, J., and MacDonald, M. (1993). Trinucleotide repeat length instability and age of onset in Huntington's disease. *Nat. Genet.* 4, 387–392.
- Duyao, M. P., Auerbach, A. B., Ryan, A., Persichetti, F., Barnes, G. T., Mcneil, S. M., Ge, P., Vonsattel, J. P., Gusella, J. F., Joyner, A. L., and MacDonald, M. E. (1995). Inactivation of the mouse Huntington's disease gene homolog Hdh. *Science* 269, 407–410.
- Fan, J., Cowan, C. M., Zhang, L. Y., Hayden, M. R., and Raymond, L. A. (2009). Interaction of post-synaptic density protein-95 with NMDA receptors influences excitotoxicity in the yeast artificial chromosome mouse model of Huntington's disease. *J. Neurosci.* 29, 10928–10938.
- Fan, M. M., Fernandes, H. B., Zhang, L. Y., Hayden, M. R., and Raymond, L. A. (2007). Altered NMDA receptor trafficking in a yeast artificial chromosome transgenic mouse model of Huntington's disease. *J. Neurosci.* 27, 3768–3779.
- Fedoroff, J. P., Peyser, C., Franz, M. L., and Folstein, S. E. (1994). Sexual disorders in Huntington's disease. *J. Neuropsychiatry Clin. Neurosci.* 6, 147–153.
- Ferrante, R. J., Kowall, N. W., Beal, M. F., Martin, J. B., Bird, E. D., and Richardson, E. P. Jr. (1987). Morphologic and histochemical characteristics of a spared subset of striatal neurons in Huntington's disease. *J. Neuropathol. Exp. Neurol.* 46, 12–27.
- Ferrante, R. J., Kowall, N. W., Beal, M. F., Richardson, E. P. Jr., Bird, E. D., and Martin, J. B. (1985). Selective sparing of a class of striatal neurons in Huntington's disease. *Science* 230, 561–563.
- Ferrante, R. J., Kowall, N. W., Cipolioni, P. B., Storey, E., and Beal, M. F. (1993). Excitotoxin lesions in primates as a model for Huntington's disease: histopathologic and neurochemical characterization. *Exp. Neurol.* 119, 46–71.
- Ferrante, R. J., Kowall, N. W., and Richardson, E. P. Jr. (1991). Proliferative and degenerative changes in striatal spiny neurons in Huntington's disease: a combined study using the section-Golgi method and calbindin D28k immunocytochemistry. *J. Neurosci.* 11, 3877–3887.
- Ferrante, R. J., Kubilus, J. K., Lee, J., Ryu, H., Beesen, A., Zucker, B., Smith, K., Kowall, N. W., Ratan, R. R., Luthi-Carter, R., and Hersch, S. M. (2003). Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. *J. Neurosci.* 23, 9418–9427.
- Ferrer, I., Goutan, E., Marin, C., Rey, M. J., and Ribalta, T. (2000). Brain-derived neurotrophic factor in Huntington disease. *Brain Res.* 866, 257–261.
- Folstein, S. E., Franz, M. L., Jensen, B. A., Chase, G. A., and Folstein, M. F. (1983). Conduct disorder and affective disorder among the offspring of patients with Huntington's disease. *Psychol. Med.* 13, 45–52.
- Gambardella, A., Muglia, M., Labate, A., Magariello, A., Gabriele, A. L., Mazzei, R., Pirritano, D., Conforti, F. L., Patitucci, A., Valentino, P., Zappia, M., and Quattrone, A. (2001). Juvenile Huntington's disease presenting as progressive myoclonic epilepsy. *Neurology* 57, 708–711.
- Garcia, M., Charvin, D., and Caboche, J. (2004). Expanded huntingtin activates the c-Jun terminal kinase/c-Jun pathway prior to aggregate formation in striatal neurons in culture. *Neuroscience* 127, 859–870.
- Garcia, M., Vanhoutte, P., Pages, C., Besson, M. J., Brouillet, E., and Caboche, J. (2002). The mitochondrial toxin 3-nitropropionic acid induces striatal neurodegeneration via a c-Jun N-terminal kinase/c-Jun module. *J. Neurosci.* 22, 2174–2184.
- Gardian, G., Browne, S. E., Choi, D. K., Klivenyi, P., Gregorio, J., Kubilus, J. K., Ryu, H., Langley, B., Ratan, R. R., Ferrante, R. J., and Beal, M. F. (2005). Neuroprotective effects of phenylbutyrate in the N171-82Q transgenic mouse model of Huntington's disease. *J. Biol. Chem.* 280, 556–563.
- Gauthier, L. R., Charrin, B. C., Borrell-Pages, M., Dompierre, J. P., Rangone, H., Cordelieres, F. P., De Mey, J., Macdonald, M. E., Lessmann, V., Humbert, S., and Saudou, F. (2004). Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell* 118, 127–138.
- Gharani, K., Xie, Y., An, J. J., Tonegawa, S., and Xu, B. (2008). Brain-derived neurotrophic factor over-expression in the forebrain ameliorates Huntington's disease phenotypes in mice. *J. Neurochem.* 105, 369–379.

- Goldberg, Y. P., Rommens, J. M., Andrew, S. E., Hutchinson, G. B., Lin, B., Theilmann, J., Graham, R., Graves, M. L., Starr, E., McDonald, H., Nasir, J., Schappert, K., Kalchman, M. A., Clark, L. A., Hayden, M. R. (1993). Identification of an Alu retrotransposition event in close proximity to a strong candidate gene for Huntington's disease. *Nature* 362, 370–373.
- Gong, B., Lim, M. C., Wanderer, J., Wytenbach, A., and Morton, A. J. (2008). Time-lapse analysis of aggregate formation in an inducible PC12 cell model of Huntington's disease reveals time-dependent aggregate formation that transiently delays cell death. *Brain Res. Bull.* 75, 146–157.
- Gonzalez-Alegre, P., and Afifi, A. K. (2006). Clinical characteristics of childhood-onset (juvenile) Huntington disease: report of 12 patients and review of the literature. *J. Child Neurol.* 21, 223–229.
- Goto, S., Hirano, A., and Rojas-Corona, R. R. (1989). Immunohistochemical visualization of afferent nerve terminals in human globus pallidus and its alteration in neostriatal neurodegenerative disorders. *Acta Neuropathol.* 78, 543–550.
- Grafton, S. T., Mazziotta, J. C., Pahl, J. J., St George-Hyslop, P., Haines, J. L., Gusella, J., Hoffman, J. M., Baxter, L. R., and Phelps, M. E. (1992). Serial changes of cerebral glucose metabolism and caudate size in persons at risk for Huntington's disease. *Arch. Neurol.* 49, 1161–1167.
- Graham, R. K., Pouladi, M. A., Joshi, P., Lu, G., Deng, Y., Wu, N.-P., Figueroa, B. E., Metzler, M., André, V. M., Slow, E. J., Raymond, L., Friedlander, R., Levine, M. S., Leavitt, B. R., and Hayden, M. R. (2009). Differential susceptibility to excitotoxic stress in YAC128 mouse models of HD between initiation and progression of disease. *J. Neurosci.* 29, 2193–2204.
- Graveland, G. A., Williams, R. S., and DiFiglia, M. (1985). Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science* 227, 770–773.
- Gu, X., Andre, V. M., Cepeda, C., Li, S. H., Li, X. J., Levine, M. S., and Yang, X. W. (2007). Pathological cell-cell interactions are necessary for striatal pathogenesis in a conditional mouse model of Huntington's disease. *Mol. Neurodegener.* 2, 8.
- Gunawardena, S., Her, L. S., Brusch, R. G., Laymon, R. A., Niesman, I. R., Gordesky-Gold, B., Sintasath, L., Bonini, N. M., and Goldstein, L. S. (2003). Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in *Drosophila*. *Neuron* 40, 25–40.
- Gutekunst, C. A., Li, S. H., Yi, H., Mulroy, J. S., Kuemmerle, S., Jones, R., Rye, D., Ferrante, R. J., Hersch, S. M., and Li, X. J. (1999). Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. *J. Neurosci.* 19, 2522–2534.
- Hamilton, J. M., Wolfson, T., Peavy, G. M., Jacobson, M. W., and Corey-Bloom, J. (2004). Rate and correlates of weight change in Huntington's disease. *J. Neurol. Neurosurg. Psychiatr.* 75, 209–212.
- Hardingham, G. E., Arnold, F. J., and Bading, H. (2001). Nuclear calcium signaling controls CREB-mediated gene expression triggered by synaptic activity. *Nat. Neurosci.* 4, 261–267.
- Hardingham, G. E., and Bading, H. (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat. Rev. Neurosci.* 11, 682–696.
- Hardingham, G. E., Fukunaga, Y., and Bading, H. (2002). Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat. Neurosci.* 5, 405–414.
- Harjes, P., and Wanker, E. E. (2003). The hunt for huntingtin function: interaction partners tell many different stories. *Trends Biochem. Sci.* 28, 425–433.
- Harper, P. S. (1992). The epidemiology of Huntington's disease. *Hum. Genet.* 89, 365–376.
- Heng, M. Y., Detloff, P. J., Wang, P. L., Tsien, J. Z., and Albin, R. L. (2009). In vivo evidence for NMDA receptor-mediated excitotoxicity in a murine genetic model of Huntington disease. *J. Neurosci.* 29, 3200–3205.
- Herbst, M., and Wanker, E. E. (2007). Small molecule inducers of heat-shock response reduce polyQ-mediated huntingtin aggregation. A possible therapeutic strategy. *Neurodegener. Dis.* 4, 254–260.
- Ho, A. K., Sahakian, B. J., Brown, R. G., Barker, R. A., Hodges, J. R., Ane, M. N., Snowden, J., Thompson, J., Esmonde, T., Gentry, R., Moore, J. W., and Bodner, R. (2003). Profile of cognitive progression in early Huntington's disease. *Neurology* 61, 1702–1706.
- Hockly, E., Richon, V. M., Woodman, B., Smith, D. L., Zhou, X., Rosa, E., Sathasivam, K., Ghazi-Noori, S., Mahal, A., Lowden, P. A., Steffan, J. S., Marsh, J. L., Thompson, L. M., Lewis, C. M., Marks, P. A., and Bates, G. P. (2003). Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. *Proc. Natl. Acad. Sci. U.S.A.* 100, 2041–2046.
- Hodges, J. R., Salmon, D. P., and Butters, N. (1990). Differential impairment of semantic and episodic memory in Alzheimer's and Huntington's diseases: a controlled prospective study. *J. Neurol. Neurosurg. Psychiatr.* 53, 1089–1095.
- Hoffner, G., Island, M. L., and Djian, P. (2005). Purification of neuronal inclusions of patients with Huntington's disease reveals a broad range of N-terminal fragments of expanded huntingtin and insoluble polymers. *J. Neurochem.* 95, 125–136.
- Huang, C. C., Faber, P. W., Persichetti, F., Mittal, V., Vonsattel, J. P., Macdonald, M. E., and Gusella, J. F. (1998). Amyloid formation by mutant huntingtin: threshold, progressivity and recruitment of normal polyglutamine proteins. *Somat. Cell Mol. Genet.* 24, 217–233.
- Hunter, J. M., Lesort, M., and Johnson, G. V. (2007). Ubiquitin-proteasome system alterations in a striatal cell model of Huntington's disease. *J. Neurosci. Res.* 85, 1774–1788.
- Jakel, R. J., and Maragos, W. F. (2000). Neuronal cell death in Huntington's disease: a potential role for dopamine. *Trends Neurosci.* 23, 239–245.
- Jana, N. R., Tanaka, M., Wang, G., and Nukina, N. (2000). Polyglutamine length-dependent interaction of Hsp40 and Hsp70 family chaperones with truncated N-terminal huntingtin: their role in suppression of aggregation and cellular toxicity. *Hum. Mol. Genet.* 9, 2009–2018.
- Jana, N. R., Zemskov, E. A., Wang, G., and Nukina, N. (2001). Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome c release. *Hum. Mol. Genet.* 10, 1049–1059.
- Jankovic, J., Beach, J., and Ashizawa, T. (1995). Emotional and functional impact of DNA testing on patients with symptoms of Huntington's disease. *J. Med. Genet.* 32, 516–518.
- Jenkins, B. G., Koroshetz, W. J., Beal, M. F., and Rosen, B. R. (1993). Evidence for impairment of energy metabolism in vivo in Huntington's disease using localized ¹H NMR spectroscopy. *Neurology* 43, 2689–2695.
- Jenkins, B. G., Rosas, H. D., Chen, Y. C., Makabe, T., Myers, R., Macdonald, M., Rosen, B. R., Beal, M. F., and Koroshetz, W. J. (1998). ¹H NMR spectroscopy studies of Huntington's disease: correlations with CAG repeat numbers. *Neurology* 50, 1357–1365.
- Jensen, P., Fenger, K., Bolwig, T. G., and Sorensen, S. A. (1998). Crime in Huntington's disease: a study of registered offences among patients, relatives, and controls. *J. Neurol. Neurosurg. Psychiatr.* 65, 467–471.
- Jocoy, E. L., Andre, V. M., Cummings, D. M., Rao, S. P., Wu, N., Ramsey, A. J., Caron, M. G., Cepeda, C., and Levine, M. S. (2011). Dissecting the contribution of individual receptor subunits to the enhancement of N-methyl-D-aspartate currents by dopamine D1 receptor activation in striatum. *Front. Syst. Neurosci.* 5:28. doi: 10.3389/fnsys.2011.00004
- Kaltschmidt, B., Widera, D., and Kaltschmidt, C. (2005). Signaling via NF- κ B in the nervous system. *Biochim. Biophys. Acta* 1745, 287–299.
- Kaminosono, S., Saito, T., Oyama, F., Ohshima, T., Asada, A., Nagai, Y., Nukina, N., and Hisanaga, S. (2008). Suppression of mutant Huntingtin aggregate formation by Cdk5/p35 through the effect on microtubule stability. *J. Neurosci.* 28, 8747–8755.
- Karpuj, M. V., Garren, H., Slunt, H., Price, D. L., Gusella, J., Becher, M. W., and Steinman, L. (1999). Transglutaminase aggregates huntingtin into nonamyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei. *Proc. Natl. Acad. Sci. U.S.A.* 96, 7388–7393.
- Kazantsev, A., Preisinger, E., Dranovsky, A., Goldgaber, D., and Housman, D. (1999). Insoluble detergent-resistant aggregates form between pathological and nonpathological lengths of polyglutamine in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* 96, 11404–11409.
- Kegel, K. B., Meloni, A. R., Yi, Y., Kim, Y. J., Doyle, E., Cuiffo, B. G., Sapp, E., Wang, Y., Qin, Z. H., Chen, J. D., Nevins, J. R., Aronin, N., and DiFiglia, M. (2002). Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. *J. Biol. Chem.* 277, 7466–7476.

- Kegel, K. B., Sapp, E., Yoder, J., Cuiffo, B., Sobin, L., Kim, Y. J., Qin, Z. H., Hayden, M. R., Aronin, N., Scott, D. L., Isenberg, G., Goldmann, W. H., and DiFiglia, M. (2005). Huntingtin associates with acidic phospholipids at the plasma membrane. *J. Biol. Chem.* 280, 36464–36473.
- Kenney, C., Powell, S., and Jankovic, J. (2007). Autopsy-proven Huntington's disease with 29 trinucleotide repeats. *Mov. Disord.* 22, 127–130.
- Kereshian, J., Burd, L., Leech, C., and Rorabaugh, A. (1991). Huntington disease and childhood-onset Tourette syndrome. *Am. J. Med. Genet.* 39, 1–3.
- Kiechle, T., Dedeoglu, A., Kubilus, J., Kowall, N. W., Beal, M. F., Friedlander, R. M., Hersch, S. M., and Ferrante, R. J. (2002). Cytochrome C and caspase-9 expression in Huntington's disease. *Neuromolecular Med.* 1, 183–195.
- Kim, M. O., Chawla, P., Overland, R. P., Xia, E., Sadri-Vakili, G., and Cha, J. H. (2008). Altered histone monoubiquitylation mediated by mutant huntingtin induces transcriptional dysregulation. *J. Neurosci.* 28, 3947–3957.
- Kirkwood, S. C., Siemers, E., Viken, R., Hodes, M. E., Conneally, P. M., Christian, J. C., and Foroud, T. (2002). Longitudinal personality changes among presymptomatic Huntington disease gene carriers. *Neuropsychiatry Neuropsychol. Behav. Neurol.* 15, 192–197.
- Kremer, B., Weber, B., and Hayden, M. R. (1992). New insights into the clinical features, pathogenesis and molecular genetics of Huntington disease. *Brain Pathol.* 2, 321–335.
- Kuhn, A., Goldstein, D. R., Hodges, A., Strand, A. D., Sengstag, T., Kooperberg, C., Becanovic, K., Pouladi, M. A., Sathasivam, K., Cha, J. H., Hannan, A. J., Hayden, M. R., Leavitt, B. R., Dunnett, S. B., Ferrante, R. J., Albin, R., Shelbourne, P., Delorenzi, M., Augood, S. J., Faull, R. L., Olson, J. M., Bates, G. P., Jones, L., and Luthi-Carter, R. (2007). Mutant huntingtin's effects on striatal gene expression in mice recapitulate changes observed in human Huntington's disease brain and do not differ with mutant huntingtin length or wild-type huntingtin dosage. *Hum. Mol. Genet.* 16, 1845–1861.
- Kuppenbender, K. D., Standaert, D. G., Feuerstein, T. J., Penney, J. B. Jr., Young, A. B., and Landwehrmeyer, G. B. (2000). Expression of NMDA receptor subunit mRNAs in neurochemically identified projection and interneurons in the human striatum. *J. Comp. Neurol.* 419, 407–421.
- Kuwert, T., Lange, H. W., Boecker, H., Titz, H., Herzog, H., Aulich, A., Wang, B. C., Nayak, U., and Feineiden, L. E. (1993). Striatal glucose consumption in chorea-free subjects at risk of Huntington's disease. *J. Neurol.* 241, 31–36.
- Landwehrmeyer, G. B., Standaert, D. G., Testa, C. M., Penney, J. B. Jr., and Young, A. B. (1995). NMDA receptor subunit mRNA expression by projection neurons and interneurons in rat striatum. *J. Neurosci.* 15, 5297–5307.
- Lange, H., Thorner, G., Hopf, A., and Schroder, K. F. (1976). Morphometric studies of the neuropathological changes in choreatic diseases. *J. Neurol. Sci.* 28, 401–425.
- Lee, W. C., Yoshihara, M., and Littleton, J. T. (2004). Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3224–3229.
- Lesort, M., Chun, W., Johnson, G. V., and Ferrante, R. J. (1999). Tissue transglutaminase is increased in Huntington's disease brain. *J. Neurochem.* 73, 2018–2027.
- Levine, M. S., Klapstein, G. J., Koppel, A., Gruen, E., Cepeda, C., Vargas, M. E., Jokel, E. S., Carpenter, E. M., Zanjani, H., Hurst, R. S., Efstratiadis, A., Zeitlin, S., and Chesselet, M. F. (1999). Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knockin mouse models of Huntington's disease. *J. Neurosci. Res.* 58, 515–532.
- Li, H., Li, S. H., Johnston, H., Shelbourne, P. F., and Li, X. J. (2000). Amino-terminal fragments of mutant huntingtin show selective accumulation in striatal neurons and synaptic toxicity. *Nat. Genet.* 25, 385–389.
- Li, H., Li, S. H., Yu, Z. X., Shelbourne, P., and Li, X. J. (2001). Huntingtin aggregate-associated axonal degeneration is an early pathological event in Huntington's disease mice. *J. Neurosci.* 21, 8473–8481.
- Li, S. H., Cheng, A. L., Zhou, H., Lam, S., Rao, M., Li, H., and Li, X. J. (2002). Interaction of Huntington disease protein with transcriptional activator Sp1. *Mol. Cell. Biol.* 22, 1277–1287.
- Li, S. H., and Li, X. J. (2004). Huntingtin-protein interactions and the pathogenesis of Huntington's disease. *Trends Genet.* 20, 146–154.
- Liévens, J. C., Rival, T., Iche, M., Chneiweiss, H., and Birman, S. (2006). Expanded polyglutamine peptides disrupt EGF receptor signaling and glutamate transporter expression in Drosophila. *Hum. Mol. Gen.* 14, 713–724.
- Liévens, J. C., Woodman, B., Mahal, A., Spasic-Bosovic, O., Samuel, D., Kerkerian-Le Goff, L., and Bates, G. P. (2001). Impaired glutamate uptake in the R6 Huntington's disease transgenic mice. *Neurobiol. Dis.* 8, 807–821.
- Lin, J., Wu, P. H., Tarr, P. T., Lindenberg, K. S., St-Pierre, J., Zhang, C. Y., Mootha, V. K., Jager, S., Vianna, C. R., Reznick, R. M., Cui, L., Manieri, M., Donovan, M. X., Wu, Z., Cooper, M. P., Fan, M. C., Rohas, L. M., Zavacki, A. M., Cinti, S., Shulman, G. I., Lowell, B. B., Kraic, D., and Spiegelman, B. M. (2004). Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell* 119, 121–135.
- Lipsky, R. H., Xu, K., Zhu, D., Kelly, C., Terhakopian, A., Novelli, A., and Marini, A. M. (2001). Nuclear factor kappaB is a critical determinant in N-methyl-D-aspartate receptor-mediated neuroprotection. *J. Neurochem.* 78, 254–264.
- Liu, Y., Wong, T. P., Aarts, M., Rooyackers, A., Liu, L., Lai, T. W., Wu, D. C., Lu, J., Tymianski, M., Craig, A. M., and Wang, Y. T. (2007). NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. *J. Neurosci.* 27, 2846–2857.
- Luo, S., Vacher, C., Davies, J. E., and Rubinshtein, D. C. (2005). Cdk5 phosphorylation of huntingtin reduces its cleavage by caspases: implications for mutant huntingtin toxicity. *J. Cell Biol.* 169, 647–656.
- Luthi-Carter, R., Strand, A., Peters, N. L., Solano, S. M., Hollingsworth, Z. R., Menon, A. S., Frey, A. S., Spector, B. S., Penney, E. B., Schilling, G., Ross, C. A., Borchelt, D. R., Tapscott, S. J., Young, A. B., Cha, J. H., and Olson, J. M. (2000). Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. *Hum. Mol. Genet.* 9, 1259–1271.
- Luthi-Carter, R., Strand, A. D., Hanson, S. A., Kooperberg, C., Schilling, G., La Spada, A. R., Merry, D. E., Young, A. B., Ross, C. A., Borchelt, D. R., and Olson, J. M. (2002). Polyglutamine and transcription: gene expression changes shared by DRPLA and Huntington's disease mouse models reveal context-independent effects. *Hum. Mol. Genet.* 11, 1927–1937.
- Lynch, G., Kramar, E. A., Rex, C. S., Jia, Y., Chappas, D., Gall, C. M., and Simmons, D. A. (2007). Brain-derived neurotrophic factor restores synaptic plasticity in a knock-in mouse model of Huntington's disease. *J. Neurosci.* 27, 4424–4434.
- Martel, M. A., Wyllie, D. J., and Hardingham, G. E. (2009). In developing hippocampal neurons, NR2B-containing N-methyl-D-aspartate receptors (NMDARs) can mediate signaling to neuronal survival and synaptic potentiation, as well as neuronal death. *Neuroscience* 158, 334–343.
- Martin, E., Betuing, S., Pages, C., Cambron, K., Auregan, G., Deglon, N., Roze, E., and Caboche, J. (2011). Mitogen- and stress-activated protein kinase 1-induced neuroprotection in Huntington's disease: role on chromatin remodeling at the PGC-1-alpha promoter. *Hum. Mol. Genet.* 20, 2422–2434.
- Martindale, D., Hackam, A., Wiczorek, A., Ellerby, L., Wellington, C., Mccutcheon, K., Singaraja, R., Kazemi-Esfarjani, P., Devon, R., Kim, S. U., Bredeisen, D. E., Tufaro, F., and Hayden, M. R. (1998). Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. *Nat. Genet.* 18, 150–154.
- Martinez-Mir, M. I., Probst, A., and Palacios, J. M. (1991). Adenosine A2 receptors: selective localization in the human basal ganglia and alterations with disease. *Neuroscience* 42, 697–706.
- Mazarei, G., Neal, S. J., Becanovic, K., Luthi-Carter, R., Simpson, E. M., and Leavitt, B. R. (2010). Expression analysis of novel striatal-enriched genes in Huntington disease. *Hum. Mol. Genet.* 19, 609–622.
- McConoughey, S. J., Basso, M., Niatsetskaya, Z. V., Sleiman, S. F., Smirnova, N. A., Langle, B. C., Mahishi, L., Cooper, A. J., Antonyak, M. A., Cerione, R. A., Li, B., Starkov, A., Chaturvedi, R. K., Beal, M. F., Coppola, G., Geschwind, D. H., Ryu, H., Xia, L., Iismaa, S. E., Pallos, J., Pastermack, R., Hils, M., Fan, J., Raymond, L. A., Marsh, J. L., Thompson, L. M., and Ratan, R. R. (2010). Inhibition of transglutaminase 2 mitigates transcriptional dysregulation in models of Huntington disease. *EMBO Mol. Med.* 2, 349–370.
- Meffert, M. K., and Baltimore, D. (2005). Physiological functions for brain NF-kappaB. *Trends Neurosci.* 28, 37–43.
- Mendez, M. F. (2000). Mania in neurologic disorders. *Curr. Psychiatry Rep.* 2, 440–445.

- Metzler, M., Gan, L., Mazarei, G., Graham, R. K., Liu, L., Bissada, N., Lu, G., Leavitt, B. R., and Hayden, M. R. (2010). Phosphorylation of huntingtin at Ser421 in YAC128 neurons is associated with protection of YAC128 neurons from NMDA-mediated excitotoxicity and is modulated by PP1 and PP2A. *J. Neurosci.* 30, 14318–14329.
- Metzler, M., Gan, L., Wong, T. P., Liu, L., Helm, J., Liu, L., Georgiou, J., Wang, Y., Bissada, N., Cheng, K., Roder, J. C., Wang, Y. T., and Hayden, M. R. (2007). NMDA receptor function and NMDA receptor-dependent phosphorylation of huntingtin is altered by the endocytic protein HIP1. *J. Neurosci.* 27, 2298–2308.
- Milnerwood, A. J., Gladding, C. M., Pouladi, M. A., Kaufman, A. M., Hines, R. M., Boyd, J. D., Ko, R. W., Vasuta, O. C., Graham, R. K., Hayden, M. R., Murphy, T. H., and Raymond, L. A. (2010). Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron* 65, 178–190.
- Mitra, S., Tsvetkov, A. S., and Finkbeiner, S. (2009). Single neuron ubiquitin-proteasome dynamics accompanying inclusion body formation in huntington disease. *J. Biol. Chem.* 284, 4398–4403.
- Mochel, F., Charles, P., Seguin, F., Barriault, J., Coussieu, C., Perin, L., Le Bouc, Y., Gervais, C., Carcelain, G., Vassault, A., Feingold, J., Rabier, D., and Durr, A. (2007). Early energy deficit in Huntington disease: identification of a plasma biomarker traceable during disease progression. *PLoS ONE* 2, e647. doi: 10.1371/journal.pone.0000647
- Mochel, F., and Haller, R. G. (2011). Energy deficit in Huntington disease: why it matters. *J. Clin. Invest.* 121, 493–499.
- Morfini, G. A., You, Y. M., Pollema, S. L., Kaminska, A., Liu, K., Yoshioka, K., Bjorkblom, B., Coffey, E. T., Bagnato, C., Han, D., Huang, C. F., Banker, G., Pigino, G., and Brady, S. T. (2009). Pathogenic huntingtin inhibits fast axonal transport by activating JNK3 and phosphorylating kinesin. *Nat. Neurosci.* 12, 864–871.
- Morton, A. J., Wood, N. I., Hastings, M. H., Hurelbrink, C., Barker, R. A., and Maywood, E. S. (2005). Disintegration of the sleep-wake cycle and circadian timing in Huntington's disease. *J. Neurosci.* 25, 157–163.
- Myers, R. H., Macdonald, M. E., Koroshetz, W. J., Duyao, M. P., Ambrose, C. M., Taylor, S. A., Barnes, G., Srinidhi, J., Lin, C. S., Whaley, W. L., Lazzarini, A. M., Schwarz, M., Wolff, G., Bird, E. D., Vonsattel, J.-P., G., and Gusella, J. F. (1993). De novo expansion of a (CAG)_n repeat in sporadic Huntington's disease. *Nat. Genet.* 5, 168–173.
- Nekooki-Machida, Y., Kurosawa, M., Nukina, N., Ito, K., Oda, T., and Tanaka, M. (2009). Distinct conformations of in vitro and in vivo amyloids of huntingtin-exon1 show different cytotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9679–9684.
- Nucifora, F. C. Jr., Sasaki, M., Peters, M. F., Huang, H., Cooper, J. K., Yamada, M., Takahashi, H., Tsuji, S., Troncoso, J., Dawson, V. L., Dawson, T. M., and Ross, C. A. (2001). Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* 291, 2423–2428.
- Ohshima, T., Verstreken, P., Ly, C. V., Rosenmund, T., Rajan, A., Tien, A. C., Haueter, C., Schulze, K. L., and Bellen, H. J. (2007). Huntingtin-interacting protein 14, a palmitoyl transferase required for exocytosis and targeting of CSP to synaptic vesicles. *J. Cell Biol.* 179, 1481–1496.
- Okamoto, S., Pouladi, M. A., Talantova, M., Yao, D., Xia, P., Ehrnhoefer, D. E., Zaidi, R., Clemente, A., Kaul, M., Graham, R. K., Zhang, D., Vincent Chen, H. S., Tong, G., Hayden, M. R., and Lipton, S. A. (2009). Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. *Nat. Med.* 15, 1407–1413.
- Ordway, J. M., Tallaksen-Greene, S., Gutekunst, C. A., Bernstein, E. M., Cearley, J. A., Wiener, H. W., Dure, L. S. T., Lindsey, R., Hersch, S. M., Johe, R. S., Albin, R. L., and Detloff, P. J. (1997). Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse. *Cell* 91, 753–763.
- Pallos, J., Bodai, L., Lukacsovich, T., Purcell, J. M., Steffan, J. S., Thompson, L. M., and Marsh, J. L. (2008). Inhibition of specific HDACs and sirtuins suppresses pathogenesis in a Drosophila model of Huntington's disease. *Hum. Mol. Genet.* 17, 3767–3775.
- Paoletti, P., Vila, I., Rife, M., Lizcano, J. M., Alberch, J., and Gines, S. (2008). Dopaminergic and glutamatergic signaling crosstalk in Huntington's disease neurodegeneration: the role of p25/cyclin-dependent kinase 5. *J. Neurosci.* 28, 10090–10101.
- Papadia, S., Soriano, F. X., Leveille, F., Martel, M. A., Dakin, K. A., Hansen, H. H., Kaundl, A., Siffringer, M., Fowler, J., Stefovskaya, V., McKenzie, G., Craigon, M., Corriveau, R., Ghazal, P., Horsburgh, K., Yankner, B. A., Wyllie, D. J., Ikonomidou, C., and Hardingham, G. E. (2008). Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nat. Neurosci.* 11, 476–487.
- Paradisi, I., Hernandez, A., and Arias, S. (2008). Huntington disease mutation in Venezuela: age of onset, haplotype analyses and geographic aggregation. *J. Hum. Genet.* 53, 127–135.
- Patzold, T., and Brune, M. (2002). Obsessive compulsive disorder in Huntington disease: a case of isolated obsessions successfully treated with sertraline. *Neuropsychiatry Neuropsychol. Behav. Neurol.* 15, 216–219.
- Paulsen, J. S., Ready, R. E., Hamilton, J. M., Mega, M. S., and Cummings, J. L. (2001). Neuropsychiatric aspects of Huntington's disease. *J. Neurol. Neurosurg. Psychiatr.* 71, 310–314.
- Peinemann, A., Schuller, S., Pohl, C., Jahn, T., Weindl, A., and Kassubek, J. (2005). Executive dysfunction in early stages of Huntington's disease is associated with striatal and insular atrophy: a neuropsychological and voxel-based morphometric study. *J. Neurol. Sci.* 239, 11–19.
- Penney, J. B. Jr., Young, A. B., Shoulson, I., Starosta-Rubenstein, S., Snodgrass, S. R., Sanchez-Ramos, J., Ramos-Arroyo, M., Gomez, F., Penchaszadeh, G., Alvir, J., Esteves, J., DeQuiroz, I., Marsol, N., Moreno, H., Conneally, P. M., Bonilla, E., Wexler, N. S. (1990). Huntington's disease in Venezuela: 7 years of follow-up on symptomatic and asymptomatic individuals. *Mov. Disord.* 5, 93–99.
- Perrin, V., Regulier, E., Abbas-Terki, T., Hassig, R., Brouillet, E., Aebischer, P., Luthi-Carter, R., and Deglon, N. (2007). Neuroprotection by Hsp104 and Hsp27 in lentiviral-based rat models of Huntington's disease. *Mol. Ther.* 15, 903–911.
- Perutz, M. F., Pope, B. J., Owen, D., Wanker, E. E., and Scherzinger, E. (2002). Aggregation of proteins with expanded glutamine and alanine repeats of the glutamine-rich and asparagine-rich domains of Sup35 and of the amyloid beta-peptide of amyloid plaques. *Proc. Natl. Acad. Sci. U.S.A.* 99, 5596–5600.
- Petersen, A. A., Larsen, K. E., Behr, G. G., Romero, N., Przedborski, S., Brundin, P., and Sulzer, D. (2001). Brain-derived neurotrophic factor inhibits apoptosis and dopamine-induced free radical production in striatal neurons but does not prevent cell death. *Brain Res. Bull.* 56, 331–335.
- Pflanz, S., Besson, J. A., Ebmeier, K. P., and Simpson, S. (1991). The clinical manifestation of mental disorder in Huntington's disease: a retrospective case record study of disease progression. *Acta Psychiatr. Scand.* 83, 53–60.
- Pillon, B., Deweer, B., Agid, Y., and Dubois, B. (1993). Explicit memory in Alzheimer's, Huntington's, and Parkinson's diseases. *Arch. Neurol.* 50, 374–379.
- Pillon, B., Deweer, B., Michon, A., Malapani, C., Agid, Y., and Dubois, B. (1994). Are explicit memory disorders of progressive supranuclear palsy related to damage to striatofrontal circuits? Comparison with Alzheimer's, Parkinson's, and Huntington's diseases. *Neurology* 44, 1264–1270.
- Quinn, N., and Schrag, A. (1998). Huntington's disease and other choreas. *J. Neurol.* 245, 709–716.
- Rammes, G., Zieglsanger, W., and Parsons, C. G. (2008). The fraction of activated N-methyl-D-aspartate receptors during synaptic transmission remains constant in the presence of the glutamate release inhibitor riluzole. *J. Neural Transm.* 115, 1119–1126.
- Ranen, N. G., Stine, O. C., Abbott, M. H., Sherr, M., Codori, A. M., Franz, M. L., Chao, N. I., Chung, A. S., Pleasant, N., Callahan, C., Kasch, L. M., Ghaffari, M., Chase, G. A., Kazanian, H. H., Brandt, J., Folstein, S. E., and Ross, C. A. (1995). Anticipation and instability of IT-15 (CAG)_n repeats in parent-offspring pairs with Huntington disease. *Am. J. Hum. Genet.* 57, 593–602.
- Ravache, M., Weber, C., Merienne, K., and Trotter, Y. (2010). Transcriptional activation of REST by Sp1 in Huntington's disease models. *PLoS ONE* 5, e14311. doi: 10.1371/journal.pone.0014311
- Ravikumar, B., Berger, Z., Vacher, C., O'Kane, C. J., and Rubinsztein, D. C. (2006). Rapamycin pre-treatment protects against apoptosis. *Hum. Mol. Genet.* 15, 1209–1216.
- Reynolds, D. S., Carter, R. J., and Morton, A. J. (1998). Dopamine modulates the susceptibility of striatal neurons to 3-nitropropionic acid in the rat model of Huntington's disease. *J. Neurosci.* 18, 10116–10127.
- Reynolds, N. (2008). Re: autopsy-proven Huntington's disease with 29 trinucleotide repeats. *Mov. Disord.* 23, 1795–1796; author reply 1793.
- Richfield, E. K., and Herkenham, M. (1994). Selective vulnerability in Huntington's disease: preferential loss of cannabinoid receptors in lateral globus pallidus. *Ann. Neurol.* 36, 577–584.

- Richfield, E. K., O'Brien, C. F., Eskin, T., and Shoulson, I. (1991). Heterogeneous dopamine receptor changes in early and late Huntington's disease. *Neurosci. Lett.* 132, 121–126.
- Roche, K. W., Standley, S., Mccallum, J., Dune Ly, C., Ehlers, M. D., and Wenthold, R. J. (2001). Molecular determinants of NMDA receptor internalization. *Nat. Neurosci.* 4, 794–802.
- Rockabrand, E., Slepko, N., Pantalone, A., Nukala, V. N., Kazantsev, A., Marsh, J. L., Sullivan, P. G., Steffan, J. S., Sensi, S. L., and Thompson, L. M. (2007). The first 17 amino acids of Huntingtin modulate its sub-cellular localization, aggregation and effects on calcium homeostasis. *Hum. Mol. Genet.* 16, 61–77.
- Rosenblatt, A., and Leroi, I. (2000). Neuropsychiatry of Huntington's disease and other basal ganglia disorders. *Psychosomatics* 41, 24–30.
- Rothman, S. M., and Olney, J. W. (1995). Excitotoxicity and the NMDA receptor – still lethal after eight years. *Trends Neurosci.* 18, 57–58.
- Roze, E., Betuing, S., Deyts, C., Marcon, E., Brami-Cherrier, K., Pages, C., Humbert, S., Merienne, K., and Caboche, J. (2008). Mitogen- and stress-activated protein kinase-1 deficiency is involved in expanded-huntingtin-induced transcriptional dysregulation and striatal death. *FASEB J.* 22, 1083–1093.
- Runne, H., Kuhn, A., Wild, E. J., Pratyaksha, W., Kristiansen, M., Isaacs, J. D., Regulier, E., Delorenzi, M., Tabrizi, S. J., and Luthi-Carter, R. (2007). Analysis of potential transcriptomic biomarkers for Huntington's disease in peripheral blood. *Proc. Natl. Acad. Sci. U.S.A.* 104, 14424–14429.
- Ryu, H., Lee, J., Hagerty, S. W., Soh, B. Y., Mcalpin, S. E., Cormier, K. A., Smith, K. M., and Ferrante, R. J. (2006). ESET/SETDB1 gene expression and histone H3 (K9) trimethylation in Huntington's disease. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19176–19181.
- Sadri-Vakili, G., Bouzou, B., Benn, C. L., Kim, M. O., Chawla, P., Overland, R. P., Glajch, K. E., Xia, E., Qiu, Z., Hersch, S. M., Clark, T. W., Yohrling, G. J., and Cha, J. H. (2007). Histones associated with downregulated genes are hypo-acetylated in Huntington's disease models. *Hum. Mol. Genet.* 16, 1293–1306.
- Sanberg, P. R., Calderon, S. F., Gior-dano, M., Tew, J. M., and Norman, A. B. (1989). The quinolinic acid model of Huntington's disease: locomotor abnormalities. *Exp. Neurol.* 105, 45–53.
- Sarkar, S., Perlstein, E. O., Imarisio, S., Pineau, S., Cordenier, A., Maglathlin, R. L., Webster, J. A., Lewis, T. A., O'Kane, C. J., Schreiber, S. L., and Rubinsztein, D. C. (2007). Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nat. Chem. Biol.* 3, 331–338.
- Saudou, F., Finkbeiner, S., Devys, D., and Greenberg, M. E. (1998). Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* 95, 55–66.
- Sawada, H., Ishiguro, H., Nishii, K., Yamada, K., Tsuchida, K., Takahashi, H., Goto, J., Kanazawa, I., and Nagatsu, T. (2007). Characterization of neuron-specific huntingtin aggregates in human huntingtin knock-in mice. *Neurosci. Res.* 57, 559–573.
- Scherzinger, E., Lurz, R., Turmaine, M., Mangiarini, L., Hollenbach, B., Hasenbank, R., Bates, G. P., Davies, S. W., Leirach, H., and Wanker, E. E. (1997). Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. *Cell* 90, 549–558.
- Semaka, A., Warby, S., Leavitt, B. R., and Hayden, M. R. (2008). Re: autopsy-proven Huntington's disease with 29 trinucleotide repeats. *Mov. Disord.* 23, 1794–1795; author reply 1793.
- Seo, H., Sonntag, K. C., Kim, W., Cattaneo, E., and Isacson, O. (2007). Proteasome activator enhances survival of Huntington's disease neuronal model cells. *PLoS ONE* 2, e238. doi: 10.1371/journal.pone.0000238
- Seong, I. S., Woda, J. M., Song, J. J., Lloret, A., Abeyrathne, P. D., Woo, C. J., Gregory, G., Lee, J. M., Wheeler, V. C., Walz, T., Kingston, R. E., Gusella, J. F., Conlon, R. A., and Macdonald, M. E. (2010). Huntingtin facilitates polycomb repressive complex 2. *Hum. Mol. Genet.* 19, 573–583.
- Shimohata, T., Nakajima, T., Yamada, M., Uchida, C., Onodera, O., Naruse, S., Kimura, T., Koide, R., Nozaki, K., Sano, Y., Ishiguro, H., Sakoe, K., Ooshima, T., Sato, A., Ikeuchi, T., Oyake, M., Sato, T., Aoyagi, Y., Hozumi, I., Nagatsu, T., Takiyama, Y., Nishizawa, M., Goto, J., Kanazawa, I., Davidson, I., Tanese, N., Takahashi, H., and Tsuji, S. (2000). Expanded polyglutamine stretches interact with TAFII130, interfering with CREB-dependent transcription. *Nat. Genet.* 26, 29–36.
- Shiwach, R. S., and Norbury, C. G. (1994). A controlled psychiatric study of individuals at risk for Huntington's disease. *Br. J. Psychiatry* 165, 500–505.
- Siesling, S., Vegter-Van Der Vlis, M., and Roos, R. A. (1997). Juvenile Huntington disease in the Netherlands. *Pediatr. Neurol.* 17, 37–43.
- Sinadinos, C., Burbidge-King, T., Soh, D., Thompson, L. M., Marsh, J. L., Wyttenbach, A., and Mudher, A. K. (2009). Live axonal transport disruption by mutant huntingtin fragments in Drosophila motor neuron axons. *Neurobiol. Dis.* 34, 389–395.
- Sipione, S., Rigamonti, D., Valenza, M., Zuccato, C., Conti, L., Pritchard, J., Kooperberg, C., Olson, J. M., and Cattaneo, E. (2002). Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses. *Hum. Mol. Genet.* 11, 1953–1965.
- Slow, E. J., Graham, R. K., Osmand, A. P., Devon, R. S., Lu, G., Deng, Y., Pearson, J., Vaid, K., Bissada, N., Wetzel, R., Leavitt, B. R., and Hayden, M. R. (2005). Absence of behavioral abnormalities and neurodegeneration in vivo despite widespread neuronal huntingtin inclusions. *Proc. Natl. Acad. Sci. U.S.A.* 102, 11402–11407.
- Snell, R. G., Macmillan, J. C., Cheadle, J. P., Fenton, I., Lazarou, L. P., Davies, P., Macdonald, M. E., Gusella, J. F., Harper, P. S., and Shaw, D. J. (1993). Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nat. Genet.* 4, 393–397.
- Stack, E. C., Dedeoglu, A., Smith, K. M., Cormier, K., Kubilus, J. K., Bogdanov, M., Matson, W. R., Yang, L., Jenkins, B. G., Luthi-Carter, R., Kowall, N. W., Hersch, S. M., Beal, M. F., and Ferrante, R. J. (2007). Neuroprotective effects of synaptic modulation in Huntington's disease R6/2 mice. *J. Neurosci.* 27, 12908–12915.
- Starling, A. J., Andre, V. M., Cepeda, C., De Lima, M., Chandler, S. H., and Levine, M. S. (2005). Alterations in N-methyl-D-aspartate receptor sensitivity and magnesium blockade occur early in development in the R6/2 mouse model of Huntington's disease. *J. Neurosci. Res.* 82, 377–386.
- Steffan, J. S., Bodai, L., Pallos, J., Poelman, M., Mccampbell, A., Apostol, B. L., Kazantsev, A., Schmidt, E., Zhu, Y. Z., Greenwald, M., Kurokawa, R., Housman, D. E., Jackson, G. R., Marsh, J. L., and Thompson, L. M. (2001). Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. *Nature* 413, 739–743.
- Steffan, J. S., Kazantsev, A., Spasic-Boskovic, O., Greenwald, M., Zhu, Y. Z., Gohler, H., Wanker, E. E., Bates, G. P., Housman, D. E., and Thompson, L. M. (2000). The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc. Natl. Acad. Sci. U.S.A.* 97, 6763–6768.
- Steigerwald, F., Schulz, T. W., Schenker, L. T., Kennedy, M. B., Seeburg, P. H., and Kohr, G. (2000). C-Terminal truncation of NR2A subunits impairs synaptic but not extrasynaptic localization of NMDA receptors. *J. Neurosci.* 20, 4573–4581.
- Strand, A. D., Baquet, Z. C., Aragaki, A. K., Holmans, P., Yang, L., Cleren, C., Beal, M. F., Jones, L., Kooperberg, C., Olson, J. M., and Jones, K. R. (2007). Expression profiling of Huntington's disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. *J. Neurosci.* 27, 11758–11768.
- Strehlow, A. N., Li, J. Z., and Myers, R. M. (2007). Wild-type huntingtin participates in protein trafficking between the Golgi and the extracellular space. *Hum. Mol. Genet.* 16, 391–409.
- Subramaniam, S., Sixt, K. M., Barrow, R., and Snyder, S. H. (2009). Rhes, a striatal specific protein, mediates mutant-huntingtin cytotoxicity. *Science* 324, 1327–1330.
- Sugars, K. L., and Rubinsztein, D. C. (2003). Transcriptional abnormalities in Huntington disease. *Trends Genet.* 19, 233–238.
- Suhr, S. T., Senut, M. C., Whitelegge, J. P., Faull, K. F., Cuizon, D. B., and Gage, F. H. (2001). Identities of sequestered proteins in aggregates from cells with induced polyglutamine expression. *J. Cell Biol.* 153, 283–294.
- Sun, Y., Savanenin, A., Reddy, P. H., and Liu, Y. F. (2001). Polyglutamine-expanded huntingtin promotes sensitization of N-methyl-D-aspartate receptors via post-synaptic density 95. *J. Biol. Chem.* 276, 24713–24718.

- Szebenyi, G., Morfini, G. A., Babcock, A., Gould, M., Selkoe, K., Stenoien, D. L., Young, M., Faber, P. W., Macdonald, M. E., McPhaul, M. J., and Brady, S. T. (2003). Neuropathogenic forms of huntingtin and androgen receptor inhibit fast axonal transport. *Neuron* 40, 41–52.
- Tallaksen-Greene, S. J., Janiszewska, A., Benton, K., Ruprecht, L., and Albin, R. L. (2010). Lack of efficacy of NMDA receptor-NR2B selective antagonists in the R6/2 model of Huntington disease. *Exp. Neurol.* 225, 402–407.
- Tang, T. S., Chen, X., Liu, J., and Bezprozvanny, I. (2007). Dopaminergic signaling and striatal neurodegeneration in Huntington's disease. *J. Neurosci.* 27, 7899–7910.
- Tang, T. S., Guo, C., Wang, H., Chen, X., and Bezprozvanny, I. (2009). Neuroprotective effects of inositol 1,4,5-trisphosphate receptor C-terminal fragment in a Huntington's disease mouse model. *J. Neurosci.* 29, 1257–1266.
- Tang, T. S., Tu, H., Orban, P. C., Chan, E. Y., Hayden, M. R., and Bezprozvanny, I. (2004). HAP1 facilitates effects of mutant huntingtin on inositol 1,4,5-trisphosphate-induced Ca release in primary culture of striatal medium spiny neurons. *Eur. J. Neurosci.* 20, 1779–1787.
- Taniura, H., Sng, J. C., and Yoneda, Y. (2007). Histone modifications in the brain. *Neurochem. Int.* 51, 85–91.
- The Huntington Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72, 971–983.
- Thomas, E. A., Coppola, G., Desplats, P. A., Tang, B., Soragni, E., Burnett, R., Gao, E., Fitzgerald, K. M., Borok, J. F., Herman, D., Geschwind, D. H., and Gottesfeld, J. M. (2008). The HDAC inhibitor 4b ameliorates the disease phenotype and transcriptional abnormalities in Huntington's disease transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15564–15569.
- Thomas, E. A., Coppola, G., Tang, B., Kuhn, A., Kim, S., Geschwind, D. H., Brown, T. B., Luthi-Carter, R., and Ehrlich, M. E. (2011). In vivo cell-autonomous transcriptional abnormalities revealed in mice expressing mutant huntingtin in striatal but not cortical neurons. *Hum. Mol. Genet.* 20, 1049–1060.
- Thompson, P. D., Bhatia, K. P., Brown, P., Davis, M. B., Pires, M., Quinn, N. P., Luthert, P., Honovar, M., O'Brien, M. D., Marsden, C. D., and Harding, A. E. (1994). Cortical myoclonus in Huntington's disease. *Mov. Disord.* 9, 633–641.
- Tovar, K. R., and Westbrook, G. L. (1999). The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *J. Neurosci.* 19, 4180–4188.
- Trushina, E., Dyer, R. B., Badger, J. D. II, Ure, D., Eide, L., Tran, D. D., Vrieze, B. T., Legendre-Guillemain, V., McPherson, P. S., Mandavilli, B. S., Van Houten, B., Zeitlin, S., Mcniven, M., Aebersold, R., Hayden, M., Parisi, J. E., Seeborg, E., Dragatsis, I., Doyle, K., Bender, A., Chacko, C., and McMurray, C. T. (2004). Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. *Mol. Cell. Biol.* 24, 8195–8209.
- Turner, C., Cooper, J. M., and Schapira, A. H. (2007). Clinical correlates of mitochondrial function in Huntington's disease muscle. *Mov. Disord.* 22, 1715–1721.
- Tydlacka, S., Wang, C. E., Wang, X., Li, S., and Li, X. J. (2008). Differential activities of the ubiquitin-proteasome system in neurons versus glia may account for the preferential accumulation of misfolded proteins in neurons. *J. Neurosci.* 28, 13285–13295.
- Vacher, C., Garcia-Oroz, L., and Rubinshtein, D. C. (2005). Overexpression of yeast hsp104 reduces polyglutamine aggregation and prolongs survival of a transgenic mouse model of Huntington's disease. *Hum. Mol. Genet.* 14, 3425–3433.
- Vanhoutte, P., and Bading, H. (2003). Opposing roles of synaptic and extrasynaptic NMDA receptors in neuronal calcium signalling and BDNF gene regulation. *Curr. Opin. Neurobiol.* 13, 366–371.
- Venkatraman, P., Wetzel, R., Tanaka, M., Nukina, N., and Goldberg, A. L. (2004). Eukaryotic proteasomes cannot digest polyglutamine sequences and release them during degradation of polyglutamine-containing proteins. *Mol. Cell* 14, 95–104.
- Verhoef, L. G., Lindsten, K., Masucci, M. G., and Dantuma, N. P. (2002). Aggregate formation inhibits proteasomal degradation of polyglutamine proteins. *Hum. Mol. Genet.* 11, 2689–2700.
- Videnovic, A., Leurgans, S., Fan, W., Jaglin, J., and Shannon, K. M. (2009). Daytime somnolence and nocturnal sleep disturbances in Huntington disease. *Parkinsonism Relat. Disord.* 15, 471–474.
- Vogel, C. M., Drury, I., Terry, L. C., and Young, A. B. (1991). Myoclonus in adult Huntington's disease. *Ann. Neurol.* 29, 213–215.
- Vonsattel, J. P., Myers, R. H., Stevens, T. J., Ferrante, R. J., Bird, E. D., and Richardson, E. P. Jr. (1985). Neuropathological classification of Huntington's disease. *J. Neuropathol. Exp. Neurol.* 44, 559–577.
- Wexler, N. S., Lorimer, J., Porter, J., Gomez, F., Moskowitz, C., Shackell, E., Marder, K., Penchaszadeh, G., Roberts, S. A., Gayan, J., Brocklebank, D., Cherny, S. S., Cardon, L. R., Gray, J., Dlouhy, S. R., Wiktorski, S., Hodes, M. E., Conneally, P. M., Penney, J. B., Gusella, J., Cha, J. H., Irizarry, M., Rosas, D., Hersch, S., Hollingsworth, Z., Macdonald, M., Young, A. B., Andresen, J. M., Housman, D. E., De Young, M. M., Bonilla, E., Stillings, T., Negrette, A., Snodgrass, S. R., Martinez-Jaurrieta, M. D., Ramos-Arroyo, M. A., Bickham, J., Ramos, J. S., Marshall, F., Shoulson, I., Rey, G. J., Feigin, A., Arnheim, N., Acevedo-Cruz, A., Acosta, L., Alvir, J., Fischbeck, K., Thompson, L. M., Young, A., Dure, L., O'Brien, C. J., Paulsen, J., Brickman, A., Krch, D., Peery, S., Hogarth, P., Higgins, D. S. Jr., and Landwehrmeyer, B. (2004). Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3498–3503.
- Weydt, P., Pineda, V. V., Torrence, A. E., Libby, R. T., Satterfield, T. F., Lazarowski, E. R., Gilbert, M. L., Morton, G. J., Bammler, T. K., Strand, A. D., Cui, L., Beyer, R. P., Easley, C. N., Smith, A. C., Krainc, D., Luquet, S., Sweet, I. R., Schwartz, M. W., and La Spada, A. R. (2006). Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. *Cell Metab.* 4, 349–362.
- Wheelock, V. L., Tempkin, T., Marder, K., Nance, M., Myers, R. H., Zhao, H., Kayson, E., Orme, C., and Shoulson, I. (2003). Predictors of nursing home placement in Huntington disease. *Neurology* 60, 998–1001.
- Xie, Y., Hayden, M. R., and Xu, B. (2010). BDNF overexpression in the forebrain rescues Huntington's disease phenotypes in YAC128 mice. *J. Neurosci.* 30, 14708–14718.
- Yanai, A., Huang, K., Kang, R., Singaraja, R. R., Arstikaitis, P., Gan, L., Orban, P. C., Mullard, A., Cowan, C. M., Raymond, L. A., Drisdell, R. C., Green, W. N., Ravikumar, B., Rubinshtein, D. C., El-Husseini, A., and Hayden, M. R. (2006). Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. *Nat. Neurosci.* 9, 824–831.
- Yang, L., Mei, Q., Zielinska-Kwiatkowska, A., Matsui, Y., Blackburn, M. L., Benedetti, D., Krumm, A. A., Taborsky, G. J. Jr., and Chansky, H. A. (2003). An ERG (ets-related gene)-associated histone methyltransferase interacts with histone deacetylases 1/2 and transcription co-repressors mSin3A/B. *Biochem. J.* 369, 651–657.
- Zeitlin, S., Liu, J. P., Chapman, D. L., Papaioannou, V. E., and Efstratiadis, A. (1995). Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. *Nat. Genet.* 11, 155–163.
- Zeron, M. M., Fernandes, H. B., Krebs, C., Shehadeh, J., Wellington, C. L., Leavitt, B. R., Baimbridge, K. G., Hayden, M. R., and Raymond, L. A. (2004). Potentiation of NMDA receptor-mediated excitotoxicity linked with intrinsic apoptotic pathway in YAC transgenic mouse model of Huntington's disease. *Mol. Cell. Neurosci.* 25, 469–479.
- Zeron, M. M., Hansson, O., Chen, N., Wellington, C. L., Leavitt, B. R., Brundin, P., Hayden, M. R., and Raymond, L. A. (2002). Increased sensitivity to N-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. *Neuron* 33, 849–860.
- Zhai, W., Jeong, H., Cui, L., Krainc, D., and Tjian, R. (2005). In vitro analysis of huntingtin-mediated transcriptional repression reveals multiple transcription factor targets. *Cell* 123, 1241–1253.
- Zhang, H., Li, Q., Graham, R. K., Slow, E., Hayden, M. R., and Bezprozvanny, I. (2008). Full length mutant huntingtin is required for altered Ca²⁺ signaling and apoptosis of striatal neurons in the YAC mouse model of Huntington's disease. *Neurobiol. Dis.* 31, 80–88.

- Zhang, S. J., Steijaert, M. N., Lau, D., Schutz, G., Delucinge-Vivier, C., Descombes, P., and Bading, H. (2007). Decoding NMDA receptor signaling: identification of genomic programs specifying neuronal survival and death. *Neuron* 53, 549–562.
- Zuccato, C., Belyaev, N., Conforti, P., Ooi, L., Tartari, M., Papadimou, E., Macdonald, M., Fossale, E., Zeitlin, S., Buckley, N., and Cattaneo, E. (2007). Widespread disruption of repressor element-1 silencing transcription factor/neuron-restrictive silencer factor occupancy at its target genes in Huntington's disease. *J. Neurosci.* 27, 6972–6983.
- Zuccato, C., and Cattaneo, E. (2009). Brain-derived neurotrophic factor in neurodegenerative diseases. *Nat. Rev. Neurol.* 5, 311–322.
- Zuccato, C., Ciammola, A., Rigamonti, D., Leavitt, B. R., Goffredo, D., Conti, L., Macdonald, M. E., Friedlander, R. M., Silani, V., Hayden, M. R., Timmusk, T., Sipione, S., and Cattaneo, E. (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 293, 493–498.
- Zuccato, C., Tartari, M., Crotti, A., Goffredo, D., Valenza, M., Conti, L., Cataudella, T., Leavitt, B. R., Hayden, M. R., Timmusk, T., Rigamonti, D., and Cattaneo, E. (2003). Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nat. Genet.* 35, 76–83.
- Zuhlke, C., Riess, O., Bockel, B., Lange, H., and Thies, U. (1993). Mitotic stability and meiotic variability of the (CAG)_n repeat in the Huntington disease gene. *Hum. Mol. Genet.* 2, 2063–2067.
- Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
- Received: 12 May 2011; accepted: 04 August 2011; published online: 23 August 2011.
- Citation: Roze E, Cahill E, Martin E, Bonnet C, Vanhoutte P, Betuing S and Caboche J (2011) Huntington's disease and striatal signaling. *Front. Neuroanat.* 5:55. doi: 10.3389/fnana.2011.00055
- Copyright © 2011 Roze, Cahill, Martin, Bonnet, Vanhoutte, Betuing and Caboche. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.



Basal ganglia disorders associated with imbalances in the striatal striosome and matrix compartments

Jill R. Crittenden* and Ann M. Graybiel

Brain and Cognitive Sciences Department and McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA, USA

Edited by:

Emmanuel Valjent, Institut de
Génomique Fonctionnelle, INSERM
U661, UMR 5203 CNRS, Université
Montpellier 1 & 2, France

Reviewed by:

Ricardo Insausti, University of Castilla
la Mancha, Spain
James M. Tepper, Rutgers The State
University of New Jersey, USA

*Correspondence:

Jill R. Crittenden, McGovern Institute
for Brain Research, Massachusetts
Institute of Technology, 46-6133,
77 Massachusetts Avenue,
Cambridge, MA 02139, USA
e-mail: jrc@mit.edu

The striatum is composed principally of GABAergic, medium spiny striatal projection neurons (MSNs) that can be categorized based on their gene expression, electrophysiological profiles, and input–output circuits. Major subdivisions of MSN populations include (1) those in ventromedial and dorsolateral striatal regions, (2) those giving rise to the direct and indirect pathways, and (3) those that lie in the striosome and matrix compartments. The first two classificatory schemes have enabled advances in understanding of how basal ganglia circuits contribute to disease. However, despite the large number of molecules that are differentially expressed in the striosomes or the extra-striosomal matrix, and the evidence that these compartments have different input–output connections, our understanding of how this compartmentalization contributes to striatal function is still not clear. A broad view is that the matrix contains the direct and indirect pathway MSNs that form parts of sensorimotor and associative circuits, whereas striosomes contain MSNs that receive input from parts of limbic cortex and project directly or indirectly to the dopamine-containing neurons of the substantia nigra, pars compacta. Striosomes are widely distributed within the striatum and are thought to exert global, as well as local, influences on striatal processing by exchanging information with the surrounding matrix, including through interneurons that send processes into both compartments. It has been suggested that striosomes exert and maintain limbic control over behaviors driven by surrounding sensorimotor and associative parts of the striatal matrix. Consistent with this possibility, imbalances between striosome and matrix functions have been reported in relation to neurological disorders, including Huntington's disease, L-DOPA-induced dyskinesias, dystonia, and drug addiction. Here, we consider how signaling imbalances between the striosomes and matrix might relate to symptomatology in these disorders.

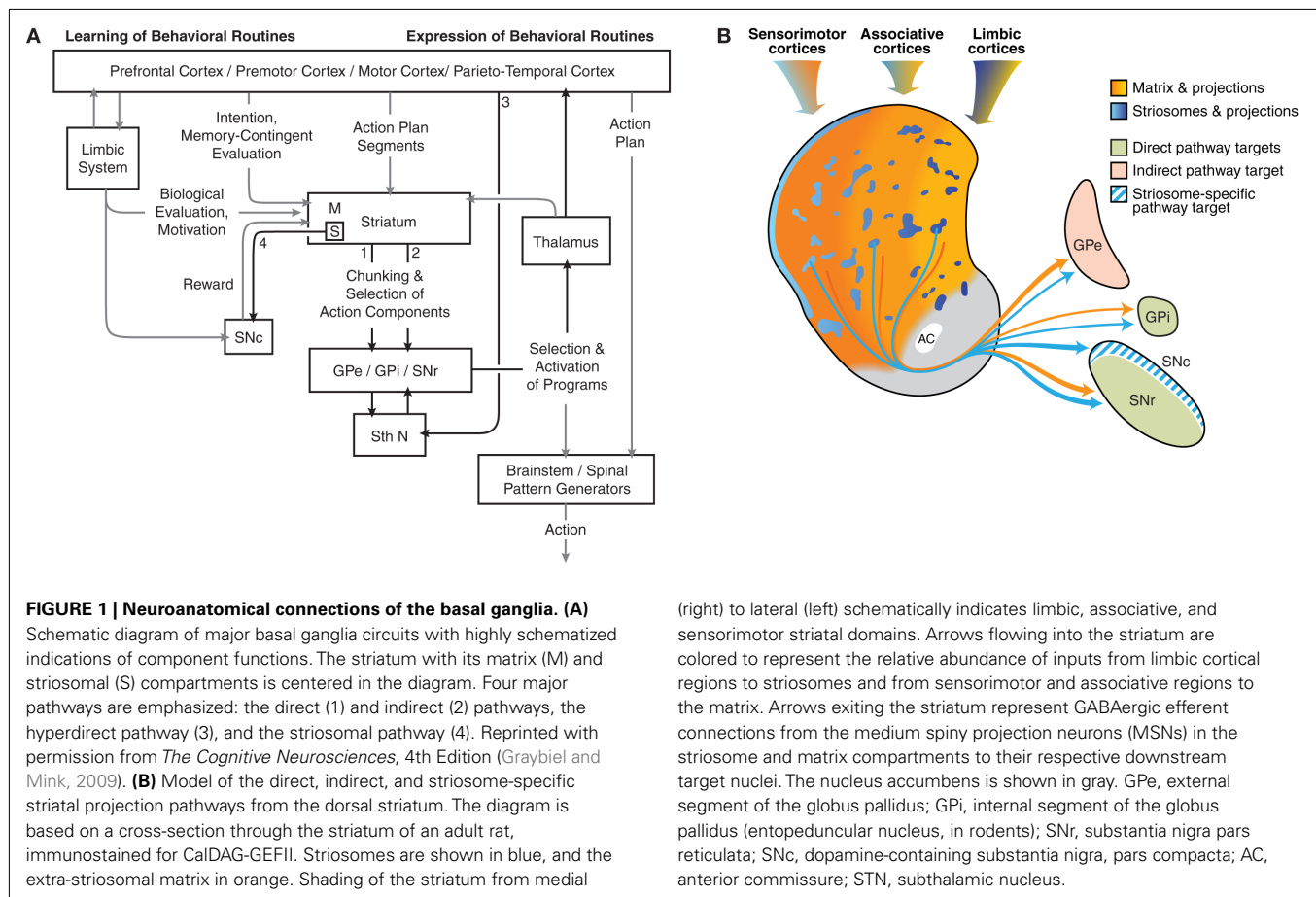
Keywords: striatum, medium spiny neuron, Huntington's disease, Parkinson's disease, dystonia, dyskinesia, substantia nigra, CalDAG-GEF

INTRODUCTION

The striatum is the primary input side of the basal ganglia, a set of subcortical brain regions that are important for the control of voluntary movement and our ability to learn patterns of behavior that maximize reward. The striatum integrates incoming information from all regions of the cerebral cortex in order to build motor patterns based on the current environment and desires as well as on past experience (Graybiel, 2008; **Figure 1A**). Distinct, largely parallel cortico-basal ganglia-thalamo-cortico loops have been proposed to control different aspects of behavior (Alexander et al., 1986) and different stages of behavioral learning (Yin et al., 2008). There are marked changes in activity across the striatum as animals undergo the transition from early-stage, goal-directed motor learning to habitual, goal-independent behaviors (Thorn et al., 2010). Lesion studies suggest that the ventromedial striatum drives the early stages of motor learning and that the dorsolateral striatum is more important for the development of habitual behaviors (Yin et al., 2004; Atallah et al., 2007). Considering its key function in reward-based learning, it is no surprise that the striatum is implicated in a wide variety of behaviors. This breadth

of function is strikingly exhibited in the range of clinical disorders for which abnormal functioning of the striatum has been found, including not only classical motor disorders: Parkinson's disease (PD), Huntington's disease (HD), and dystonia, but also habit formation and drug addiction, impulsivity and attention-deficit hyperactivity disorder (ADHD), compulsivity and obsessive compulsive disorder, emotional control, and depression (for review, see Graybiel and Mink, 2009).

Incoming cortical activity is modulated in the striatum by neurochemicals that reflect mood, motivation, and expectation. The striatum is heavily enriched in a number of neurochemical receptor systems including those for dopamine, acetylcholine, endocannabinoids, and endogenous opiates (Graybiel, 1990). Striatal circuits for appropriate behaviors can thus be engaged according to the neurochemical receptors that they express. For example, medium spiny striatal projection neurons (MSNs) that are part of a circuit that promotes movement express the Drd1 (D1) dopamine receptor, which boosts MSN cell excitability. MSNs that are part of a circuit that suppresses movement express the Drd2 (D2) dopamine receptor, which diminishes excitability of this MSN



cell-type. Thus, dopamine has a pro-movement effect by simultaneously promoting and disinhibiting movement through the so-called direct and indirect pathways, respectively (Albin et al., 1989; Delong, 1990).

Balanced regulation of the direct and indirect pathways is important for motor control, as evidenced by deficits associated with selective disruption of either the direct pathway (D1-enriched), or the indirect pathway (D2-enriched). The direct pathway MSNs target primarily two nuclei of the basal ganglia – the internal segment of the globus pallidus (GPi), known in rodents as the entopeduncular nucleus (EP), and the substantia nigra pars reticulata (SNr; **Figure 1B**). The SNr and GPi in turn send their outputs to brainstem nuclei and to components of the motor thalamus, which excite cortical areas related to motor control. The indirect pathway MSNs project to the external pallidal segment (GPe, often known simply as the globus pallidus in rodents), which inversely controls these same thalamo-cortical motor circuits. Support for this model, oversimplified as it undoubtedly is, has flourished with the development of genetically engineered mice and cell-type specific viral vectors in which the direct and indirect pathways can be separately characterized and controlled, including by optogenetic intervention (Valjent et al., 2009; Kravitz and Kreitzer, 2011). Such studies have contributed to our understanding of symptoms and therapies in movement disorders, including PD and HD, in which there is neurodegeneration of basal ganglia nuclei (Albin et al., 1989, 1995).

Studies of how the striatum controls movement have focused heavily on dopamine and the direct and indirect pathways. A less well-studied but equally distinct pair of striatal circuits originates in striatal compartments that are known as the striosomes (or patch) and matrix. These compartments can be defined by their pronounced segregation of numerous neurotransmitter-related signaling molecules (Graybiel, 1990). The striosomal compartment of the dorsal striatum forms a labyrinthine, interconnected structure that is embedded in the surrounding striatal matrix (Pert et al., 1976; Graybiel and Ragsdale, 1978; Herkenham and Pert, 1981; Gerfen, 1984). Whereas the main afferent connections of the matrix are related to associative and sensorimotor regions, striosomes have predominant limbic-related connections (Jimenez-Castellanos and Graybiel, 1987; Gerfen, 1989; Eblen and Graybiel, 1995; Kincaid and Wilson, 1996). Both striosomes and matrix contain MSNs that project to the direct and indirect pathway target nuclei but, in some documented cases, to separate subregions. Most, but not all, of the local connectivity of these MSNs is with other neurons within their own compartment or with interneurons located near the compartment borders (Somogyi et al., 1981; Gerfen, 1984; Walker et al., 1993; Kincaid and Wilson, 1996; Tokuno et al., 2002; Levesque and Parent, 2005; Fujiyama et al., 2006, 2011). The striosome compartment is thought to contain the only striatal neurons that have direct projections to the substantia nigra, pars compacta (SNC), which contains dopamine-producing neurons that project back to the entire dorsal striatum

(Gerfen, 1984; Jimenez-Castellanos and Graybiel, 1989; Tokuno et al., 2002; Fujiyama et al., 2011).

The striosome and matrix compartments are typically defined by their gene expression profiles. Upward of 60 genes are reported to be differentially expressed in the striosomes and matrix (Table 1). This review is based on the proposition that these distinct molecular expression patterns are likely to reflect specialized processing by striosome-based circuits of the basal ganglia. As evidence already suggests that striosomes and matrix have differential vulnerability patterns in some basal ganglia-associated disorders, there is a clear need to understand how these compartments influence behavior in health and disease. Until very recently, the functions of striosomes and matrix have been surmised primarily on the basis of their activation profiles, mainly by immediate early gene (IEG) induction assays, and on the basis of their distinct input–output connections. The creation of genetic tools by which these two compartments can be selectively manipulated will certainly shed light on their function. Here, we review differences between the striosomes and matrix at the anatomical and gene expression level and differences between these two compartments in a wide variety of diseases associated with abnormal movement and emotion.

DISTINGUISHING FEATURES OF THE STRIOSOME AND MATRIX COMPARTMENTS OF THE DORSAL STRIATUM

DEVELOPMENT OF THE STRIOSOME AND MATRIX COMPARTMENTS

A striosome-substantia nigra-striatum circuit is established relatively early in the developing striatum. The striosomal neurons are born in a discrete time-window, during the time that layer 6 neurons of the neocortex are born (Graybiel and Hickey, 1982), as though forming an ontogenetic unit. They begin to migrate out from the lateral ganglionic eminence earlier in development than most matrix neurons, which are mainly born later in embryogenesis. The developing striosomes correspond to the “dopamine islands” formed by incoming dopamine-containing nigrostriatal fibers (Olson et al., 1972; Graybiel, 1984; Moon Edley and Herkenham, 1984; Fishell and Van Der Kooy, 1987). Anatomical studies suggest that the clusters of MSNs in the proto-striosomes begin to mature just in advance of the dopamine-containing fibers that demarcate the clusters (Newman-Gage and Graybiel, 1988). The dopamine-containing fiber input to striosomes is nevertheless required for the expression of some striosomal markers, including the mu opioid receptor (Van Der Kooy and Fishell, 1992).

The protostriosomal neurons project to developing dopamine-containing neurons in the ventral mesencephalon and secrete Reelin (Nishikawa et al., 1999), a protein that is essential for the lateral migration of dopamine-containing neurons that come to form the SNc (Nishikawa et al., 2003). The neurotrophic factor, GDNF, is also enriched in striosomes, and its expression in the striatum is important for survival of the dopamine-containing neurons (Lopez-Martin et al., 1999; Oo et al., 2005). As later-born matrix neurons migrate out into the striatum, they form caudal-to-rostral and lateral-to-medial gradients of migration, beginning around E20 in rats (Liu and Graybiel, 1992) and at comparable times in cats (Newman-Gage and Graybiel, 1988). Peri-natally, cell-type specific expression of ephrins and their receptors serve to

compartmentalize the MSNs into striosome and matrix compartments (Janis et al., 1999; Passante et al., 2008). Finally, extracellular matrix proteins and peri-neuronal nets have dynamic compartmental distributions that may contribute to the consolidation of synaptic circuits and the mature appearance of the striatum by about P20 (Liu and Graybiel, 1992; Lee et al., 2008).

In adulthood, striosomes make up roughly 15% of the volume in the dorsal striatum (Johnston et al., 1990) of humans, monkeys, rats, and mice and they are largest in the rostral and medial parts of the striatum – to be precise, the medial and anterior caudoputamen in rodents and the caudate nucleus and anterior putamen in primates (Graybiel and Ragsdale, 1978). Striosome–matrix compartmentalization is largely, but not entirely, observed by the dendrite and local axon collaterals of striatal neurons. MSNs have axon collaterals that mainly synapse onto neighboring MSNs within the same compartment, and the dendritic trees of MSNs tend to be compartmentally confined (Walker et al., 1993; Kincaid and Wilson, 1996; Yung et al., 1996; Fujiyama et al., 2011). It is thought that differential activation of striosomes and matrix could be modulated, in part, by differentially distributed striatal interneurons, including cholinergic interneurons and somatostatin/nitric oxide synthase-positive interneurons that tend to be located at compartment borders (Graybiel et al., 1986; Kubota and Kawaguchi, 1993; Aosaki et al., 1994; Saka et al., 2002; Miura et al., 2007).

A number of transcription factors, including Ctip2 (Arlotta et al., 2008), RAR β (Liao et al., 2008), and Dlx1/2 (Anderson et al., 1997) are required for the differentiation of MSNs. The compartment-enriched expression of multiple genes has been found to change not only prenatally, but also during the period between birth and maturity, as the compartmental pattern matures. Remarkably, despite the limitation of anatomical marking of post-mortem human brain sections, evidence suggests that the outlines of this developmental pattern are visible in humans. Striosomes have been identified in prenatal striatum by histochemical (acetylcholinesterase) staining methods and a switch in the compartmental staining with this marker occurs during the postnatal period (Graybiel and Ragsdale, 1980). There are also cross-species variations in compartmental gene enrichment. Table 1 summarizes molecules reported to be differentially expressed in striosomes or extra-striosomal matrix, with a bias toward differences reported in adult animals.

COMMON AND DISTINCT EFFERENT CONNECTIONS OF THE STRIOSOME AND MATRIX COMPARTMENTS

Definitive evidence about the connections of striosomal MSNs has been difficult to obtain for technical reasons, but a recent study based on single-neuron labeling now suggests that in adult rats, striosomal MSNs may be unique among striatal MSNs in projecting directly to the SNc, which contains dopamine-producing neurons (Fujiyama et al., 2011). This single-neuron evidence is in accord with earlier work with classical anterograde and retrograde tracing methods (Gerfen, 1985; Jimenez-Castellanos and Graybiel, 1989; Tokuno et al., 2002). One such retrograde labeling experiment indicated that the densocellular zone of the SNc, which exhibits low levels of acetylcholinesterase (AChE) immunoreactivity relative to the surrounding region, is preferentially targeted by striosomes, which are also AChE-poor (Jimenez-Castellanos and

Table 1 | Molecules with differential expression between the striosome and matrix compartments of the dorsal striatum.

Gene/protein abbreviation	Full name	Species	Reference
STRIOSOME-ENRICHED			
5HT2a	Serotonin receptor type 2a	Human; monkey; mouse	Lopez-Gimenez et al. (1999, 2001, 2002)
5HT2c	Serotonin receptor type 2c	Rat	Ward and Dorsa (1996)
AADC	Aromatic, l-amino acid decarboxylase	Early postnatal mouse	Kim et al. (2002)
Adora2A	Adenosine receptor type 2A	Early postnatal rat	Schiffmann and Vanderhaeghen (1992)
Bcl-2	B-cell CLL/lymphoma 2	Monkey	Bernier and Parent (1998)
C3HC4	Membrane-associated ring finger 4	Mouse	Gensat (Gong et al., 2003)
CaLDAG-GEFII/RasGRP	Calcium/diacylglycerol-regulated guanine nucleotide exchange factor II	Rat	Pierret et al. (2002), Crittenden et al. (2009)
CALB2	Calretinin	Mouse	Davis and Puhl (2011)
CHRM	Cholinergic receptors, muscarinic (ligand binding assay)	Fetal human; E50 and adult cat; Ferret; Rat	Nastuk and Graybiel (1985)
Cxcr4	Chemokine (C-X-C motif) receptor 4	P7 mouse	Gensat (Gong et al., 2003)
DARPP-32	Dopamine and cAMP-regulated phosphoprotein 32 kDa	P0 mouse	Arlotta et al. (2008)
Drd1	Dopamine receptor D1	Human; monkey; cat; rat; early postnatal mouse	Besson et al. (1988), Murrin and Zeng (1989), Kim et al. (2002)
Drd4	Dopamine receptor D4	Monkey; rat; mouse	Rivera et al. (2002)
CD73	Ecto-5'-nucleotidase (enzymatic assay)	Rat	Schoen and Graybiel (1992)
Egr1	Early growth response 1	P7 mouse	Gensat (Gong et al., 2003)
EphA7	Ephrin receptor A7	P7 mouse	Gensat (Gong et al., 2003)
Fam131	Family with sequence similarity 131, member B	Mouse	Gensat (Gong et al., 2003)
FoxP2	Forkhead box P2	Prenatal monkey; P3-P20	Takahashi et al. (2003, 2008)
Gαolf	Olfactory type G-protein, alpha subunit	Mouse	Sako et al. (2010)
Gad2/Gad65	Glutamate decarboxylase 2	Mouse	Gensat (Gong et al., 2003)
GNF	Glial cell line-derived neurotrophic factor	Early postnatal rat	Lopez-Martin et al. (1999)
GluR1	Glutamate receptor, ionotropic, AMPA 1	P0 mouse	Martin et al. (1993a,b), Arlotta et al. (2008)
GRIK	Glutamate receptor, ionotropic, kainate (ligand binding assay)	Human	Dure et al. (1992)
HTT	Huntingtin protein	Rat	Kosinski et al. (1997)
IP3R	Inositol 1,4,5-triphosphate receptor	Monkey	Fotuhi et al. (1993)
Kcnp2	Kv channel-interacting protein 2	P0 mouse	Arlotta et al., 2008)
KChIP1	Kv4 potassium channel subunit potassium channel-interacting protein 1	Monkey	Mikula et al. (2009)
LSAMP	Limbic system-associated membrane protein	Cat	Chesselet et al. (1991)
MAP2	Microtubule-associated protein 2	P0 mouse	Arlotta et al. (2008)
MOR1	Opioid receptor, mu 1	Rat; mouse; P0 mouse	Herkenham and Pert (1981), Arlotta et al. (2008)
Nnat	Neuronatin	Mouse	Gensat (Gong et al., 2003)
NPY2R	Neuropeptide Y receptor Y2	P7 mouse	Gensat (Gong et al., 2003)
N-TAF1	TBP-associated RNA polymerase II	Rat	Sako et al. (2011)
Ntrk3	Neurotrophic tyrosine kinase receptor type 3	Mouse	Gensat (Gong et al., 2003)
Nur77/NR4a1	Nuclear hormone receptor	Mouse	Davis and Puhl (2011), Gensat (Gong et al., 2003)
PBX3	Pre-B-cell leukemia homeobox 3	Prenatal monkey	Takahashi et al. (2008)
PCDH	OL-Protocadherin	P2 mouse	Redies et al. (2002)
Pdyn	Prodynorphin	Mouse	Gensat (Gong et al., 2003)
Phactr1	Phosphatase and actin regulator	P7 mouse	Gensat (Gong et al., 2003)
PIK3R1	Phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	P7 mouse	Gensat (Gong et al., 2003)
PLCgamma	Phospholipase C, gamma	Primate	Fotuhi et al. (1993)

(Continued)

Table 1 | Continued

Gene/protein abbreviation	Full name	Species	Reference
PLCbeta	Phospholipase C, beta	Primate	Fotuhi et al. (1993)
RELN	Reelin	P3 rat; P0 mouse	Nishikawa et al. (1999), Arlotta et al. (2008)
SCG2	Secretogranin II	P7 mouse	Gensat (Gong et al., 2003)
SpdS	Spermidine synthase	Rat	Krauss et al. (2006)
MATRIX-ENRICHED			
5HT2a	Serotonin receptor type 2a	Rat	Ward and Dorsa (1996)
AchE	Acetylcholinesterase	Monkey; cat; rat	Herkenham and Pert (1981), Graybiel (1984), Graybiel et al. (1986)
CaBP	Calcium-binding protein	Monkey; rat	Gerfen et al. (1985)
CALB1	Calbindin 1, 28 kDa	Human; monkey; rat; embryonic rat; mouse	Martin et al. (1991), Ito et al. (1992), Liu and Graybiel (1992), Davis and Puhl (2011)
CalDAG-GEFI/RasGRP2	Calcium/diacylglycerol-regulated guanine nucleotide exchange factor I	Rat; mouse	Kawasaki et al. (1998), Crittenden et al. (2010a)
Cb1	Cannabinoid receptor 1	Human	Villares (2007)
CDH8	Cadherin-8	P0–P8 rat	Korematsu et al. (1998)
Cdk5-pY15	Cyclin-dependent kinase 5, phosphorylated at Y15	Mouse	Morigaki et al. (2011)
ChAT	Choline O-acetyltransferase	Human; monkey; cat	Graybiel et al. (1986), Hirsch et al. (1989)
Ebf1	Early B-cell factor 1	P0 mouse	Liao et al. (2005), Arlotta et al. (2008)
EphA4	Ephrin receptor A4	Rat	Martone et al. (1997)
GluR	Glutamate receptors, AMPA (ligand binding assay)	Human	Dure et al. (1992)
Htr4	5-hydroxytryptamine (serotonin) receptor 4	Mouse	Gensat (Gong et al., 2003)
NADPH diaphorase	NADPH diaphorase (enzymatic activity assay)	Human; cat	Sandell et al. (1986), Morton et al. (1993)
Preproenk	Enkephalin	GFP reporter mice	Koshimizu et al. (2008)
SOD2	Superoxide dismutase 2, mitochondrial	Human; monkey	Zhang et al. (1994), Medina et al. (1996)
SST	Somatostatin	Rat	Gerfen et al. (1985)
TH	Tyrosine hydroxylase	Human; monkey; early postnatal cat; early postnatal mouse	Graybiel (1984), Graybiel et al. (1987)
VGLUT2	Vesicular glutamate transporter 2	Rat	Fujiyama et al. (2006)

Differential immunoreactivity can result from differential expression in MSNs, striatal interneurons or afferents that preferentially target one compartment. When developmental stage is not indicated, the finding was made in adults. P, postnatal day; E, embryonic day.

Graybiel, 1989). In the rat, the striatonigral fibers have been suggested to form symmetric synapses on both the dendrites and cell bodies of the dopamine-containing neurons (Somogyi et al., 1981; Gerfen, 1984; Tokuno et al., 2002; Fujiyama et al., 2011). A monosynaptic connection between MSNs and dopamine-producing cells of the SNc is also consistent with electrophysiological recording assays in rats (Lee and Tepper, 2009). If confirmed by further work, the direct striosome-SNc projection would parallel a ventral striatal input to the SNc (Somogyi et al., 1981). The striosomes and ventral striatum are in a position to exert global control over dopamine signaling in the dorsal striatum.

Aside from this connection, both striosomes and matrix share in projecting to the main output nuclei of the basal ganglia, but in different proportions. These projections are more abundant from the large matrix compartment (Gerfen and Young, 1988; Jimenez-Castellanos and Graybiel, 1989; Rajakumar et al., 1993; Tokuno et al., 2002; Levesque and Parent, 2005; Fujiyama et al., 2011).

Efferents from MSNs in the matrix have consistently been found not to project to the SNc but, rather, to project to the GPe, GPi, and SNr (Gerfen, 1984; Kawaguchi et al., 1990; Gimenez-Amaya and Graybiel, 1991; Levesque and Parent, 2005; Chuhma et al., 2011; Fujiyama et al., 2011). Likewise, a juxtacellular labeling experiment identified six MSNs, all located within the same striosome in the striatum of a squirrel monkey, that had projections to the GPi, GPe, and non-dopaminergic SNr, rather than to the dopamine-containing SNc (Levesque and Parent, 2005). Striosomal MSNs targeting the GPi, GPe, and SNr have also been reported in the rat. Striosomal MSNs that target the GPi/EP are reported to innervate specifically the regions of the GPi that in turn project to the lateral habenula, whereas the matrix MSNs targeting GPi/EP project to regions that are interconnected to motor regions of the thalamus (Rajakumar et al., 1993). This pathway to the habenula is notable, because the lateral habenula, itself interconnected with the prefrontal cortex, can inhibit dopamine cell firing in the SNc (Herkenham

and Nauta, 1979; Christoph et al., 1986; Ji and Shepard, 2007; Matsumoto and Hikosaka, 2007; Bromberg-Martin et al., 2010). Thus, the striosomes appear to represent a unique dopamine- and limbic system-related circuit that is situated to influence the associative and sensorimotor regions of the striatum.

Based on these findings, a three-compartment model of striatal efferent connectivity has been proposed, including: (1) the direct pathway targeting the SNr with collaterals in the GPi/EP and GPe, (2) the indirect pathway targeting only the GPe, and (3) the striosome-specific pathway targeting the SNc, with collaterals in the GPi/EP and GPe (**Figure 1B**; Graybiel et al., 2000; Fujiyama et al., 2011). The apparently unique projection of striosomes to the SNc, if confirmed, would place striosomes in a functionally pivotal position to influence the dopamine system of the midbrain.

RELATIONSHIP BETWEEN THE DIRECT-INDIRECT PATHWAY DIVISIONS AND STRIOSOME-MATRIX COMPARTMENTS

MSNs can be classified according to whether they give rise to either the direct or indirect pathways, and they can also be classified according to whether they are in striosomes or in the extra-striosomal matrix. These are two distinct classificatory schemes. Most of the neurons of direct and indirect pathways lie in the matrix, which makes up approximately 80% of the volume of the striatum. There is evidence that some neurons in striosomes project to direct pathway targets, and others (fewer in number) project to indirect pathway target nuclei. Single-fiber tracing methods have allowed definitive identification of the collaterals of efferent axons of neurons identified as belonging to either striosomes or matrix. Fujiyama et al. (2011) labeled 11 striosomal neurons that were distributed across the striatum and in different rats, and found that 8 projected to the SNc, with collaterals in one or more of the direct pathway targets (SNr, GPi/EP, and GPe) and 3 exhibited an indirect pathway projection pattern, solely to the GPe. Similarly, in the monkey labeling experiment by Parent and colleagues, all six of the labeled striosomal neurons projected to the direct pathway nuclei (Levesque and Parent, 2005). Thus, at least some striosomes have an overabundance of neurons that target GPi/EP and SNr, but they may target specific regions of these nuclei, relative to matrix MSNs. In the matrix, as a whole, the MSNs are equally divided between the direct and indirect pathway projection types. MSNs that target the SNc appear to be restricted to the striosomes and are not found in the matrix.

Direct and indirect pathway MSNs are often identified by their expression of peptides in addition to their expression of dopamine receptors. Direct pathway D1-positive MSNs express substance P and prodynorphin, whereas the indirect pathway D2-positive MSNs express proenkephalin/enkephalin (Gerfen and Young, 1988). In adult rats, an estimated 5% of MSNs in the dorsal striatum express both D1/substance P and D2/enkephalin, whereas co-expression in the ventral striatum may be slightly higher (Surmeier et al., 1996; Wang et al., 2006a; Bertran-Gonzalez et al., 2008; Perreault et al., 2010). D1/D2 heteromers have been discovered and are thought to signal through phospholipase C, a calcium-dependent signaling cascade, rather than through the canonical adenylyl cyclase, cAMP-dependent dopamine receptor signaling cascade (Lee et al., 2004; Perreault et al., 2010). Neurons that co-express substance P and enkephalin have been found

in both striosomes and matrix, but may represent a greater proportion of the MSNs in the striosomal compartment than in the matrix (Besson et al., 1990; Wang et al., 2007). Furthermore, co-expression of substance P and enkephalin was abundant in terminals in the SNc, but not in the GPe, GPi, or SNr (Wang et al., 2006a). Thus, it is possible that some of the unique subset of striosomal neurons that target the SNc co-express D1 and D2. This would stand in contrast to the direct pathway neurons of the matrix, which express primarily D1. However, the expression profiles of the striosome-specific pathway MSNs, which target the SNc, have not yet been thoroughly analyzed. Such analysis should take into account that the expression levels of the proteins that are typically used to define the pathways and compartments, including prodynorphin, mu opioid receptor, and dopamine receptors, can change with experience (Moratalla et al., 1996; Cenci et al., 1998; Adams et al., 2003; Jabourian et al., 2005; Perreault et al., 2010).

In summary, data support a model in which MSNs projecting to the SNc are confined to striosomes (striosome-specific pathway), whereas MSNs contributing to the direct and indirect pathway are present mainly in the matrix compartment but also in striosomes. The direct and indirect pathway target nuclei (GPi, SNr, and GPe/EP) receive more inputs from MSNs in the matrix than those in striosomes, because the matrix compartment is much larger than the striosomal compartment. The balance of signaling between the direct and indirect pathways is strongly influenced by dopamine and, therefore, striosomal MSNs are in a position to control this balance through their projection to the dopamine-containing neurons of the SNc. Cholinergic interneurons, which are often located at the border between striosomes and matrix, can also control dopamine release (Threlfell and Cragg, 2011) and the balance of direct vs. indirect pathway activation (Ding et al., 2011).

Amemori et al. (2011) propose a model whereby striosomes and indirect pathway MSNs select modules of previously learned behaviors that are appropriate for the given environmental context. In this model, the direct pathway selects specific actions within each striatal module, whereas the indirect pathway serves to select among modules. Based on contextual prediction errors originating in the limbic system, striosomes provide “responsibility signals” that regulate the activity of adjacent matrix MSNs within their respective modules. This modular regulation is amplified through the indirect pathway by virtue of funneling of representations, which serves to select modules (rather than specific actions) based on contextual information. This model is supported by computational modeling experiments and would support context-dependent learning and faster behavioral adaptation in a fluctuating environment.

CEREBRAL CORTEX AFFERENTS TO STRIOSOME AND MATRIX COMPARTMENTS

Although a majority of neocortical areas may project to both striosomes and matrix, there are major differences in the strength of the projections to the two compartments. In keeping with the concept that ontogeny recapitulates phylogeny, striosomal MSNs not only begin differentiating before matrix MSNs, but also are innervated

by neurons in more evolutionarily conserved regions of the cerebral cortex, especially the limbic medial and lateral margins of the cortical plate (Ragsdale and Graybiel, 1988; Gerfen, 1989). The orbitofrontal, anterior cingulate, and insular cortices preferentially innervate striosomes, whereas projections from the somatosensory, motor, and association cortices terminate mainly in the matrix (Ragsdale and Graybiel, 1990; Flaherty and Graybiel, 1994; Eblen and Graybiel, 1995; Kincaid and Wilson, 1996; Levesque and Parent, 1998). The amygdala, hippocampus, and nucleus accumbens (part of the ventral striatum) are interconnected with the orbitofrontal, anterior cingulate, and insular cortices, supporting the idea that the striosomes are part of a limbic circuit embedded in the sensorimotor and associative striatum (Graybiel, 2008). Indeed, in the cat, part of the amygdala itself also projects to part of the striosomal system (Ragsdale and Graybiel, 1988).

The strong innervation of medial striosomes by limbic neocortical regions that have reduced superficial layers is consistent with the fact that striosomal MSNs receive preferential inputs from deep cortical layer Vb (Gerfen, 1989; Eblen and Graybiel, 1995; Kincaid and Wilson, 1996; Levesque and Parent, 1998). MSNs in the matrix receive input from ontogenically younger, and putatively evolutionarily younger, cortical layers (III–Va). This is consistent with the finding that the somatosensory cortex, which is part of the neocortex that has a well-defined upper layer Va, projects to the matrix and not to the striosomes (Wilson, 1987; Gerfen, 1989). Nevertheless, the correlation between evolutionary age and compartment-specific targeting of corticostriatal neurons is speculative and does not fully represent the types of compartmentalized targeting that have been observed. For example, labeling of efferents from individual cortical regions was found to preferentially fill striosomes dorsally but to avoid striosomes ventrally, within their striatal target zones (Ragsdale and Graybiel, 1990). The striatal target zones themselves progressed dorso-ventrally according to the neighboring cortical regions from which they arose and thus, neighboring striosome–matrix compartments received input from neighboring cortical regions.

Corticostriatal projection neurons send efferents to other brain regions, including the brainstem, thalamus, claustrum, and contralateral cortex (Wilson, 1987; Kincaid and Wilson, 1996; Levesque et al., 1996). Upper layer V is enriched in intratelencephalic-projecting (IT-type) neurons that can bifurcate to both hemispheres and synapse preferentially onto D1-positive MSNs. By contrast, deep layer V is enriched in pyramidal tract-projecting (PT-type) neurons that send collaterals preferentially to D2-positive MSNs in the ipsilateral striatum (Reiner et al., 2003). Axon terminals from PT-type cortical neurons were found to be larger and more often contacted a perforated post-synaptic density, compared to the IT-type corticostriatal terminals that contacted direct pathway MSNs (Reiner et al., 2010). This is consistent with the preferential activation of IEGs in enkephalin-containing neurons found in response to activation of the motor cortex (Berretta et al., 1997; Parthasarathy and Graybiel, 1997). Based on evidence that afferents to striosomes arise preferentially from deep layers of limbic cortex, one might expect striosomes to receive preferential PT-type inputs. However, the properties of corticostriatal synapses, such as terminal area and post-synaptic density length, were found to be similarly distributed between striosomes and

matrix (Fujiyama et al., 2006) and both IT-type and PT-type projections have been detected in striosomes (Donoghue and Herkenham, 1986; Levesque and Parent, 1998; Wang and Pickel, 1998). These findings, together with the evidence that striosomes and matrix both have D1- and D2-dopamine receptor-expressing MSNs, even if not in equal proportion, indicate that each compartment receives input from both IT-type and PT-type cortical neurons. Reiner et al. (2010) point out that BAC mice expressing EGFP in either the PT neurons or the IT neurons will help to resolve this issue of whether there is preferential compartment targeting of one projection type.

In summary, each striatal compartment receives preferential inputs from different cortical regions and individual corticostriatal axons tend to innervate MSNs that are confined to a single compartment (Kincaid and Wilson, 1996). Nevertheless, subregions of the striatum that encompass both striosomes and neighboring clusters of cells in the matrix (matrisomes) are innervated by related cortical regions (Ragsdale and Graybiel, 1990; Flaherty and Graybiel, 1995; Kincaid and Wilson, 1996). Thus, MSNs in striosomes and matrisomes are thought to process related information and to communicate selectively with surrounding regions, likely via interneurons that have dendrites that cross compartmental borders. Such striatal interneurons include NOS/somatostatin-positive interneurons and parvalbumin-positive interneurons, which receive strong input from the cerebral cortex, and cholinergic interneurons, which receive strong input from the thalamus (Graybiel et al., 1986; Sandell et al., 1986; Lapper and Bolam, 1992; Lapper et al., 1992; Kubota and Kawaguchi, 1993; Bennett and Bolam, 1994; Aosaki et al., 1995; Kawaguchi, 1997; Saka et al., 2002; Miura et al., 2008).

THALAMIC AND NIGRAL AFFERENTS TO STRIOSOME AND MATRIX COMPARTMENTS

Thalamic and cortical neurons make excitatory, asymmetric synaptic connections onto MSNs in similar proportions (Doig et al., 2010). These inputs can be distinguished by immunostaining for the vesicular glutamate transporters VGluT1 (present in cortical terminals and also in less abundant amygdaloid terminals) and VGluT2 (present in thalamic terminals), according to evidence from numerous laboratories (Freneau et al., 2001; Lacey et al., 2005; Raju et al., 2006). With these markers, it was found that inputs from the thalamus terminate on both dendritic spines and dendritic shafts of striatal neurons (Lacey et al., 2005; Raju et al., 2006), but the inputs to shafts are largely confined to the matrix (Fujiyama et al., 2006; Raju et al., 2006). This compartmental selectivity is likely due to the fact that inputs from the parafascicular nucleus, one of the intralaminar nuclei of the thalamus, terminate onto the shafts of cholinergic interneurons (Lapper and Bolam, 1992), which are abundant in the striatal matrix. The parafascicular inputs also contact spines and shafts of MSNs (Lacey et al., 2007). The intralaminar thalamic nuclei are highly interconnected with sensorimotor cortical regions and preferentially target the matrix (Herkenham and Pert, 1981; Ragsdale and Graybiel, 1991; Sadikot et al., 1992). By contrast, the midline paraventricular and rhomboid nuclei of the thalamus, which have strong connections with the nucleus accumbens and the amygdala, have been shown in the cat to target striosomes preferentially (Ragsdale and Graybiel,

1991). This pattern is consistent with striosomes participating in cortico-striato-thalamic circuits with limbic connections and the extra-striosomal matrix participating in cortico-striato-thalamic circuits with sensorimotor connections.

Striosomes and matrix receive dopamine-containing inputs preferentially from distinct groups of neurons in the SNc. The distinct localization of the striosome vs. matrix targeting nigrostriatal neurons has been better documented in rats than in primates (Joel and Weiner, 2000), but differences have been documented in multiple species. The striosomal MSNs are innervated by a group of densely packed dopamine-containing neurons in the ventral tier of the SNc (the A9 cell group) and groups of clustered dopamine-containing neurons that are intermingled with neurons of the SNr (densocellular zone; Gerfen et al., 1987; Jimenez-Castellanos and Graybiel, 1987; Langer and Graybiel, 1989; Prensa and Parent, 2001). These results suggested that there may be reciprocal connections between some of the striosomal system, at least of the so-called subcallosal streak in the lateral striatum, and the densocellular zone of the SNc (Jimenez-Castellanos and Graybiel, 1989; Prensa and Parent, 2001). Matrix neurons are preferentially innervated by dopaminergic neurons in the dorsal tier of the SNc, the retrorubral area (A8 cell group) and the ventral tegmental area (VTA; A10 cell group; Gerfen et al., 1987; Jimenez-Castellanos and Graybiel, 1987; Prensa and Parent, 2001). Nevertheless, single dopamine-containing fibers can innervate both striosomes and matrix (Matsuda et al., 2009) and, for example, ventral tier SNc neurons are reported to innervate the matrix surrounding a target striosome (Prensa and Parent, 2001). The dopamine-containing axons innervating striosomes are reported to be thicker and to have more varicosities than those innervating the matrix, even when the axons are simply different branches from the same innervating neuron (Gerfen et al., 1987; Prensa and Parent, 2001). The ultrastructure of the dopamine-containing afferents is equivalent between compartments, however, and situated so as to modulate both cortical and thalamic excitatory inputs to MSNs (Hanley and Bolam, 1997; Moss and Bolam, 2008). In addition to this compartmental organization of the nigrostriatal system is the well-known gradient of dopamine-containing inputs whereby the medial VTA projects to the ventral striatum and the SNc, located more laterally, projects to the dorsal striatum.

STRIOSOMES, SIGNALING, AND DISEASE

MSNs in the striosomes and matrix exhibit remarkably similar morphology and baseline electrophysiological profiles despite their divergent expression of numerous signaling molecules (Kawaguchi et al., 1989; Graybiel, 1990; Trytek et al., 1996; Miura et al., 2007). However, drug treatments that engage signaling cascades linked to dopamine, acetylcholine, and opioid receptors evoke strikingly different patterns of IEG induction and electrophysiologically recorded activity in the two compartments (Graybiel et al., 1990b; Krebs et al., 1991; Moratalla et al., 1996; Canales and Graybiel, 2000; Adams et al., 2003; Saka et al., 2004; Miura et al., 2007). Disruption or activation of these same neurotransmitter signaling cascades can lead to neurologic and neuropsychiatric disorders. Hence, the compartmentalized patterning of activity is likely to delineate important distinctions in the operation of the basal ganglia.

The best documented differences in activation of striosomes and matrix are for models of drug addiction and models of L-DOPA-induced dyskinesia (LIDs), two hyper-kinetic disorders in which there is hyper-responsivity of dopamine receptor signaling. In clinical studies, differences in neurodegeneration between striosome and matrix compartments have been reported for HD and dystonia, two additional disorders characterized by hyperactivation of the motor system. Differential degeneration has also been reported in a few cases of multiple system atrophy with predominantly parkinsonian features (MSA-P) and in animal models of PD, diseases characterized by hypo-kinesia. All of these disorders can be accompanied by mood disturbances. Differential degeneration of either the striosomes or the surrounding matrix would be expected to result in an imbalance in the numerous neurotransmitter signaling cascades that are differentially expressed between the two compartments.

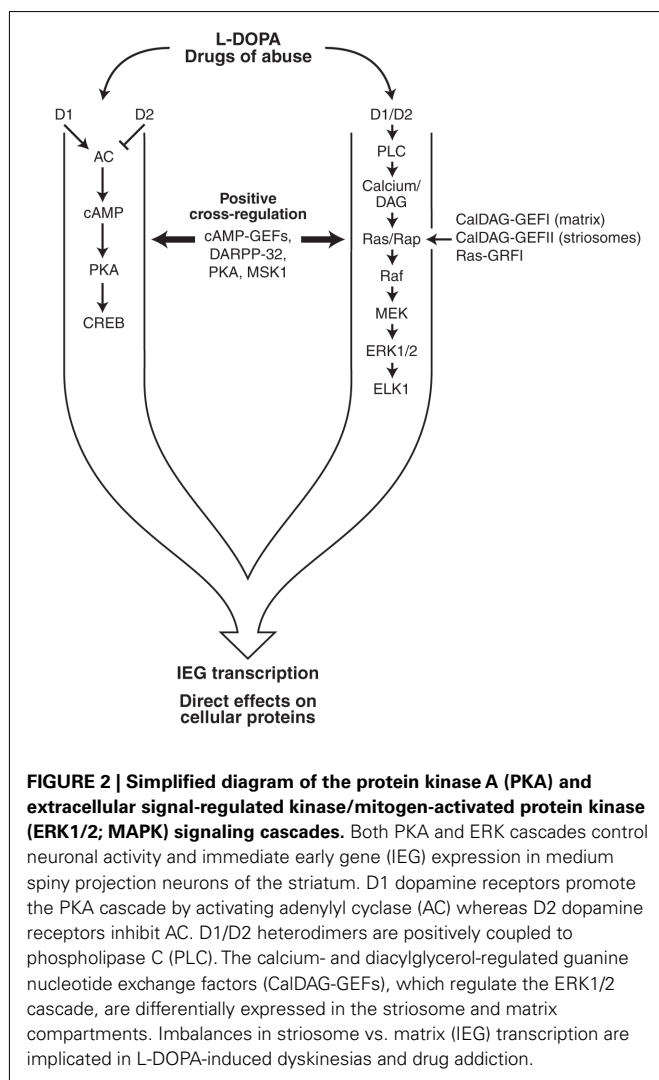
It is notable that drug treatments and disease states can also induce differential activation of D1- vs. D2-expressing MSNs. We have reviewed some of these findings here for comparative purposes, but how these observations relate to such compartmentalized activation is still unclear. It is worth keeping in mind evidence that striosomes contain a greater abundance of MSNs that project to the direct pathway nuclei than MSNs projecting to indirect pathway nuclei. However, preferential activation of D1-expressing MSNs cannot always account for predominant striosomal activation because, in some cases, there is wide-spread suppression of the matrix compartment, which does not have an overabundance of D2-expressing MSNs. Furthermore, the identification of SNc-targeting MSNs in the striosomes suggests that some of the striosomal activation is related to this special pathway.

Here, we focus on prominent neuro-molecular aspects of each of these diseases, with an emphasis on extracellular signal-regulated kinases 1 and 2 (ERK1/2) and protein kinase A (PKA) signaling cascades (Figure 2), and we relate these to the striosome/matrix imbalances that have been reported (Table 2; Figure 3).

HUNTINGTON'S DISEASE

Huntington's disease is a fatal neurodegenerative disease caused by an expanded CAG repeat in the IT15 gene for Huntingtin protein (Htt; The Huntington's Disease Collaborative Research Group, 1993). The CAG expansion encodes a polyglutamine tract that contributes to aggregation of the mutant Htt. HD is typified by the death of striatal MSNs and neocortical neurons and by symptoms of emotional disturbances and uncontrollable, choreic motor movements (Bates et al., 2002). HD hyper-kinesia may be linked in part to the preferential degeneration of D2 dopamine receptor-expressing MSNs of the indirect pathway, which normally inhibit movement (Reiner et al., 1988; Delong, 1990; Glass et al., 2000). Experiments regarding selective vulnerability of either striosome or matrix compartments have yielded variable results, however (Table 2).

Early reports indicated that tissue from individuals with early-stage HD have more severe loss of neurons and immunomarkers in the striosomes than in the matrix, whereas tissue from late-stage cases have equivalent neurodegeneration between compartments (Morton et al., 1993; Hedreen and Folstein, 1995). In mouse



models, in which death of MSNs is minimal but neurite pathology is evident, there is more Htt aggregate burden and preferential volume loss in striosomes, relative to matrix (Menalled et al., 2002; Lawhorn et al., 2008). These patterns are not universal across individuals or models, and other clinical studies even found preferential loss of matrix markers (Ferrante et al., 1987; Seto-Ohshima et al., 1988). Some clarity to this controversy was provided by a study of 35 HD cases correlating symptomatology and compartmental-specific cell loss analyzed in post-mortem specimens (Tippett et al., 2007). In this study, reduced cell density in striosomes is more frequently observed in striatal sections from the brains of individuals who had presented early on with mood, rather than motor, symptoms. The emotional symptoms in the HD cases included depression, anxiety, and compulsively repetitive behaviors. Furthermore, in a follow-up study, it was shown that cell loss in the anterior cingulate cortex is more severe in post-mortem tissue samples from the individuals that presented with primarily emotional symptoms (Thu et al., 2010). Thus, neurodegeneration of components of the striosome limbic loop is associated with the emotional symptoms that characterize HD.

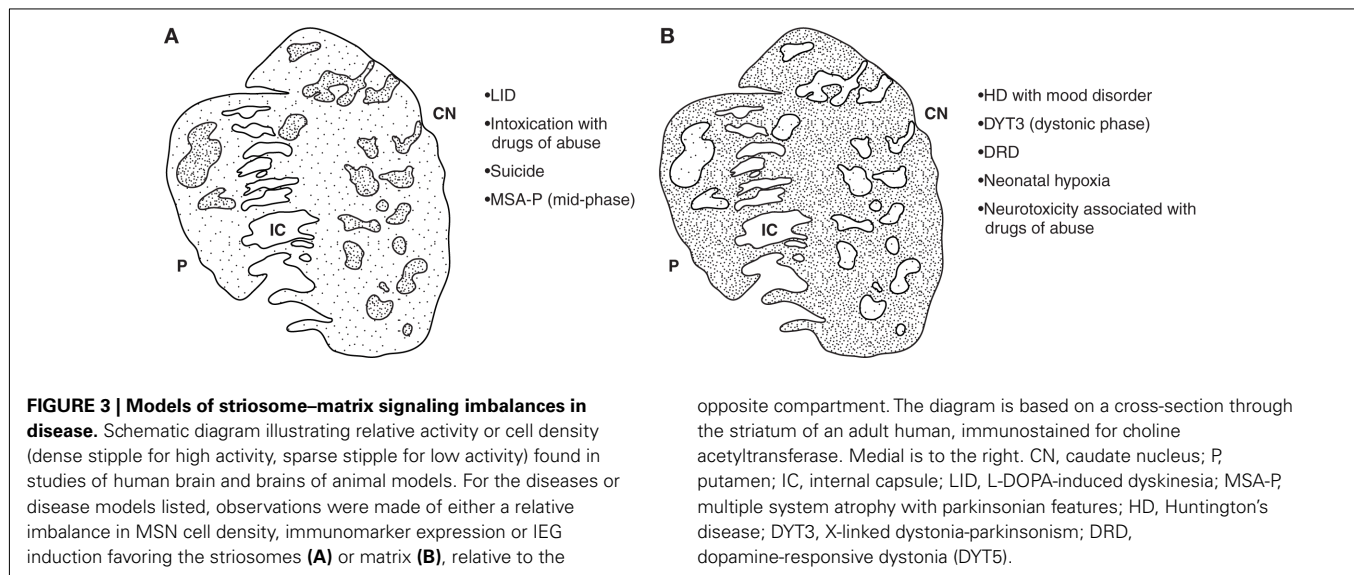
Considering that post-mortem analysis is typically restricted to late-stage HD when nearly the entire brain is affected, longitudinal imaging studies, including of pre-symptomatic individuals, are providing more insight into the correlations between the complex symptomatology and neuronal degeneration patterns that typify HD (Rosas et al., 2008; Politis et al., 2011).

Despite the wide-spread expression of the mutant isoform of Htt in HD, there is disproportionate cell death of striatal MSNs and cortical pyramidal neurons, relative to loss of local interneurons or to neuronal loss in other brain regions. HD leads to a large array of cellular changes, including oxidative stress responses, transcriptional dysregulation, and excitotoxicity. Excitotoxicity is thought to be evoked, at least in part, by an impaired capacity to buffer the calcium rise that follows the stimulation of glutamate and dopamine receptors (Beal, 1998; Bezprozvanny and Hayden, 2004; Tang et al., 2007). Accordingly, disruption of either the cortical (glutamatergic) or nigral (dopamine-containing) afferents to the striatum is protective in the R6/2 mouse model of HD (Stack et al., 2007). Neocortical stimulation, D1 dopamine receptor activation and D2 receptor antagonism can all activate striatal ERK1/2, which exhibits altered activation patterns in HD models (Lievens et al., 2002; Roze et al., 2008). ERK1/2 signaling leads to phosphorylation and activation of the transcription factors ELK1 and CREB (Figure 2) and, thereby, could contribute to, or compensate for, the massive transcriptional dysregulation that occurs in HD (Cha, 2007). Other relevant changes in transcription factors are not discussed here, but even wildtype Htt, as well as mutant Htt, can directly control transcription (Benn et al., 2008).

In view of evidence that there are striosome-predominant and matrix-predominant patterns of cell loss in individual cases of HD, it is of special interest that two striatum-enriched molecules that signal through ERK1/2, CalDAG-GEFI, and CalDAG-GEFII (Figure 2), have strongly compartmentalized patterns of expression. CalDAG-GEFII (aka RasGRP) is expressed especially in striosomes, and CalDAG-GEFI is expressed preferentially in the matrix compartment. The CalDAG-GEFs are severely down-regulated in the R6/2 mouse model of HD and in post-mortem striatal tissue from individuals with HD (Crittenden et al., 2010a,b). This down-regulation is not a function of the preferential loss of D2-positive neurons in HD, considering that CalDAG-GEFI and CalDAG-GEFII are expressed in both prodynorphin and preproenkephalin-positive striatal neurons (Toki et al., 2001). The CalDAG-GEFs contain calcium and diacylglycerol binding domains and signal to the ERK1/2 phosphorylation cascade (Kawasaki et al., 1998) following the activation of cell surface receptors (Dower et al., 2000; Guo et al., 2001; Crittenden et al., 2004). For CalDAG-GEFI, it is known from work in a cortical slice model of HD that knockdown of CalDAG-GEFI expression blocks at least some of the deleterious effects of over-expressing a mutant fragment of the Htt protein (Crittenden et al., 2010a). Thus, the down-regulation of CalDAG-GEFI that is observed in HD may be compensatory. The consequences of CalDAG-GEFII dysregulation in HD models still need to be evaluated. Interestingly, in contrast to CalDAG-GEFI, another matrix-enriched calcium-binding protein, calbindin-D28K, is up-regulated in HD and HD animal models, and this change has been suggested to be protective against

Table 2 | Diseases and disease models with disparate striosome vs. matrix properties.

Disorder/model	Species	Change observed	Reference
Huntington's disease	Human	Preferential loss of striosomal volume at early stage, more equivalent at later stages of disease.	Morton et al. (1993)
Huntington's disease	Human	Preferential loss of MSNs in striosomes in early stage of disease.	Hedreen and Folstein (1995)
Huntington's disease	Human	Preferential loss of neuronal markers in striosomes of individuals with predominant mood disorder.	Tippett et al. (2007)
Huntington's disease	Human	Preferential volume or cell loss in matrix.	Ferrante et al. (1987), Seto-Ohshima et al. (1988)
Huntington's disease/excitotoxic insult	Rat	Preferential survival of MSNs expressing matrix immunomarker.	Figueredo-Cardenas et al. (1998)
Huntington's disease/94Q expanded repeat knock-in	Mouse	Increased aggregate formation in striosomal MSNs.	Menalled et al. (2002)
Huntington's disease/YAC128, full-length human huntingtin with 128Q	Mouse	Preferential volume loss in striosomes with cell loss in both compartments.	Lawhorn et al. (2008)
Parkinson's disease/idiopathic	Human	Preferential loss of cells in nigrosomes.	Damier et al. (1999b)
Parkinson's disease/MPTP	Monkey	Preferential loss of cells in nigrosomes and dopamine terminals in striosomes.	Iravani et al. (2005)
Parkinson's disease/MPTP	Monkey	Preferential loss of <i>Nur77</i> expression in striosomes.	Mahmoudi et al. (2009)
Parkinson's disease/MPTP	Monkey	Preferential loss of dopamine terminals in matrix.	Moratalla et al. (1992)
Parkinson's disease/MPTP	Monkey	Preferential preservation of 5-HT1A receptor immunostaining in striosomes.	Frechilla et al. (2001)
Parkinson's disease/MPTP	Dog	Preferential loss of dopamine terminals in matrix.	Turner et al. (1988)
Dopamine deficiency/ <i>weaver</i> mutation	Mouse	Preferential loss of dopamine terminals in striosomes.	Graybiel et al. (1990c)
Parkinson's disease with l-DOPA-induced dyskinesia	Human	Up-regulation of prodynorphin in striosomes.	Henry et al. (2003)
l-DOPA-induced dyskinesia	Monkey	Up-regulation of prodynorphin in striosomes	Henry et al. (2003)
l-DOPA-induced dyskinesia	Monkey	Up-regulation of <i>Nur77</i> in rostral striosomes.	Mahmoudi et al. (2009)
Multiple System Atrophy	Human	Preferential loss of MSNs in the matrix compartment in mid-phase of disease.	Goto and Hirano (1990), Ito et al. (1992), Sato et al. (2007)
Dystonia DYT3	Human	Preferential loss of MSNs in the striosome compartment in dystonic phase of disease.	Goto et al. (2005)
Dopamine-responsive dystonia/ <i>Pts</i> mutation	Mouse	Preferential loss of tyrosine hydroxylase in striosomal compartment.	Sato et al. (2008)
Drug addiction/cocaine	Human	Increased dynorphin in striosomes of caudate nucleus.	Hurd and Herkenham (1993)
Drug addiction/cocaine	Monkey	Increased striosome to matrix ratio of IEG induction.	Hurd and Herkenham (1993), Saka et al. (2004)
Drug addiction/psychomotor stimulants	Rat	Increased striosome to matrix ratio of IEG induction.	Graybiel et al. (1990b), Moratalla et al. (1996), Canales and Graybiel (2000)
Drug addiction/methamphetamine	Rat	Increased preprodynorphin expression in striosomes of rostral striatum.	Graybiel et al. (1990b), Moratalla et al. (1996), Canales and Graybiel (2000), Adams et al. (2003)
Drug addiction/MDMA	Mouse	Preferential loss of dopamine terminals in striosomes.	Adams et al. (2003), Granado et al. (2008)
Drug addiction/methamphetamine	Mouse	Preferential loss of dopamine terminals in striosomes.	Granado et al. (2008), Granado et al. (2010)
Neonatal hypoxic ischemia	Rat	Preferential survival of striatal neurons that express matrix marker.	Burke and Baimbridge (1993)
Suicide	Human	Increased dynorphin in striosomes of caudate nucleus.	Hurd et al. (1997)
Schizophrenia	Human	Increased asymmetrical axospinous synapses in striosomes of putamen and in matrix of caudate nucleus.	Roberts et al. (2005)



calcium-induced excitotoxicity (Huang et al., 1995; Sun et al., 2005). Most striatum-enriched genes are down-regulated in HD however (Desplats et al., 2006), which may be a compensatory response to the differential vulnerability of the MSN cell-type to mutant Htt expression.

PARKINSON'S DISEASE AND PARKINSONISM

Parkinsonian disorders are characterized by difficulty initiating movements, bradykinesia, muscle rigidity, and postural instability. Idiopathic PD is an age-related neurodegenerative disease second only to Alzheimer's disease in prevalence. PD is typified by degeneration of dopamine-containing neurons in the SNc, which results in the loss of the dopamine-containing innervation of the striatum, usually with a progression of loss from posterior regions (putamen) that are important for the execution of voluntary movements toward anterior regions (caudate nucleus) that are more associative and limbic in function (Kish et al., 1988). PD is now thought to arise from an interaction between the environment and genetic susceptibility. Exposure to mitochondrial toxins, such as the pesticide rotenone or MPTP-tainted drugs of abuse, are well-documented risk factors (Shulman et al., 2011). A number of genetic mutations can lead to PD, including mutations in *SNCA*, which encodes alpha-synuclein and for which there is a gene dosage effect that correlates with the severity of disease (Singleton et al., 2003; Chartier-Harlin et al., 2004; Farrer et al., 2004). The pathologic SNc hallmark of PD has long been identified as the large cytoplasmic inclusions known as Lewy bodies, and these are highly enriched in alpha-synuclein protein.

Different groups of dopaminergic neurons within the SNc appear to have different vulnerability to cell death in PD. For example, the loss of neurons within the densely packed cell groups called nigrosomes correlates with disease duration, whereas dopamine-containing neurons in surrounding parts of the SNc are less vulnerable (Damier et al., 1999b). The "nigrosomes" of the substantia nigra share with the striosomes of the dorsal striatum the characteristic of being weakly immunoreactive for calbindin-D28k, relative to other nigral regions (Damier et al., 1999a). A study

assessing neuronal loss in a single section through the caudal SNc of 20 PD cases and 36 control cases found preferential loss of pigmented cells in the ventro-lateral SNc in PD, relative to age-related loss of more dorsally located pigmented neurons in controls (Fearnley and Lees, 1991). A second study also reported increased vulnerability of neurons located in the ventro-lateral SNc that are calbindin-D28k-negative (Yamada et al., 1990), but this pattern is not uniform across studies and is likely dependent on the individual and how the SNc subregions are defined (Hirsch et al., 1988; Gibb, 1992). Direct measurements of differential striatal loss of dopamine-containing terminals in striosomes or matrix have not yet been possible because in nearly all PD cases that come to autopsy, the depletion of dopamine is so extensive that markers of the nigrostriatal innervation yield little or no signal.

Differential loss of dopamine terminals in the striosomes, relative to matrix, is observed in a mouse model of dopamine deficiency, *weaver*, which carries a mutation in the *GIRK2/Kir3.2/KCNJ6* gene that encodes a G-protein-activated inwardly rectifying potassium channel (Graybiel et al., 1990c). However, systemic deficits in *weaver* mice, including in the cerebellum, preclude simple interpretation of motor effects from their striosomal dopamine deficiency. Overall, results from neurotoxin-induced dopamine depletion in animal models of PD have been mixed, with some reporting increased dopamine terminal loss from striosomes and others increased loss from matrix (Table 2). This inconsistency may be related to the fact that the site of intracerebral neurotoxin injection influences the pattern of compartment-selective terminal loss (Zahm, 1991). These studies are further confounded by the fact that the immunomarker typically used to label dopamine terminals, anti-tyrosine hydroxylase (TH) immunoserum, shows higher immunoreactivity in the matrix than in striosomes of normative animals in some species. This differential anti-TH immunoreactivity is observed prominently in primates, including in humans, and weakly in rodents (Graybiel et al., 1987). This mosaic pattern of TH immunostaining was found to be preserved in several post-mortem PD cases, but could only be assessed in the most ventral parts of the caudate

nucleus and putamen that were not fully depleted of this marker (Graybiel et al., 1990a). Immunostains for the matrix or striosome MSNs themselves also show grossly normal mosaic staining patterns in striatal sections from individuals with idiopathic PD or in cases of parkinsonism-dementia complex of Guam (Ito et al., 1992, 1993).

In a rare form of parkinsonism, MSA-P, differential matrix vs. striosome MSN degeneration has been reported. In MSA-P, there is degeneration of multiple neuronal types, including MSNs and nigral neurons. Analysis of brain sections through the striatum obtained from individuals that died with MSA-P and control cases showed preferential loss of the matrix marker, calbindin-D28k, and maintenance of a striosome immunomarker in the individuals with MSA-P (Goto and Hirano, 1990; Ito et al., 1992; Sato et al., 2007). It was suggested that, in MSA-P, there is selective loss of matrix MSNs in early stages of disease that progresses to loss of MSNs in both compartments by late stages of disease (Sato et al., 2007). Early loss of matrix signaling in MSA-P is consistent with the course of dopamine terminal loss in more common forms of PD, in which terminal loss progresses forward from the caudal putamen, which is relatively poor in striosomes (Graybiel and Ragsdale, 1978).

Considering the heterogeneity of PD-related disorders, it is tempting to speculate that a consistent compartmentalized pattern of dopamine terminal degeneration might be found if patients were classified according to detailed symptomatology, as has been reported for HD (Tippett et al., 2007; Rosas et al., 2008; Thu et al., 2010). As we discuss in the following section, the best evidence to date for the status of striosomes and matrix in PD brains comes from work on the expression of preproenkephalin opioids in post-mortem sections from PD brains. In these, striosomes are clearly preserved and over-activated in the brains of patients who experienced dyskinesias as side effects of treatment (Henry et al., 2003).

L-DOPA-INDUCED DYSKINESIAS

The most widely available treatment for PD is dopamine replacement therapy by oral administration of the dopamine precursor, L-3,4-dihydroxyphenylalanine (L-DOPA). However, as the disease progresses and the dopamine depletion becomes increasingly more severe, L-DOPA treatment often produces debilitating involuntary movements referred to as LIDs. LIDs are likely evoked, at least in part, by hyper-responsivity of MSNs to pulsatile dopamine receptor stimulation during ongoing corticostriatal activation of glutamate receptors (Jenner, 2008). Thus, treatment for these side effects typically requires reducing the L-DOPA dosage to suboptimal levels.

In rodent and non-human primate models, the occurrence of LIDs is correlated with activation of both D1 and D2 dopamine receptors and hyperactivation of the pro-movement direct pathway (Gerfen et al., 1995, 2002, 2008; Jenner, 2008; Berthet et al., 2009; Meurers et al., 2009; Cenci and Konradi, 2010). Stimulation of D1 and glutamate receptors leads to the activation of two key protein kinase cascades, denoted by their central kinase mediators: PKA and ERK1/2 (Figure 2). D1 is known to signal through G-protein coupled receptors to activate adenylyl cyclase for the generation of cAMP. cAMP, in turn, activates PKA,

which site-specifically phosphorylates the dopamine and cAMP-regulated phosphoprotein 32 kDa (DARPP-32). DARPP-32 intersects with the ERK1/2 cascade by blocking protein phosphatases that would normally deactivate ERK1/2 (Valjent et al., 2005; Santini et al., 2007). PKA and ERK1/2 control chromatin remodeling and phosphorylate the CREB and ELK transcription factors that converge to drive expression of numerous IEGs, including, for example, c-Fos (Valjent et al., 2005; Santini et al., 2007, 2009). The induction of numerous IEGs has been observed in D1-positive MSNs in normal animals treated with dopamine receptor agonists, and this induction is dramatically increased in the dopamine-depleted striatum of animals treated with L-DOPA (Valjent et al., 2005; Santini et al., 2007, 2009). Protein translation is also activated in D1-positive MSNs of animal models of LIDs, via activation of mammalian target of rapamycin (mTOR) and ERK1/2 (Santini et al., 2009). Several studies indicate that in models of LIDs, there is a switch from PKA- to ERK1/2-mediated signaling in driving IEG expression (Andersson et al., 2001; Gerfen et al., 2002). Nevertheless, inhibition of ERK1/2, PKA, or mTOR in the striatum attenuates the development of LIDs in animal models (Gerfen et al., 2002; Westin et al., 2007; Darmopil et al., 2009; Santini et al., 2009; Lebel et al., 2010). The wide-spread expression and functions of ERK1/2, PKA, and mTOR make them poor targets for therapeutic intervention, however. Evidence from IEG induction assays in human and animal models suggests that there is a striosome to matrix imbalance in signaling cascades that parallels the development of LIDs. Strikingly striosome-predominant increases in the expression of prodynorphin (also called preproenkephalin B) have been documented in post-mortem striatal sections from parkinsonian humans with LIDs (Henry et al., 2003). In monkeys, the IEG *Nur77* is dysregulated in striosomes following dopamine depletion with and without dopamine replacement treatments (Mahmoudi et al., 2009). In the anterior caudate nucleus of monkeys with dopamine depletion only, *Nur77* is down-regulated in striosomes, relative to levels in control monkeys. By contrast, following dopamine depletion with subsequent L-DOPA treatment, *Nur77* is strongly up-regulated in striosomal neurons. The degree of *Nur77* up-regulation is inversely correlated with the severity of the LIDs, suggesting that the *Nur77* modulation is compensatory. Striosome-predominant IEG induction is also observed in the dopamine-depleted striatum, relative to control striatum, in rat models of LIDs (Cenci et al., 1999; Saka et al., 1999).

This heightened IEG activity in striosomes may be related to activation of the striosome-specific pathway to the SNc (Figure 1B) or to the predominance of D1-positive neurons in striosomes of some species, relative to matrix (Table 1). These possibilities have not been directly assessed, but gene induction studies show increased IEG expression in D1-positive neurons, relative to changes in D2-positive neurons, in rat models of LIDs (Gerfen, 2003; Santini et al., 2009; Bateup et al., 2010). Furthermore, there may be increased signal transduction from D1 receptors in striosomes in LIDs, considering that G α olf, the G-protein that is coupled to the D1 receptor, is elevated in post-mortem tissue from individuals who died with a diagnosis of PD (Corvol et al., 2004). G α olf is enriched in striosomes in normal mice (Sako et al., 2010). An imbalance in striosome to matrix activity may

also be related to the finding that selective antagonists of mu opioid receptors dramatically reduce LIDs in non-human primate models (Koprach et al., 2011). Mu opioid receptor activation contributes to differential activation of striosomes over matrix (Miura et al., 2007), but whether mu opioid receptor antagonists reduce excessive striosome to matrix activity in LIDs has not yet been reported.

Increasing evidence suggests that ERK1/2-mediated IEG induction in LIDs may involve the striosome-enriched gene, CalDAG-GEFII (Kawasaki et al., 1998; Dower et al., 2000; Pierret et al., 2002; Crittenden et al., 2009). CalDAG-GEFII, an ERK1/2 activator, is strongly up-regulated in a rat model of LIDs, and has a striking striosome-enriched pattern. By contrast, the matrix-enriched gene, CalDAG-GEFI, is down-regulated in the same model. The dysregulation of both of these genes was found to occur in proportion to the severity of LIDs that were expressed pre-mortem in rats (Crittenden et al., 2009). Genetic deletion of another ERK1/2 activator, Ras-GRF1, partially alleviates the development of LIDs in a mouse model, and combined Ras-GRF1 and ERK1/2 knockdown reduces LIDs in a monkey model (Fasano et al., 2010). LIDs are further reduced in the Ras-GRF1 knockout mice by treatment with doses of ERK1/2 antagonists that are too low to be effective in control mice. These results suggest that there are multiple pathways to ERK1/2 activation in LIDs and that Ras-GRF1 and CalDAG-GEFII might, together, fully activate this pathway.

Recent studies highlight evidence that ERK1/2 activation in MSNs is high in the early stages of LIDs induction but decreases with prolonged treatment, despite the maintenance of the dyskinetic behaviors (Santini et al., 2010; Ding et al., 2011). By contrast, striatal DARPP-32 phosphorylation is highest in dopamine-depleted animals that received prolonged L-DOPA treatment. These results led to the proposal that ERK1/2 cascades are important for the priming that constitutes excessive responses of dopamine-depleted animals to the first-ever L-DOPA treatment, whereas cAMP/PKA/DARPP-32 signaling cascades are important for the maintenance of LIDs. However, activated ERK1/2 has been found in cholinergic interneurons of parkinsonian rodent models after prolonged L-DOPA treatment, indicating that ERK1/2 may be critical in MSNs for LID priming and in a sparsely distributed striatal interneuron for LID maintenance (Ding et al., 2011).

Taken together, this evidence strongly suggests that MSNs in striosomes are over-activated, relative to matrix MSNs, in LIDs. This differential activation is likely related to PKA and ERK1/2 activation in striatal MSNs (likely involving the CalDAG-GEFs and Ras-GRF1), and in striatal cholinergic interneurons. Strikingly, increased IEG activation of striosomes relative to matrix is also evoked by over-stimulation of the dopamine system with drugs of abuse. Such over-stimulation elicits repetitive motor and cognitive behaviors that share features with LIDs that are observed in a subpopulation of individuals with PD (Voon et al., 2009). There is a strong and direct correlation between the severity of repetitive behaviors induced by dopamine receptor agonists and the ratio of striosome to matrix IEG up-regulation, further suggesting a link between these different hyper-kinetic states (Canales and Graybiel, 2000; Graybiel et al., 2000).

DYSTONIA

Dystonia presents as twisting limb movements or sustained abnormal body postures that are attributed to failures in motor control and sensory integration by the cortico-basal ganglia-thalamic loop (Kaji et al., 2005). Electromyographic recordings from the muscles of patients with dystonia have shown that, during a dystonic event, there is co-contraction of opposing muscles or activation of muscle groups neighboring those that are normally used (Cohen and Hallett, 1988; Fahn, 1989). Dystonia can appear in childhood or in adulthood and can involve a single body part (focal), a group of adjacent body parts (segmental) or the whole body (generalized). As with PD, the susceptibility to disease genes and risk factors is variable (Roze et al., 2009; Schmidt et al., 2009). Dystonia can be evoked by a wide variety of phenomena, including repetitive motor tasks, anti-psychotic medications, brain damage, environmental factors, neurological disease (e.g., PD and HD) and genetic mutations. Intense repetition of a specific motor task, such as playing a musical instrument or typing, can predispose individuals to focal dystonias, including writer's cramp (Byl et al., 1996; Roze et al., 2009). In a review of 158 hemidystonia cases involving stroke, head trauma, or perinatal hypoxia as precipitating factors, 60% were found to have lesions in the contralateral striatum (Chuang et al., 2002). However, disruptions to the striatum, neocortex, GPi, VTA, thalamus, cerebellum, and brain stem can all produce forms of dystonia-like symptoms in animal models (Guehl et al., 2009). Dystonia is also associated with complications of childbirth (Bressman, 2000), presumably because of the hypersensitivity of the neonatal basal ganglia to hypoxia. The latency between the precipitating event and the onset of dystonia is often months to years, supporting the hypothesis that aberrant neuroplasticity in the striatum and/or neocortex contributes to dystonia (Byl et al., 1996; Quartarone et al., 2003; Peterson et al., 2010). Disorganization of the somatotopy in the somatosensory and motor cortices and in the basal ganglia has been observed in dystonia (Lenz et al., 1998; Vitek et al., 1999; Quartarone et al., 2008; Delmaire et al., 2009; Tamura et al., 2009). This may bear relevance to the fact that touching a body region near the affected muscle groups can sometimes alleviate symptoms – a long-recognized phenomenon referred to as a “sensory trick.”

Given the heterogeneity in the etiology of dystonia, it is to be expected that there are multiple cellular signaling abnormalities. However, there is a scarcity of reports on signaling mechanisms in dystonia, relative to the many such studies in models of PD, LID, and HD. Dopamine and acetylcholine signaling are known to be important in several forms of dystonia. The clearest example of this is dopamine-responsive dystonia (DRD; also known as Segawa's disease or DYT5). DRD is an autosomal dominant genetic disorder that is usually produced by loss of function mutations in *GTP cyclohydrolase 1* (*GCH1*; Ichinose et al., 1994; Ludecke et al., 1996; Thony and Blau, 1997, 2006). *GCH1* is in the pathway for synthesis of tetrahydrobiopterin (BH₄), an essential cofactor for the dopamine-synthesizing protein, TH. Therefore, in DRD, there is likely a deficiency in dopamine, and related biogenic amines, throughout development. Age of onset is variable but often early, and symptoms range from focal to generalized dystonia to developmental delay (Segawa, 2011). Many of the symptoms

are alleviated by L-DOPA administration, and LIDs are typically not a complication.

Loss of dopamine in adulthood is also associated with dystonia, as a secondary symptom of PD. In addition, the classical anti-psychotic medications that antagonize D2 receptor signaling precipitate acute or tardive dystonia in a significant proportion of patients and in animal models (Kiriakakis et al., 1998). Moderate abnormalities in D2 receptor binding and expression are observed in cases of focal and generalized dystonia (Tanabe et al., 2009), but whether the defects are in post-synaptic D2 receptors on MSNs, in presynaptic D2 autoreceptors that inhibit dopamine release or in D2-positive striatal interneurons remains controversial (Playford et al., 1993; Naumann et al., 1998; Napolitano et al., 2010).

Dopamine and acetylcholine signaling are tightly interrelated in the striatum and anticholinergic drugs improve dystonic symptoms in some patients and animal models (Bressman, 2000). Clues to the relevance of acetylcholine signaling in dystonia come from animal models of DYT1. DYT1 is produced by a mutation in *torsinA* (Ozelius et al., 1997), which is expressed widely but is enriched in the cholinergic interneurons of the striatum (thought to correspond mainly to the physiologically identified tonically active neurons, or TANs) and in dopaminergic neurons of the SNc (Shashidharan et al., 2000; Oberlin et al., 2004). D2 is expressed in cholinergic interneurons and can, under certain conditions, inhibit their activation and thereby permit long-term depression (LTD) in MSNs (Wang et al., 2006b). However, in slice recordings from the mouse model of DYT1, the effect is reversed such that D2 agonists activate cholinergic interneurons and MSNs fail to undergo LTD (Pisani et al., 2003). This failure of MSN neuroplasticity is rescued by blocking acetylcholine receptors. Thus, anticholinergics may serve to restore normal plasticity of MSNs in DYT1 models. Because cholinergic interneurons control striosome to matrix activity ratios (Saka et al., 2002), it is possible that anticholinergic therapies change the communication between these striatal compartments as well.

The most direct case made for a striosome to matrix imbalance in some types of dystonia comes from post-mortem analyses of striatal tissue from individuals with X-linked dystonia-parkinsonism (DYT3). DYT3 is caused by mutations in the transcription factor TAF1 and manifests as adult-onset dystonia, often generalized, that progresses to parkinsonism (Makino et al., 2007). Histological analyses of post-mortem striatal sections from five people with DYT3 who died during the dystonic phase and two people who died during the parkinsonian phase led to the discovery of severe striosome degeneration in the individuals with dystonia (Goto et al., 2005). Analyses suggested that gliosis and MSN loss is confined to the striosomal compartment in the individuals in the dystonic phase whereas MSN degeneration spreads to both compartments in the individuals in the parkinsonian phase of the disease. Striatal projections into the GPi, GPe, and substantia nigra all appear to be significantly depleted in a presented DYT3 case. TH staining for dopaminergic neurons in the substantia nigra was patchy, suggesting that there was also ventral midbrain pathology. Thus, DYT3 appears to cause degeneration of MSNs in the striosome compartment in the early phase of the disease and then progresses to cause degeneration of MSNs in the matrix compartment as well. This preferential loss of striosomal MSNs fits with

the expression of N-TAF1, the neuron-specific isoform of TAF1 that is implicated as causative in DYT3. In the rat, N-TAF1 expression is strikingly enriched in striosomal MSNs, relative to the low expression in the extra-striosomal matrix (Sako et al., 2011).

Support for the possibility that striosomes are differentially affected in other forms of dystonia comes from a mouse model of an unusual form of DRD. To generate this mouse line, a mutation was introduced into 6-pyruvoyl tetrahydropterin synthase, an enzyme that is required for BH₄ synthesis and that was found to be mutated in a human case of DRD (Hanihara et al., 1997). These mutant mice exhibit abnormal hindlimb claspings and slow beam-crossing behavior, suggestive of dystonic and parkinsonian symptoms. A postnatal, striosome-predominant loss of TH protein is observed. The pattern of TH depletion also exhibits similarities to that observed in PD in that it was depleted in a caudal-to-rostral gradient and preserved in the nucleus accumbens (Sato et al., 2008). Unlike in PD however, this DRD mouse model shows preservation of SNc integrity, based on immunostaining of brain sections for markers of dopaminergic neurons. Thus, it appears that 6-pyruvoyl tetrahydropterin synthase is required for TH expression in the terminals of dopaminergic neurons targeting the striosomes, suggesting that preferential dopamine loss in the striosomes may occur in DRD. Clinical studies have yet to test this idea, however.

DRUG ADDICTION

Drug addiction is generally agreed to involve the repeated and escalating use of psychoactive drugs, despite adverse consequences such as loss of employment, friends, and family ties (Diagnostic and Statistical Manual of Mental Disorders IV). Only ~15% of people and animals who experience a highly addictive drug escalate their use to the point of addiction (Deroche-Gamonet et al., 2004). Susceptibility to drug addiction has environmental components (in particular, repeated drug administration) and genetic components. Twin studies suggest that as much as 50% of the susceptibility may be genetic (Kreek et al., 2005). Relapse after withdrawal is associated, in both humans and animal models, with stress, re-exposure to the drug, or re-exposure to environmental cues associated with the drug (Sinha, 2008). Cues and experiences that promote reinstatement of drug use can activate the same brain regions as the drugs themselves, suggesting that they share neurocircuitry and underlying molecular mechanisms (Childress et al., 1999; Volkow et al., 2006).

It is widely agreed that there are differential effects of drugs of abuse on signaling pathways in the ventromedial and dorsolateral striatum, in the direct and indirect pathways, and in striosome and matrix compartments. Human brain imaging studies have shown that acute drug exposure activates limbic structures including the nucleus accumbens and amygdala and that repeated use expands the region of activation to include the dorsal striatum and neocortex (Breiter et al., 1997; Volkow et al., 2006; Porrino et al., 2007). This changing pattern of activation might be related to the change in self-reported effects of the drug: in the early phases, individuals describe hedonic effects of the drug but in late phases of addiction the effects are more likely to be described as “filling a hole” (Robinson and Berridge, 2008). This contrast fits with the notion that the ventromedial striatum and nucleus accumbens mediate

goal-oriented behaviors (e.g., early, hedonia-seeking drug use) whereas the dorsolateral striatum and some neocortical regions mediate habitual behaviors (e.g., late, compulsive drug use; Yin et al., 2004; Barnes et al., 2005; Atallah et al., 2007). Considering that drugs of abuse and associated cues activate the dopamine system (Di Chiara and Imperato, 1988), the transition from ventral to dorsal striatum activity with repeated drug use may be influenced by indirectly recursive corticostriatal circuitry and an apparently progressive connectivity of the striatonigral loop (Haber et al., 2000; Ikemoto, 2007). This possibility has been supported by studies in rats in which physical disruption of this ventral to dorsal striatal connectivity pattern was shown to interfere with the development of habitual responding for drug access (Belin and Everitt, 2008). Thus, the development of a drug-taking habit may rely on the dorsal striatum, and other brain regions, that normally mediate the acquisition of motor and cognitive habits.

Locomotor and highly repetitive and idiosyncratic motor behaviors (stereotypies) are elicited in humans and animals under the influence psychomotor stimulants such as cocaine and amphetamine. These motoric responses to drugs of abuse escalate with repeated treatments, a phenomenon that is termed sensitization and that is thought to be related to drug addiction (Vezina and Leyton, 2009). There is strong evidence that both PKA and ERK1/2 cascades in MSNs mediate acute and sensitized responses to psychomotor stimulants (Ferguson and Robinson, 2004; Shi and McGinty, 2006; Girault et al., 2007; Russo et al., 2010). Genetic deletion of DARPP-32 (a key effector of the PKA cascade) and inhibitors of MEK1 (an upstream activator of ERK1/2) each block sensitization to drugs of abuse (Valjent et al., 2005). Immunohistochemistry for phosphorylation of activating sites on various members of the PKA and ERK1/2 cascades show that drugs of abuse activate these cascades primarily in D1-expressing neurons (Valjent et al., 2005; Bertran-Gonzalez et al., 2008). Likewise, Delta FosB, an IEG that is downstream of both signaling cascades, is activated preferentially in D1-expressing neurons following drug treatments (Berretta et al., 1992; Hope et al., 1994; Moratalla et al., 1996; Zachariou et al., 2006; Fasano et al., 2009). Activation of D2-positive MSNs is not observed in most reports of MSN activation by drugs of abuse (Bertran-Gonzalez et al., 2008), but this finding may depend on the context in which the drug is taken, given that the activation of D2-positive MSNs is observed when drugs are administered in a novel cage (Badiani et al., 1999; Ferguson and Robinson, 2004; Vezina and Leyton, 2009). Furthermore, it is widely recognized that normal acute and sensitized responses to drugs of abuse are dependent on both D1- and D2-type dopamine receptor activation (Xu et al., 1994; Caine et al., 2002, 2007; Capper-Loup et al., 2002; Barrett et al., 2004; Bateup et al., 2010). Pharmacologic or genetic studies indicating a D2-dependence of drug responses do not typically specify the cell-type of action. Therefore, it may be that D2 activity is required in non-MSN cell types such as cholinergic interneurons, which are sparsely distributed in the striatum. Cholinergic interneurons are implicated in the development of LIDs (Ding et al., 2011) and are required for the increased striosome over matrix activity ratios associated with drugs of abuse (Saka et al., 2002). Psychomotor stimulants have also been shown to signal through D1/D2 heterodimers (Perreault et al., 2010). Thus, D2 expression in MSNs,

striatal interneurons, dopamine-producing axon terminals, and D1/D2 heteromers may all be important for particular aspects of drug responsivity. Histochemical examination of post-mortem brains from cocaine addicts who tested positive for cocaine at autopsy, showed that mRNA for dynorphin was selectively elevated in striosomes in the caudate nucleus (Hurd and Herkenham, 1993). In animal models, strong behavioral responses to psychomotor stimulants, administered by intraperitoneal injection in the home cage, is correlated with a profound change in IEG induction in the striosome vs. matrix compartments, favoring increased activation in striosomes (Graybiel et al., 1990b; Canales and Graybiel, 2000; Vanderschuren et al., 2002; Glickstein and Schmauss, 2004; Saka et al., 2004). At very high doses, or with repeated treatments, psychomotor stimulants suppress locomotion in favor of a limited number of spatially confined stereotypies (Lyon and Robbins, 1975). In rats and monkeys, there is a direct correlation between the intensity of drug-induced stereotypy and the increased striosome to matrix ratio of IEG induction in the dorsolateral quadrant of the striatum (Canales and Graybiel, 2000; Saka et al., 2004). Based on this correlation, it was suggested that increased striosome activity and repressed matrix activity in this zone, together, could predict the degree of repetitive behavior expressed. The possibility that increased activity in the striosomes promotes stereotypies whereas activity in the matrix permits flexibility in motor behaviors is consistent with several additional studies. First, rats with a self-stimulation electrode implanted in or near a striosome learn to bar-press more frequently for stimulation than rats in which the stimulating electrode is implanted in the matrix (White and Hiroi, 1998). Second, metabolic labeling studies show that rats moving freely or under gentle restraint in their home cage have preferential activation of MSNs in the matrix compartment (Brown et al., 2002). Third, knockout mice that lack CalDAG-GEFI, an ERK1/2 regulator that is selectively enriched in the matrix compartment, show enhanced stereotypy responses to amphetamine, relative to sibling controls (Crittenden et al., 2006). Importantly, the CalDAG-GEFI knockout mice have normal locomotor responses to low doses of amphetamine, and locomote normally in the absence of drug, suggesting that disruption of matrix function specifically promotes stereotypies induced by high doses of amphetamine. The possibility that differential signaling in the striosomes vs. matrix is related to the switch from goal-oriented behavior to habitual, goal-independent behaviors that compare to drug-induced stereotypy is reviewed by Canales (2005).

Vanderschuren et al. (2002) showed that increased striosome to matrix activity was also observed in adult rats treated with a single high dose, or with repeated low doses, of amphetamine and suggested that the compartmental ratio change was correlated with overall drug response rather than specifically with stereotypies. However, this study did not rate stereotypic behaviors and even low doses of stimulant drug can induce intermittent stereotypies such as sniffing. A single high dose of methylphenidate, an indirect dopamine receptor agonist that is prescribed to children for ADHD, also induces preferential IEG expression in striosomes of adolescent mice (Davis and Puhl, 2011). In follow-up cell culture studies, methylphenidate appeared to be acting through D1 and cAMP-mediated signaling cascades, which are enriched in

striosomes (Davis and Puhl, 2011). Likewise, in a study of spontaneous stereotypies in deer mice, it was found that there is an increase in direct over indirect pathway activity, relative to non-stereotypic controls (Tanimura et al., 2011). The stereotypic deer mice do not show changes in striosome to matrix activity, as measured by cytochrome oxidase histochemistry, however. Together, these studies indicate that an increased striosome to matrix activity ratio is correlated with hyper-responsivity to psychomotor stimulants.

Although one study found that deletion of a matrix-enriched signaling molecule (CalDAG-GEFI) is sufficient to promote drug-induced stereotypies (Crittenden et al., 2006), other studies show that an increase in striosome to matrix IEG activity is not necessary to elicit stereotypy. In rats, ablation of cholinergic and nitric oxide synthase-positive interneurons prevents the compartmentalized IEG induction ratio, but not the sensitization of drug-associated stereotypies (Saka et al., 2002). Additionally, mice lacking D2 show increased stereotypy responses to D1-type dopamine receptor agonists but do not exhibit increased striosome to matrix ratios of IEG induction (Glickstein and Schmauss, 2004). By contrast, mice with striosome, but not matrix, overexpression of the negative mGluR1/5 modulator, Homer1a, show enhanced locomotor and stereotypy responses to amphetamine (Tappe and Kuner, 2006). These mice may not have decreased mGluR1/5 signaling however, as they show strong and patchy (presumably striosomal) IEG induction in the striatum. Evidence that there is not a simple correlation between increased striosome function and increased responses to drugs of abuse comes from mice in which rostral striosomes fail to develop (Liao et al., 2008). Mice lacking RAR β , a transcription factor that is required for formation of rostral striosomes, exhibit enhanced repetitive behavioral responses to apomorphine. At a dose of apomorphine that does not affect behaviors of control mice, mice lacking RAR β show increased stereotypic responses. These results could be interpreted to indicate that increased striosome to matrix activity normally serves to dampen stereotypies. Alternatively, any striosome to matrix imbalance, regardless of direction, might predispose to drug-induced stereotypies. In addition, how the selective disruption of striosome or matrix function influences behavioral responses to drugs of abuse could depend on the context of drug exposure and which region of the striatum is activated. This apparent complexity is not surprising considering that different drugs of abuse induce distinct patterns of striosome and matrix IEG induction across the caudal-rostral and medial-lateral gradients of the striatum, and that there are differences in the connectivity and transmitter/modulator expression patterns of the striosomes themselves in different striatal regions (Graybiel et al., 1990b; Hurd and Herkenham, 1993; Wang et al., 1994; Tan et al., 2000; Adams et al., 2001).

Repeated treatment of rats with drugs of abuse also activates IEG expression in the cholinergic interneurons that reside at the compartment borders (Moratalla et al., 1996). This finding is likely related to the observation that prolonged L-DOPA treatment of PD models induces ERK activation in cholinergic interneurons (Ding et al., 2011). Moreover, pharmacological disruption of cholinergic signaling modulates stereotypy responses to drugs of abuse with a dependence on medial-lateral striatal gradients (Ohmori

et al., 1995; Aliane et al., 2011). Ablation of cholinergic interneurons disrupts the increased striosome to matrix activity ratio in response to the direct dopamine receptor agonist, apomorphine (Saka et al., 2002). The CalDAG-GEFI knockout mice that have exaggerated drug-induced stereotypies have defective cholinergic signaling as well (Crittenden et al., 2006). Thus, cholinergic modulation of drug responses may occur, in part, through control of striosome to matrix activity ratios.

Studies in animal models suggest that the striosome and matrix compartments are differentially vulnerable to the loss of dopamine terminals that is caused by drug abuse. Treatment of mice with high doses of either methamphetamine or MDMA ("ecstasy") causes selective degeneration of dopaminergic inputs to the striosomes, relative to the surrounding matrix (Granado et al., 2008, 2010). This selective vulnerability was suggested to be related to the relatively lower striosomal levels of superoxide dismutase 2 (SOD2; Zhang et al., 1994; Medina et al., 1996), which neutralizes the reactive oxygen species that are a byproduct of dopamine metabolism. Another possibility is that the enriched vascularization of striosomes (Breuer et al., 2005) causes them to have increased exposure to injected drugs. Striosomal immunomarkers are selectively depleted in rats treated with excitotoxic drugs or that undergo neonatal hypoxia (Burke and Baimbridge, 1993; Figueredo-Cardenas et al., 1998). The anti-apoptosis factor, Bcl-2, and the neurotrophic factor, GDNF, are enriched in striosomal neurons, suggesting that they may have compensatory anti-neurodegeneration mechanisms in place (Bernier and Parent, 1998; Lopez-Martin et al., 1999; Oo et al., 2005). There remain only a few cases in which brains of drug abusers were processed to detect striosomes, and cell death was not assayed in this study, but the selective striosomal enhancement of the opioid, dynorphin, could relate to striatum-based disruption of limbic-related behaviors in addition to effects attributable to the ventral striatum itself (Hurd and Herkenham, 1993).

In summary, normal and dopamine-depleted animals show a strong correlation between the degree of striosome to matrix ratio of MSN activation and the degree of behavioral response to dopamine agonists or drugs of abuse. New genetic tools that allow the specific activation or repression of genes in defined subsets of striosomal and matrix MSNs will allow us to better understand the meaning of this correlation.

CONCLUSION

Striosomes are heavily innervated by limbic cortices, which may be related to the preferential degeneration of striosomes and cingulate cortex in individuals with mood disorders associated with HD. Striosomes are also implicated in limbic control of motor behaviors based on findings that drug addicts and suicide victims exhibit preferentially increased striosomal expression of prodynorphin, relative to control individuals. Evidence for imbalance between striosome and matrix functions in PD is primarily associated with L-DOPA treatment that can produce dyskinesias. In some individuals, LIDs are associated with mood disruptions and compulsive behaviors that resemble behaviors seen in drug addicts. Animal models and a few clinical studies support the idea that there are similarities between the excessive dopamine signaling that occurs in MSNs of individuals with LIDs and in drug abuse.

Hyperactivation of D1-positive MSNs and increased striosome to matrix activity ratios have been observed in models of both disorders. Inhibitors of PKA and ERK signaling can alleviate LIDs and drug sensitization but more specific targets are needed for therapeutic development. Dystonic symptoms of rigidity can co-occur with the basal ganglia disorders of HD, PD, and LIDs and several small clinical studies indicate differential degeneration of striosome vs. matrix MSNs in hereditary forms of dystonia. The striosome-specific pathway to the SNc may be important for some aspects of HD, LIDs, dystonia, and drug addiction, which have overlapping symptoms of hyperactivation of the motor system and mood disturbances.

Much remains to be determined about the relevance of striosome–matrix architecture to human neurologic and neuropsychiatric disorders. First and foremost, striosomes have so far been below-threshold for reliable detection by human brain imaging methods currently in use, so that evidence is restricted to rare post-mortem specimens. Even in instances in which observations have been made on human brains, they are necessarily incomplete. And importantly, even though there is mounting evidence that some aspects of repetitive behavior may be related to excessive

striosomal signaling, there are still no studies for some human disorders in which repetitive behaviors are common symptoms, such as in autism spectrum disorders. Technical advances are close to overcoming some of these limitations, and promise insights into the functions of the highly developed striosomal system of the human striatum.

ACKNOWLEDGMENTS

We thank Drs Leif G. Gibb, Ledia F. Hernandez, Min Jung Kim, Yasuo Kubota, and Carolyn J. Lacey for comments on the manuscript and Messrs. Alexander McWhinnie and Henry F. Hall for help with the illustrations. Supported by the National Institute of Child Health and Development (R01-HD028341); a Distinguished Investigator Award from NARSAD; the James and Pat Poitras Research Fund; the Grayce B. Kerr Fund; the Stanley H. and Sheila G. Sydney Fund; the Tourette Syndrome Association; the Simons Foundation; the Stanley Center for Psychiatric Research at the Broad Institute, via a grant to Edward Scolnick from the Stanley Medical Research Institute; the MGH/MIT Morris Udall Center of Excellence in Parkinson's disease research; the National Parkinson Foundation; and the Michael J. Fox Foundation.

REFERENCES

- Adams, D. H., Hanson, G. R., and Keefe, K. A. (2001). Differential effects of cocaine and methamphetamine on neurotensin/neuromedin N and preprotachykinin messenger RNA expression in unique regions of the striatum. *Neuroscience* 102, 843–851.
- Adams, D. H., Hanson, G. R., and Keefe, K. A. (2003). Distinct effects of methamphetamine and cocaine on preprodynorphin messenger RNA in rat striatal patch and matrix. *J. Neurochem.* 84, 87–93.
- Albin, R. L., Young, A. B., and Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends Neurosci.* 12, 366–375.
- Albin, R. L., Young, A. B., and Penney, J. B. (1995). The functional anatomy of disorders of the basal ganglia. *Trends Neurosci.* 18, 63–64.
- Alexander, G. E., DeLong, M. R., and Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu. Rev. Neurosci.* 9, 357–381.
- Aliane, V., Perez, S., Bohren, Y., Deniau, J. M., and Kemel, M. L. (2011). Key role of striatal cholinergic interneurons in processes leading to arrest of motor stereotypies. *Brain* 134, 110–118.
- Amemori, K., Gibb, L. G., and Graybiel, A. M. (2011). Shifting responsibility: the importance of striatal modularity to reinforcement learning in uncertain environments. *Front. Hum. Neurosci.* 5:47. doi: 10.3389/fnhum.2011.00047
- Anderson, S. A., Qiu, M., Bulfone, A., Eisenstat, D. D., Meneses, J., Pedersen, R., and Rubenstein, J. L. (1997). Mutations of the homeobox genes *Dlx-1* and *Dlx-2* disrupt the striatal subventricular zone and differentiation of late born striatal neurons. *Neuron* 19, 27–37.
- Andersson, M., Konradi, C., and Cenci, M. A. (2001). cAMP response element-binding protein is required for dopamine-dependent gene expression in the intact but not the dopamine-denervated striatum. *J. Neurosci.* 21, 9930–9943.
- Aosaki, T., Kimura, M., and Graybiel, A. M. (1995). Temporal and spatial characteristics of tonically active neurons of the primate's striatum. *J. Neurophysiol.* 73, 1234–1252.
- Aosaki, T., Tsubokawa, H., Ishida, A., Watanabe, K., Graybiel, A. M., and Kimura, M. (1994). Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning. *J. Neurosci.* 14, 3969–3984.
- Arlotta, P., Molyneaux, B. J., Jabaudon, D., Yoshida, Y., and Macklis, J. D. (2008). *Ctip2* controls the differentiation of medium spiny neurons and the establishment of the cellular architecture of the striatum. *J. Neurosci.* 28, 622–632.
- Atallah, H. E., Lopez-Paniagua, D., Rudy, J. W., and O'Reilly, R. C. (2007). Separate neural substrates for skill learning and performance in the ventral and dorsal striatum. *Nat. Neurosci.* 10, 126–131.
- Badiani, A., Oates, M. M., Day, H. E., Watson, S. J., Akil, H., and Robinson, T. E. (1999). Environmental modulation of amphetamine-induced c-fos expression in D1 versus D2 striatal neurons. *Behav. Brain Res.* 103, 203–209.
- Barnes, T. D., Kubota, Y., Hu, D., Jin, D. Z., and Graybiel, A. M. (2005). Activity of striatal neurons reflects dynamic encoding and recoding of procedural memories. *Nature* 437, 1158–1161.
- Barrett, A. C., Miller, J. R., Dohrmann, J. M., and Caine, S. B. (2004). Effects of dopamine indirect agonists and selective D1-like and D2-like agonists and antagonists on cocaine self-administration and food maintained responding in rats. *Neuropharmacology* 47(Suppl. 1), 256–273.
- Bates, G., Harper, P., and Jones, L. (2002). *Huntington's Disease*. New York: Oxford University Press.
- Bateup, H. S., Santini, E., Shen, W., Birnbaum, S., Valjent, E., Surmeier, D. J., Fisone, G., Nestler, E. J., and Greengard, P. (2010). Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14845–14850.
- Beal, M. F. (1998). Mitochondrial dysfunction in neurodegenerative diseases. *Biochim. Biophys. Acta* 1366, 211–223.
- Belin, D., and Everitt, B. J. (2008). Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. *Neuron* 57, 432–441.
- Benn, C. L., Sun, T., Sadri-Vakili, G., McFarland, K. N., DiRocco, D. P., Yohrling, G. J., Clark, T. W., Bouzou, B., and Cha, J. H. (2008). Huntingtin modulates transcription, occupies gene promoters in vivo, and binds directly to DNA in a polyglutamine-dependent manner. *J. Neurosci.* 28, 10720–10733.
- Bennett, B. D., and Bolam, J. P. (1994). Localisation of parvalbumin-immunoreactive structures in primate caudate-putamen. *J. Comp. Neurol.* 347, 340–356.
- Bernier, P. J., and Parent, A. (1998). The anti-apoptosis bcl-2 proto-oncogene is preferentially expressed in limbic structures of the primate brain. *Neuroscience* 82, 635–640.
- Berretta, S., Parthasarathy, H. B., and Graybiel, A. M. (1997). Local release of GABAergic inhibition in the motor cortex induces immediately early gene expression in indirect pathway neurons of the striatum. *J. Neurosci.* 17, 4752–4763.
- Berretta, S., Robertson, H. A., and Graybiel, A. M. (1992). Dopamine and glutamate agonists stimulate neuron-specific expression of Fos-like protein in the striatum. *J. Neurophysiol.* 68, 767–777.
- Berthet, A., Porras, G., Doudnikoff, E., Stark, H., Cador, M., Bezard, E., and Bloch, B. (2009). Pharmacological analysis demonstrates dramatic alteration of D1 dopamine receptor neuronal distribution in the rat analog of L-DOPA-induced dyskinesia. *J. Neurosci.* 29, 4829–4835.

- Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamalas, M., Herve, D., Valjent, E., and Girault, J. A. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J. Neurosci.* 28, 5671–5685.
- Besson, M. J., Graybiel, A. M., and Nantuk, M. A. (1988). [3H]SCH 23390 binding to D1 dopamine receptors in the basal ganglia of the cat and primate: delineation of striosomal compartments and pallidal and nigral subdivisions. *Neuroscience* 26, 101–119.
- Besson, M. J., Graybiel, A. M., and Quinn, B. (1990). Co-expression of neuropeptides in the cat's striatum: an immunohistochemical study of substance P, dynorphin B and enkephalin. *Neuroscience* 39, 33–58.
- Bezprozvanny, I., and Hayden, M. R. (2004). Deranged neuronal calcium signaling and Huntington disease. *Biochem. Biophys. Res. Commun.* 322, 1310–1317.
- Breiter, H. C., Gollub, R. L., Weisskoff, R. M., Kennedy, D. N., Makris, N., Berke, J. D., Goodman, J. M., Kantor, H. L., Gastfriend, D. R., Riorden, J. P., Mathew, R. T., Rosen, B. R., and Hyman, S. E. (1997). Acute effects of cocaine on human brain activity and emotion. *Neuron* 19, 591–611.
- Bressman, S. B. (2000). Dystonia update. *Clin. Neuropharmacol.* 23, 239–251.
- Breuer, O., Lawhorn, C., Miller, T., Smith, D. M., and Brown, L. L. (2005). Functional architecture of the mammalian striatum: mouse vascular and striosome organization and their anatomic relationships. *Neurosci. Lett.* 385, 198–203.
- Bromberg-Martin, E. S., Matsumoto, M., Nakahara, H., and Hikosaka, O. (2010). Multiple timescales of memory in lateral habenula and dopamine neurons. *Neuron* 67, 499–510.
- Brown, L. L., Feldman, S. M., Smith, D. M., Cavanaugh, J. R., Ackermann, R. F., and Graybiel, A. M. (2002). Differential metabolic activity in the striosome and matrix compartments of the rat striatum during natural behaviors. *J. Neurosci.* 22, 305–314.
- Burke, R. E., and Baimbridge, K. G. (1993). Relative loss of the striatal striosome compartment, defined by calbindin-D28k immunostaining, following developmental hypoxic-ischemic injury. *Neuroscience* 56, 305–315.
- Byl, N. N., Merzenich, M. M., and Jenkins, W. M. (1996). A primate genesis model of focal dystonia and repetitive strain injury: I. Learning-induced dedifferentiation of the representation of the hand in the primary somatosensory cortex in adult monkeys. *Neurology* 47, 508–520.
- Caine, S. B., Negus, S. S., Mello, N. K., Patel, S., Bristow, L., Kulagowski, J., Vallone, D., Saiardi, A., and Borrelli, E. (2002). Role of dopamine D2-like receptors in cocaine self-administration: studies with D2 receptor mutant mice and novel D2 receptor antagonists. *J. Neurosci.* 22, 2977–2988.
- Caine, S. B., Thomsen, M., Gabriel, K. I., Berkowitz, J. S., Gold, L. H., Koob, G. F., Tonegawa, S., Zhang, J., and Xu, M. (2007). Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. *J. Neurosci.* 27, 13140–13150.
- Canales, J. J. (2005). Stimulant-induced adaptations in neostriatal matrix and striosome systems: transiting from instrumental responding to habitual behavior in drug addiction. *Neurobiol. Learn. Mem.* 83, 93–103.
- Canales, J. J., and Graybiel, A. M. (2000). A measure of striatal function predicts motor stereotypy. *Nat. Neurosci.* 3, 377–383.
- Capper-Loup, C., Canales, J. J., Kadaba, N., and Graybiel, A. M. (2002). Concurrent activation of dopamine D1 and D2 receptors is required to evoke neural and behavioral phenotypes of cocaine sensitization. *J. Neurosci.* 22, 6218–6227.
- Cenci, M. A., and Konradi, C. (2010). Maladaptive striatal plasticity in L-DOPA-induced dyskinesia. *Prog. Brain Res.* 183, 209–233.
- Cenci, M. A., Lee, C. S., and Bjorklund, A. (1998). L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *Eur. J. Neurosci.* 10, 2694–2706.
- Cenci, M. A., Tranberg, A., Andersson, M., and Hilbertson, A. (1999). Changes in the regional and compartmental distribution of FosB- and JunB-like immunoreactivity induced in the dopamine-denervated rat striatum by acute or chronic L-dopa treatment. *Neuroscience* 94, 515–527.
- Cha, J. H. (2007). Transcriptional signatures in Huntington's disease. *Prog. Neurobiol.* 83, 228–248.
- Chartier-Harlin, M. C., Kachergus, J., Roumier, C., Mouroux, V., Douay, X., Lincoln, S., Leveque, C., Larvor, L., Andrieux, J., Hulihan, M., Waucquier, N., Defebvre, L., Amouyel, P., Farrer, M., and Destee, A. (2004). Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* 364, 1167–1169.
- Chesselet, M. F., Gonzales, C., and Levitt, P. (1991). Heterogeneous distribution of the limbic system-associated membrane protein in the caudate nucleus and substantia nigra of the cat. *Neuroscience* 40, 725–733.
- Childress, A. R., Mozley, P. D., McElgin, W., Fitzgerald, J., Reivich, M., and O'Brien, C. P. (1999). Limbic activation during cue-induced cocaine craving. *Am. J. Psychiatry* 156, 11–18.
- Christoph, G. R., Leonzio, R. J., and Wilcox, K. S. (1986). Stimulation of the lateral habenula inhibits dopamine-containing neurons in the substantia nigra and ventral tegmental area of the rat. *J. Neurosci.* 6, 613–619.
- Chuang, C., Fahn, S., and Frucht, S. J. (2002). The natural history and treatment of acquired hemidystonia: report of 33 cases and review of the literature. *J. Neurol. Neurosurg. Psychiatr.* 72, 59–67.
- Chuhma, N., Tanaka, K. F., Hen, R., and Rayport, S. (2011). Functional connectome of the striatal medium spiny neuron. *J. Neurosci.* 31, 1183–1192.
- Cohen, L. G., and Hallett, M. (1988). Hand cramps: clinical features and electromyographic patterns in a focal dystonia. *Neurology* 38, 1005–1012.
- Corvol, J. C., Muriel, M. P., Valjent, E., Feger, J., Hanoun, N., Girault, J. A., Hirsch, E. C., and Herve, D. (2004). Persistent increase in olfactory type G-protein alpha subunit levels may underlie D1 receptor functional hypersensitivity in Parkinson disease. *J. Neurosci.* 24, 7007–7014.
- Crittenden, J. R., Bergmeier, W., Zhang, Y., Piffath, C. L., Liang, Y., Wagner, D. D., Housman, D. E., and Graybiel, A. M. (2004). CalDAG-GEFI integrates signaling for platelet aggregation and thrombus formation. *Nat. Med.* 10, 982–986.
- Crittenden, J. R., Cantuti-Castelvetri, I., Saka, E., Keller-McGandy, C. E., Hernandez, L. F., Kett, L. R., Young, A. B., Standaert, D. G., and Graybiel, A. M. (2009). Dysregulation of CalDAG-GEFI and CalDAG-GEFII predicts the severity of motor side-effects induced by anti-parkinsonian therapy. *Proc. Natl. Acad. Sci. U.S.A.* 106, 2892–2896.
- Crittenden, J. R., Dunn, D. E., Meralli, F. I., Woodman, B., Yim, M., Borkowska, A. E., Frosch, M. P., Bates, G. P., Housman, D. E., Lo, D. C., and Graybiel, A. M. (2010a). CalDAG-GEFI down-regulation in the striatum as a neuroprotective change in Huntington's disease. *Hum. Mol. Genet.* 19, 1756–1765.
- Crittenden, J. R., Dunn, D. E., Bowden, H. A., Woodman, B., Yim, M. J., Frosch, M. P., Bates, G. P., Housman, D. E., Lo, D. C., and Graybiel, A. M. (2010b). "Dysregulation of the striatum-enriched signaling molecules, CalDAG-GEFI and CalDAG-GEFII, in Huntington's disease," in *Poster presentation at the Hereditary Disease Foundation's HD2010 meeting: "The Milton Wexler Celebration of Life"*, Cambridge, MA.
- Crittenden, J. R., Picconi, B., Ghiglieri, V., Calabresi, P., Harlan, P., Housman, D. E., and Graybiel, A. M. (2006). "CalDAG-GEFI is required for sensitization to amphetamine-induced stereotypy and corticostriatal LTP, but not for locomotor sensitization and LTD," in *Poster presentation at the Society for Neuroscience Annual Meeting*, Atlanta, GA.
- Damier, P., Hirsch, E. C., Agid, Y., and Graybiel, A. M. (1999a). The substantia nigra of the human brain. I. Nigrosomes and the nigral matrix, a compartmental organization based on calbindin D(28K) immunohistochemistry. *Brain* 122 (Pt 8), 1421–1436.
- Damier, P., Hirsch, E. C., Agid, Y., and Graybiel, A. M. (1999b). The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain* 122 (Pt 8), 1437–1448.
- Darmopil, S., Martin, A. B., De Diego, I. R., Ares, S., and Moratalla, R. (2009). Genetic inactivation of dopamine D1 but not D2 receptors inhibits L-DOPA-induced dyskinesia and histone activation. *Biol. Psychiatry* 66, 603–613.
- Davis, M. I., and Puhl, H. L. III. (2011). Nr4a1-eGFP is a marker of striosome-matrix architecture, development and activity in the extended striatum. *PLoS ONE* 6, e16619. doi: 10.1371/journal.pone.0016619
- Delmaire, C., Vidailhet, M., Wassermann, D., Descoteaux, M., Valabregue, R., Bourdain, F., Lenglet, C., Sangla, S., Terrier, A., Deriche, R., and Lehericy, S. (2009). Diffusion abnormalities in the primary sensorimotor pathways in writer's cramp. *Arch. Neurol.* 66, 502–508.
- Delong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends Neurosci.* 13, 281–285.

- Deroche-Gamonet, V., Belin, D., and Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science* 305, 1014–1017.
- Desplats, P. A., Kass, K. E., Gilmartin, T., Stanwood, G. D., Woodward, E. L., Head, S. R., Sutcliffe, J. G., and Thomas, E. A. (2006). Selective deficits in the expression of striatal-enriched mRNAs in Huntington's disease. *J. Neurochem.* 96, 743–757.
- Di Chiara, G., and Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. U.S.A.* 85, 5274–5278.
- Ding, Y., Won, L., Britt, J. P., Lim, S. A., McGehee, D. S., and Kang, U. J. (2011). Enhanced striatal cholinergic neuronal activity mediates L-DOPA-induced dyskinesia in parkinsonian mice. *Proc. Natl. Acad. Sci. U.S.A.* 108, 840–845.
- Doig, N. M., Moss, J., and Bolam, J. P. (2010). Cortical and thalamic innervation of direct and indirect pathway medium-sized spiny neurons in mouse striatum. *J. Neurosci.* 30, 14610–14618.
- Donoghue, J. P., and Herkenham, M. (1986). Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. *Brain Res.* 365, 397–403.
- Dower, N. A., Stang, S. L., Bottorff, D. A., Ebinu, J. O., Dickie, P., Ostergaard, H. L., and Stone, J. C. (2000). RasGRP is essential for mouse thymocyte differentiation and TCR signaling. *Nat. Immunol.* 1, 317–321.
- Dure, L. S. T., Young, A. B., and Penney, J. B. Jr. (1992). Compartmentalization of excitatory amino acid receptors in human striatum. *Proc. Natl. Acad. Sci. U.S.A.* 89, 7688–7692.
- Eblen, F., and Graybiel, A. M. (1995). Highly restricted origin of prefrontal cortical inputs to striosomes in the macaque monkey. *J. Neurosci.* 15, 5999–6013.
- Fahn, S. (1989). Clinical variants of idiopathic torsion dystonia. *J. Neurol. Neurosurg. Psychiatry* 52(Suppl.), 96–100.
- Farrer, M., Kachergus, J., Forno, L., Lincoln, S., Wang, D. S., Hulihan, M., Maraganore, D., Gwinn-Hardy, K., Wszolek, Z., Dickson, D., and Langston, J. W. (2004). Comparison of kindreds with parkinsonism and alpha-synuclein genomic multiplications. *Ann. Neurol.* 55, 174–179.
- Fasano, S., Bezard, E., D'antoni, A., Francardo, V., Indrigo, M., Qin, L., Dovero, S., Cerovic, M., Cenci, M. A., and Brambilla, R. (2010). Inhibition of Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) signaling in the striatum reverts motor symptoms associated with L-dopa-induced dyskinesia. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21824–21829.
- Fasano, S., D'antoni, A., Orban, P. C., Valjent, E., Putignano, E., Vara, H., Pizzorusso, T., Giustetto, M., Yoon, B., Soloway, P., Maldonado, R., Caboche, J., and Brambilla, R. (2009). Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) controls activation of extracellular signal-regulated kinase (ERK) signaling in the striatum and long-term behavioral responses to cocaine. *Biol. Psychiatry* 66, 758–768.
- Fearnley, J. M., and Lees, A. J. (1991). Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* 114(Pt 5), 2283–2301.
- Ferguson, S. M., and Robinson, T. E. (2004). Amphetamine-evoked gene expression in striatopallidal neurons: regulation by corticostriatal afferents and the ERK/MAPK signaling cascade. *J. Neurochem.* 91, 337–348.
- Ferrante, R. J., Kowall, N. W., Beal, M. F., Martin, J. B., Bird, E. D., and Richardson, E. P. Jr. (1987). Morphologic and histochemical characteristics of a spared subset of striatal neurons in Huntington's disease. *J. Neuropathol. Exp. Neurol.* 46, 12–27.
- Figueredo-Cardenas, G., Harris, C. L., Anderson, K. D., and Reiner, A. (1998). Relative resistance of striatal neurons containing calbindin or parvalbumin to quinolinic acid-mediated excitotoxicity compared to other striatal neuron types. *Exp. Neurol.* 149, 356–372.
- Fishell, G., and Van Der Kooy, D. (1987). Pattern formation in the striatum: developmental changes in the distribution of striatonigral neurons. *J. Neurosci.* 7, 1969–1978.
- Flaherty, A. W., and Graybiel, A. M. (1994). Input-output organization of the sensorimotor striatum in the squirrel monkey. *J. Neurosci.* 14, 599–610.
- Flaherty, A. W., and Graybiel, A. M. (1995). Motor and somatosensory corticostriatal projection magnifications in the squirrel monkey. *J. Neurophysiol.* 74, 2638–2648.
- Fotuhi, M., Dawson, T. M., Sharp, A. H., Martin, L. J., Graybiel, A. M., and Snyder, S. H. (1993). Phosphoinositide second messenger system is enriched in striosomes: immunohistochemical demonstration of inositol 1,4,5-trisphosphate receptors and phospholipase C beta and gamma in primate basal ganglia. *J. Neurosci.* 13, 3300–3308.
- Frechilla, D., Cobreros, A., Saldise, L., Moratalla, R., Insausti, R., Luquin, M., and Del Rio, J. (2001). Serotonin 5-HT(1A) receptor expression is selectively enhanced in the striosomal compartment of chronic parkinsonian monkeys. *Synapse* 39, 288–296.
- Freneau, R. T. Jr., Troyer, M. D., Pahner, I., Nygaard, G. O., Tran, C. H., Reimer, R. J., Bellocchio, E. E., Fortin, D., Storm-Mathisen, J., and Edwards, R. H. (2001). The expression of vesicular glutamate transporters defines two classes of excitatory synapse. *Neuron* 31, 247–260.
- Fujiyama, F., Sohn, J., Nakano, T., Furuta, T., Nakamura, K. C., Matsuda, W., and Kaneko, T. (2011). Exclusive and common targets of neostriatofugal projections of rat striosome neurons: a single neuron-tracing study using a viral vector. *Eur. J. Neurosci.* 33, 668–677.
- Fujiyama, F., Unzai, T., Nakamura, K., Nomura, S., and Kaneko, T. (2006). Difference in organization of corticostriatal and thalamostriatal synapses between patch and matrix compartments of rat neostriatum. *Eur. J. Neurosci.* 24, 2813–2824.
- Gerfen, C. R. (1984). The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. *Nature* 311, 461–464.
- Gerfen, C. R. (1985). The neostriatal mosaic. I. Compartmental organization of projections from the striatum to the substantia nigra in the rat. *J. Comp. Neurol.* 236, 454–476.
- Gerfen, C. R. (1989). The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. *Science* 246, 385–388.
- Gerfen, C. R. (2003). D1 dopamine receptor supersensitivity in the dopamine-depleted striatum animal model of Parkinson's disease. *Neuroscientist* 9, 455–462.
- Gerfen, C. R., Baimbridge, K. G., and Miller, J. J. (1985). The neostriatal mosaic: compartmental distribution of calcium-binding protein and parvalbumin in the basal ganglia of the rat and monkey. *Proc. Natl. Acad. Sci. U.S.A.* 82, 8780–8784.
- Gerfen, C. R., Herkenham, M., and Thibault, J. (1987). The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. *J. Neurosci.* 7, 3915–3934.
- Gerfen, C. R., Keefe, K. A., and Gauda, E. B. (1995). D1 and D2 dopamine receptor function in the striatum: coactivation of D1- and D2-dopamine receptors on separate populations of neurons results in potentiated immediate early gene response in D1-containing neurons. *J. Neurosci.* 15, 8167–8176.
- Gerfen, C. R., Miyachi, S., Paletzki, R., and Brown, P. (2002). D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. *J. Neurosci.* 22, 5042–5054.
- Gerfen, C. R., Paletzki, R., and Worley, P. (2008). Differences between dorsal and ventral striatum in Drd1a dopamine receptor coupling of dopamine- and cAMP-regulated phosphoprotein-32 to activation of extracellular signal-regulated kinase. *J. Neurosci.* 28, 7113–7120.
- Gerfen, C. R., and Young, W. S. III. (1988). Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Res.* 460, 161–167.
- Gibb, W. R. (1992). Melanin, tyrosine hydroxylase, calbindin and substance P in the human midbrain and substantia nigra in relation to nigrostriatal projections and differential neuronal susceptibility in Parkinson's disease. *Brain Res.* 581, 283–291.
- Gimenez-Amaya, J. M., and Graybiel, A. M. (1991). Modular organization of projection neurons in the matrix compartment of the primate striatum. *J. Neurosci.* 11, 779–791.
- Girault, J. A., Valjent, E., Caboche, J., and Herve, D. (2007). ERK2: a logical AND gate critical for drug-induced plasticity? *Curr. Opin. Pharmacol.* 7, 77–85.
- Glass, M., Dragunow, M., and Faull, R. L. (2000). The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. *Neuroscience* 97, 505–519.
- Glickstein, S. B., and Schmauss, C. (2004). Focused motor stereotypies do not require enhanced activation of neurons in striosomes. *J. Comp. Neurol.* 469, 227–238.
- Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E., and Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425, 917–925.
- Goto, S., and Hirano, A. (1990). Inhomogeneity of the putaminal lesion in striatonigral degeneration. *Acta Neuropathol.* 80, 204–207.

- Goto, S., Lee, L. V., Munoz, E. L., Tooyama, I., Tamiya, G., Makino, S., Ando, S., Dantes, M. B., Yamada, K., Matsumoto, S., Shimazu, H., Kuratsu, J., Hirano, A., and Kaji, R. (2005). Functional anatomy of the basal ganglia in X-linked recessive dystonia-parkinsonism. *Ann. Neurol.* 58, 7–17.
- Granado, N., Ares-Santos, S., O'shea, E., Vicario-Abejon, C., Colado, M. I., and Moratalla, R. (2010). Selective vulnerability in striosomes and in the nigrostriatal dopaminergic pathway after methamphetamine administration: early loss of TH in striosomes after methamphetamine. *Neurotox. Res.* 18, 48–58.
- Granado, N., Escobedo, I., O'shea, E., Colado, I., and Moratalla, R. (2008). Early loss of dopaminergic terminals in striosomes after MDMA administration to mice. *Synapse* 62, 80–84.
- Graybiel, A. M. (1984). Correspondence between the dopamine islands and striosomes of the mammalian striatum. *Neuroscience* 13, 1157–1187.
- Graybiel, A. M. (1990). Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci.* 13, 244–254.
- Graybiel, A. M. (2008). Habits, rituals, and the evaluative brain. *Annu. Rev. Neurosci.* 31, 359–387.
- Graybiel, A. M., Baughman, R. W., and Eckenstein, F. (1986). Cholinergic neuropil of the striatum observes striosomal boundaries. *Nature* 323, 625–627.
- Graybiel, A. M., Canales, J. J., and Capper-Loup, C. (2000). Levodopa-induced dyskinesias and dopamine-dependent stereotypies: a new hypothesis. *Trends Neurosci.* 23, S71–S77.
- Graybiel, A. M., and Hickey, T. L. (1982). Chemospecificity of ontogenetic units in the striatum: demonstration by combining [3H]thymidine neuronography and histochemical staining. *Proc. Natl. Acad. Sci. U.S.A.* 79, 198–202.
- Graybiel, A. M., Hirsch, E. C., and Agid, Y. (1990a). The nigrostriatal system in Parkinson's disease. *Adv. Neurol.* 53, 17–29.
- Graybiel, A. M., Moratalla, R., and Robertson, H. A. (1990b). Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 87, 6912–6916.
- Graybiel, A. M., Ohta, K., and Roffler-Tarlov, S. (1990c). Patterns of cell and fiber vulnerability in the mesostriatal system of the mutant mouse weaver. I. Gradients and compartments. *J. Neurosci.* 10, 720–733.
- Graybiel, A. M., Hirsch, E. C., and Agid, Y. A. (1987). Differences in tyrosine hydroxylase-like immunoreactivity characterize the mesostriatal innervation of striosomes and extrastriosomal matrix at maturity. *Proc. Natl. Acad. Sci. U.S.A.* 84, 303–307.
- Graybiel, A. M., and Mink, J. W. (2009). *The Cognitive Neurosciences*. Cambridge: MIT Press.
- Graybiel, A. M., and Ragsdale, C. W. Jr. (1978). Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. *Proc. Natl. Acad. Sci. U.S.A.* 75, 5723–5726.
- Graybiel, A. M., and Ragsdale, C. W. Jr. (1980). Clumping of acetylcholinesterase activity in the developing striatum of the human fetus and young infant. *Proc. Natl. Acad. Sci. U.S.A.* 77, 1214–1218.
- Guehl, D., Cuny, E., Ghorayeb, I., Michelet, T., Bioulac, B., and Burbaud, P. (2009). Primate models of dystonia. *Prog. Neurobiol.* 87, 118–131.
- Guo, F. F., Kumahara, E., and Saffen, D. (2001). A CalDAG-GEFI/Rap1/B-Raf cassette couples M(1) muscarinic acetylcholine receptors to the activation of ERK1/2. *J. Biol. Chem.* 276, 25568–25581.
- Haber, S. N., Fudge, J. L., and McFarland, N. R. (2000). Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *J. Neurosci.* 20, 2369–2382.
- Hanihara, T., Inoue, K., Kawanishi, C., Sugiyama, N., Miyakawa, T., Onishi, H., Yamada, Y., Osaka, H., Kosaka, K., Iwabuchi, K., and Owada, M. (1997). 6-Pyruvoyl-tetrahydropterin synthase deficiency with generalized dystonia and diurnal fluctuation of symptoms: a clinical and molecular study. *Mov. Disord.* 12, 408–411.
- Hanley, J. J., and Bolam, J. P. (1997). Synaptology of the nigrostriatal projection in relation to the compartmental organization of the neostriatum in the rat. *Neuroscience* 81, 353–370.
- Hedreen, J. C., and Folstein, S. E. (1995). Early loss of neostriatal striosome neurons in Huntington's disease. *J. Neuropathol. Exp. Neurol.* 54, 105–120.
- Henry, B., Duty, S., Fox, S. H., Crossman, A. R., and Brotchie, J. M. (2003). Increased striatal pre-proenkephalin B expression is associated with dyskinesia in Parkinson's disease. *Exp. Neurol.* 183, 458–468.
- Herkenham, M., and Nauta, W. J. (1979). Efferent connections of the habenular nuclei in the rat. *J. Comp. Neurol.* 187, 19–47.
- Herkenham, M., and Pert, C. B. (1981). Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum. *Nature* 291, 415–418.
- Hirsch, E., Graybiel, A. M., and Agid, Y. A. (1988). Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature* 334, 345–348.
- Hirsch, E. C., Graybiel, A. M., Hersh, L. B., Duyckaerts, C., and Agid, Y. (1989). Striosomes and extrastriosomal matrix contain different amounts of immunoreactive choline acetyltransferase in the human striatum. *Neurosci. Lett.* 96, 145–150.
- Hope, B. T., Nye, H. E., Kelz, M. B., Self, D. W., Iadarola, M. J., Nakabeppu, Y., Duman, R. S., and Nestler, E. J. (1994). Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* 13, 1235–1244.
- Huang, Q., Zhou, D., Sapp, E., Aizawa, H., Ge, P., Bird, E. D., Vonsattel, J. P., and Difiglia, M. (1995). Quinolinic acid-induced increases in calbindin D28k immunoreactivity in rat striatal neurons in vivo and in vitro mimic the pattern seen in Huntington's disease. *Neuroscience* 65, 397–407.
- Hurd, Y. L., and Herkenham, M. (1993). Molecular alterations in the neostriatum of human cocaine addicts. *Synapse* 13, 357–369.
- Hurd, Y. L., Herman, M. M., Hyde, T. M., Bigelow, L. B., Weinberger, D. R., and Kleinman, J. E. (1997). Prodynorphin mRNA expression is increased in the patch vs matrix compartment of the caudate nucleus in suicide subjects. *Mol. Psychiatry* 2, 495–500.
- Ichinose, H., Ohye, T., Takahashi, E., Seki, N., Hori, T., Segawa, M., Nomura, Y., Endo, K., Tanaka, H., Tsuji, S., Fujita, K., and Nagatsu, T. (1994). Hereditary progressive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase I gene. *Nat. Genet.* 8, 236–242.
- Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res. Rev.* 56, 27–78.
- Iravani, M. M., Syed, E., Jackson, M. J., Johnston, L. C., Smith, L. A., and Jenner, P. (2005). A modified MPTP treatment regime produces reproducible partial nigrostriatal lesions in common marmosets. *Eur. J. Neurosci.* 21, 841–854.
- Ito, H., Goto, S., Sakamoto, S., and Hirano, A. (1992). Calbindin-D28k in the basal ganglia of patients with parkinsonism. *Ann. Neurol.* 32, 543–550.
- Ito, H., Goto, S., Sakamoto, S., and Hirano, A. (1993). Striosomal arrangement of met-enkephalin and substance P expression in parkinsonism-dementia complex on Guam. *Acta Neuropathol.* 85, 390–393.
- Jabourian, M., Venance, L., Bourgoin, S., Ozon, S., Perez, S., Godeheu, G., Glowinski, J., and Kemel, M. L. (2005). Functional mu opioid receptors are expressed in cholinergic interneurons of the rat dorsal striatum: territorial specificity and diurnal variation. *Eur. J. Neurosci.* 21, 3301–3309.
- Janis, L. S., Cassidy, R. M., and Kromer, L. F. (1999). Ephrin-A binding and EphA receptor expression delineate the matrix compartment of the striatum. *J. Neurosci.* 19, 4962–4971.
- Jenner, P. (2008). Molecular mechanisms of L-DOPA-induced dyskinesia. *Nat. Rev. Neurosci.* 9, 665–677.
- Ji, H., and Shepard, P. D. (2007). Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABA(A) receptor-mediated mechanism. *J. Neurosci.* 27, 6923–6930.
- Jimenez-Castellanos, J., and Graybiel, A. M. (1987). Subdivisions of the dopamine-containing A8-A9-A10 complex identified by their differential mesostriatal innervation of striosomes and extrastriosomal matrix. *Neuroscience* 23, 223–242.
- Jimenez-Castellanos, J., and Graybiel, A. M. (1989). Compartmental origins of striatal efferent projections in the cat. *Neuroscience* 32, 297–321.
- Joel, D., and Weiner, I. (2000). The connections of the dopaminergic system with the striatum in rats and primates: an analysis with respect to the functional and compartmental organization of the striatum. *Neuroscience* 96, 451–474.
- Johnston, J. G., Gerfen, C. R., Haber, S. N., and Van Der Kooy, D. (1990). Mechanisms of striatal pattern formation: conservation of mammalian compartmentalization. *Brain Res. Dev. Brain Res.* 57, 93–102.
- Kaji, R., Urushihara, R., Murase, N., Shimazu, H., and Goto, S. (2005). Abnormal sensory gating in basal ganglia disorders. *J. Neurol.* 252(Suppl. 4), IV13–IV16.

- Kawaguchi, Y. (1997). Neostriatal cell subtypes and their functional roles. *Neurosci. Res.* 27, 1–8.
- Kawaguchi, Y., Wilson, C. J., and Emson, P. C. (1989). Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs. *J. Neurophysiol.* 62, 1052–1068.
- Kawaguchi, Y., Wilson, C. J., and Emson, P. C. (1990). Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. *J. Neurosci.* 10, 3421–3438.
- Kawasaki, H., Springett, G. M., Toki, S., Canales, J. J., Harlan, P., Blumenstiel, J. P., Chen, E. J., Bany, I. A., Mochizuki, N., Ashbacher, A., Matsuda, M., Housman, D. E., and Graybiel, A. M. (1998). A Rap guanine nucleotide exchange factor enriched highly in the basal ganglia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 13278–13283.
- Kim, D. S., Froelick, G. J., and Palmiter, R. D. (2002). Dopamine-dependent desensitization of dopaminergic signaling in the developing mouse striatum. *J. Neurosci.* 22, 9841–9849.
- Kincaid, A. E., and Wilson, C. J. (1996). Corticostriatal innervation of the patch and matrix in the rat neostriatum. *J. Comp. Neurol.* 374, 578–592.
- Kiriakakis, V., Bhatia, K. P., Quinn, N. P., and Marsden, C. D. (1998). The natural history of tardive dystonia. A long-term follow-up study of 107 cases. *Brain* 121(Pt 11), 2053–2066.
- Kish, S. J., Shannak, K., and Hornykiewicz, O. (1988). Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiology and clinical implications. *N. Engl. J. Med.* 318, 876–880.
- Koprich, J. B., Fox, S. H., Johnston, T. H., Goodman, A., Le Boudonnet, B., Dolle, R. E., Dehaven, R. N., Dehaven-Hudkins, D. L., Little, P. J., and Brochie, J. M. (2011). The selective mu-opioid receptor antagonist adl5510 reduces levodopa-induced dyskinesia without affecting antiparkinsonian action in mptp-lesioned macaque model of Parkinson's disease. *Mov. Disord.* 26, 1225–1233.
- Korematsu, K., Goto, S., Okamura, A., and Ushio, Y. (1998). Heterogeneity of cadherin-8 expression in the neonatal rat striatum: comparison with striatal compartments. *Exp. Neurol.* 154, 531–536.
- Koshimizu, Y., Wu, S. X., Unzai, T., Hioki, H., Sonomura, T., Nakamura, K. C., Fujiyama, F., and Kaneko, T. (2008). Paucity of enkephalin production in neostriatal striosomal neurons: analysis with preproenkephalin-green fluorescent protein transgenic mice. *Eur. J. Neurosci.* 28, 2053–2064.
- Kosinski, C. M., Cha, J. H., Young, A. B., Persichetti, F., Macdonald, M., Gusella, J. F., Penney, J. B. Jr., and Standaert, D. G. (1997). Huntingtin immunoreactivity in the rat neostriatum: differential accumulation in projection and interneurons. *Exp. Neurol.* 144, 239–247.
- Krauss, M., Langnaese, K., Richter, K., Brunk, I., Wieske, M., Ahnert-Hilger, G., Veh, R. W., and Laube, G. (2006). Spermidine synthase is prominently expressed in the striatal patch compartment and in putative interneurons of the matrix compartment. *J. Neurochem.* 97, 174–189.
- Kravitz, A. V., and Kreitzer, A. C. (2011). Optogenetic manipulation of neural circuitry in vivo. *Curr. Opin. Neurobiol.* 21, 433–439.
- Krebs, M. O., Trovero, F., Desban, M., Gauchy, C., Glowinski, J., and Kemel, M. L. (1991). Distinct presynaptic regulation of dopamine release through NMDA receptors in striosome- and matrix-enriched areas of the rat striatum. *J. Neurosci.* 11, 1256–1262.
- Kreek, M. J., Nielsen, D. A., Butelman, E. R., and Laforge, K. S. (2005). Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. *Nat. Neurosci.* 8, 1450–1457.
- Kubota, Y., and Kawaguchi, Y. (1993). Spatial distributions of chemically identified intrinsic neurons in relation to patch and matrix compartments of rat neostriatum. *J. Comp. Neurol.* 332, 499–513.
- Lacey, C. J., Bolam, J. P., and Magill, P. J. (2007). Novel and distinct operational principles of intralaminar thalamic neurons and their striatal projections. *J. Neurosci.* 27, 4374–4384.
- Lacey, C. J., Boyes, J., Gerlach, O., Chen, L., Magill, P. J., and Bolam, J. P. (2005). GABA(B) receptors at glutamatergic synapses in the rat striatum. *Neuroscience* 136, 1083–1095.
- Langer, L. F., and Graybiel, A. M. (1989). Distinct nigrostriatal projection systems innervate striosomes and matrix in the primate striatum. *Brain Res.* 498, 344–350.
- Lapper, S. R., and Bolam, J. P. (1992). Input from the frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. *Neuroscience* 51, 533–545.
- Lapper, S. R., Smith, Y., Sadikot, A. F., Parent, A., and Bolam, J. P. (1992). Cortical input to parvalbumin-immunoreactive neurones in the putamen of the squirrel monkey. *Brain Res.* 580, 215–224.
- Lawhorn, C., Smith, D. M., and Brown, L. L. (2008). Striosome-matrix pathology and motor deficits in the YAC128 mouse model of Huntington's disease. *Neurobiol. Dis.* 32, 471–478.
- Lebel, M., Chagniel, L., Bureau, G., and Cyr, M. (2010). Striatal inhibition of PKA prevents levodopa-induced behavioural and molecular changes in the hemiparkinsonian rat. *Neurobiol. Dis.* 38, 59–67.
- Lee, C. R., and Tepper, J. M. (2009). Basal ganglia control of substantia nigra dopaminergic neurons. *J. Neural Transm. Suppl.* 73, 71–90.
- Lee, H., Leamey, C. A., and Sawtari, A. (2008). Rapid reversal of chondroitin sulfate proteoglycan associated staining in subcompartments of mouse neostriatum during the emergence of behaviour. *PLoS ONE* 3, e3020. doi: 10.1371/journal.pone.0003020
- Lee, S. P., So, C. H., Rashid, A. J., Varghese, G., Cheng, R., Lanca, A. J., O'dowd, B. F., and George, S. R. (2004). Dopamine D1 and D2 receptor Co-activation generates a novel phospholipase C-mediated calcium signal. *J. Biol. Chem.* 279, 35671–35678.
- Lenz, F. A., Suarez, J. I., Metman, L. V., Reich, S. G., Karp, B. I., Hallett, M., Rowland, L. H., and Dougherty, P. M. (1998). Pallidal activity during dystonia: somatosensory reorganisation and changes with severity. *J. Neurol. Neurosurg. Psychiatr.* 65, 767–770.
- Levesque, M., Charara, A., Gagnon, S., Parent, A., and Deschenes, M. (1996). Corticostriatal projections from layer V cells in rat are collaterals of long-range corticofugal axons. *Brain Res.* 709, 311–315.
- Levesque, M., and Parent, A. (1998). Axonal arborization of corticostriatal and corticothalamic fibers arising from prelimbic cortex in the rat. *Cereb. Cortex* 8, 602–613.
- Levesque, M., and Parent, A. (2005). The striatofugal fiber system in primates: a reevaluation of its organization based on single-axon tracing studies. *Proc. Natl. Acad. Sci. U.S.A.* 102, 11888–11893.
- Liao, W. L., Tsai, H. C., Wang, H. F., Chang, J., Lu, K. M., Wu, H. L., Lee, Y. C., Tsai, T. F., Takahashi, H., Wagner, M., Ghyselinck, N. B., Chambon, P., and Liu, F. C. (2008). Modular patterning of structure and function of the striatum by retinoid receptor signaling. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6765–6770.
- Liao, W. L., Tsai, H. C., Wu, C. Y., and Liu, F. C. (2005). Differential expression of RARbeta isoforms in the mouse striatum during development: a gradient of RARbeta2 expression along the rostrocaudal axis. *Dev. Dyn.* 233, 584–594.
- Lievens, J. C., Woodman, B., Mahal, A., and Bates, G. P. (2002). Abnormal phosphorylation of synapsin I predicts a neuronal transmission impairment in the R6/2 Huntington's disease transgenic mice. *Mol. Cell. Neurosci.* 20, 638–648.
- Liu, F. C., and Graybiel, A. M. (1992). Heterogeneous development of calbindin-D28K expression in the striatal matrix. *J. Comp. Neurol.* 320, 304–322.
- Lopez-Gimenez, J. F., Mengod, G., Palacios, J. M., and Vilaro, M. T. (1999). Human striosomes are enriched in 5-HT2A receptors: autoradiographical visualization with [3H]MDL100,907,[125I](±)DOI and [3H]ketanserin. *Eur. J. Neurosci.* 11, 3761–3765.
- Lopez-Gimenez, J. F., Tecott, L. H., Palacios, J. M., Mengod, G., and Vilaro, M. T. (2002). Serotonin 5-HT (2C) receptor knockout mice: autoradiographic analysis of multiple serotonin receptors. *J. Neurosci. Res.* 67, 69–85.
- Lopez-Gimenez, J. F., Vilaro, M. T., Palacios, J. M., and Mengod, G. (2001). Mapping of 5-HT2A receptors and their mRNA in monkey brain: [3H]MDL100,907 autoradiography and in situ hybridization studies. *J. Comp. Neurol.* 429, 571–589.
- Lopez-Martin, E., Caruncho, H. J., Rodriguez-Pallares, J., Guerra, M. J., and Labandeira-Garcia, J. L. (1999). Striatal dopaminergic afferents concentrate in GDNF-positive patches during development and in developing intrastriatal striatal grafts. *J. Comp. Neurol.* 406, 199–206.
- Ludecke, B., Knappskog, P. M., Clayton, P. T., Surtees, R. A., Clelland, J. D., Heales, S. J., Brand, M. P., Bartholome, K., and Flatmark, T. (1996). Recessively inherited L-DOPA-responsive parkinsonism in infancy caused by a point mutation (L205P) in the tyrosine hydroxylase gene. *Hum. Mol. Genet.* 5, 1023–1028.
- Lyon, M., and Robbins, T. W. (1975). *The Action of Central Nervous System Stimulant Drugs: A General Theory Concerning Amphetamine Effects*. New York, NY: Spectrum.

- Mahmoudi, S., Samadi, P., Gilbert, F., Ouattara, B., Morissette, M., Gregoire, L., Rouillard, C., Di Paolo, T., and Levesque, D. (2009). Nur77 mRNA levels and L-Dopa-induced dyskinesias in MPTP monkeys treated with docosahexaenoic acid. *Neurobiol. Dis.* 36, 213–222.
- Makino, S., Kaji, R., Ando, S., Tomizawa, M., Yasuno, K., Goto, S., Matsumoto, S., Tabuena, M. D., Maranon, E., Dantes, M., Lee, L. V., Ogasawara, K., Tooyama, I., Akatsu, H., Nishimura, M., and Tamiya, G. (2007). Reduced neuron-specific expression of the TAF1 gene is associated with X-linked dystonia-parkinsonism. *Am. J. Hum. Genet.* 80, 393–406.
- Martin, L. J., Blackstone, C. D., Levey, A. I., Huganir, R. L., and Price, D. L. (1993a). AMPA glutamate receptor subunits are differentially distributed in rat brain. *Neuroscience* 53, 327–358.
- Martin, L. J., Blackstone, C. D., Levey, A. I., Huganir, R. L., and Price, D. L. (1993b). Cellular localizations of AMPA glutamate receptors within the basal forebrain magnocellular complex of rat and monkey. *J. Neurosci.* 13, 2249–2263.
- Martin, L. J., Hadfield, M. G., Dellovade, T. L., and Price, D. L. (1991). The striatal mosaic in primates: patterns of neuropeptide immunoreactivity differentiate the ventral striatum from the dorsal striatum. *Neuroscience* 43, 397–417.
- Martone, M. E., Holash, J. A., Bayardo, A., Pasquale, E. B., and Ellisman, M. H. (1997). Immunolocalization of the receptor tyrosine kinase EphA4 in the adult rat central nervous system. *Brain Res.* 771, 238–250.
- Matsuda, W., Furruta, T., Nakamura, K. C., Hioki, H., Fujiyama, F., Arai, R., and Kaneko, T. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *J. Neurosci.* 29, 444–453.
- Matsumoto, M., and Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature* 447, 1111–1115.
- Medina, L., Figueredo-Cardenas, G., and Reiner, A. (1996). Differential abundance of superoxide dismutase in interneurons versus projection neurons and in matrix versus striosome neurons in monkey striatum. *Brain Res.* 708, 59–70.
- Menalled, L. B., Sison, J. D., Wu, Y., Olivieri, M., Li, X. J., Li, H., Zeitlin, S., and Chesselet, M. F. (2002). Early motor dysfunction and striosomal distribution of huntingtin microaggregates in Huntington's disease knock-in mice. *J. Neurosci.* 22, 8266–8276.
- Meurers, B. H., Dziewczapolski, G., Shi, T., Bittner, A., Kamme, F., and Shults, C. W. (2009). Dopamine depletion induces distinct compensatory gene expression changes in DARPP-32 signal transduction cascades of striatonigral and striatopallidal neurons. *J. Neurosci.* 29, 6828–6839.
- Mikula, S., Parrish, S. K., Trimmer, J. S., and Jones, E. G. (2009). Complete 3D visualization of primate striosomes by KChIP1 immunostaining. *J. Comp. Neurol.* 514, 507–517.
- Miura, M., Masuda, M., and Aosaki, T. (2008). Roles of micro-opioid receptors in GABAergic synaptic transmission in the striosome and matrix compartments of the striatum. *Mol. Neurobiol.* 37, 104–115.
- Miura, M., Saino-Saito, S., Masuda, M., Kobayashi, K., and Aosaki, T. (2007). Compartment-specific modulation of GABAergic synaptic transmission by mu-opioid receptor in the mouse striatum with green fluorescent protein-expressing dopamine islands. *J. Neurosci.* 27, 9721–9728.
- Moon Edley, S., and Herkenham, M. (1984). Comparative development of striatal opiate receptors and dopamine revealed by autoradiography and histofluorescence. *Brain Res.* 305, 27–42.
- Moratalla, R., Elibol, B., Vallejo, M., and Graybiel, A. M. (1996). Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. *Neuron* 17, 147–156.
- Moratalla, R., Quinn, B., Delanney, L. E., Irwin, I., Langston, J. W., and Graybiel, A. M. (1992). Differential vulnerability of primate caudate-putamen and striosome-matrix dopamine systems to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci. U.S.A.* 89, 3859–3863.
- Morigaki, R., Sako, W., Okita, S., Kasahara, J., Yokoyama, H., Nagahiro, S., Kaji, R., and Goto, S. (2011). Cyclin-dependent kinase 5 with phosphorylation of serine 159 residue is enriched in striatal matrix compartment in adult mice. *Neuroscience* 189, 25–31.
- Morton, A. J., Nicholson, L. F., and Faull, R. L. (1993). Compartmental loss of NADPH diaphorase in the neuropil of the human striatum in Huntington's disease. *Neuroscience* 53, 159–168.
- Moss, J., and Bolam, J. P. (2008). A dopaminergic axon lattice in the striatum and its relationship with cortical and thalamic terminals. *J. Neurosci.* 28, 11221–11230.
- Murrin, L. C., and Zeng, W. Y. (1989). Dopamine D1 receptor development in the rat striatum: early localization in striosomes. *Brain Res.* 480, 170–177.
- Napolitano, F., Pasqualetti, M., Usiello, A., Santini, E., Pacini, G., Sciamanna, G., Errico, F., Tassone, A., Di Dato, V., Martella, G., Cuomo, D., Fisone, G., Bernardi, G., Mandolesi, G., Mercuri, N. B., Standaert, D. G., and Pisani, A. (2010). Dopamine D2 receptor dysfunction is rescued by adenosine A2A receptor antagonism in a model of DYT1 dystonia. *Neurobiol. Dis.* 38, 434–445.
- Nastuk, M. A., and Graybiel, A. M. (1985). Patterns of muscarinic cholinergic binding in the striatum and their relation to dopamine islands and striosomes. *J. Comp. Neurol.* 237, 176–194.
- Naumann, M., Pirker, W., Reiners, K., Lange, K. W., Becker, G., and Brucke, T. (1998). Imaging the pre- and post-synaptic side of striatal dopaminergic synapses in idiopathic cervical dystonia: a SPECT study using [123I] epidepride and [123I] beta-CIT. *Mov. Disord.* 13, 319–323.
- Newman-Gage, H., and Graybiel, A. M. (1988). Expression of calcium/calmodulin-dependent protein kinase in relation to dopamine islands and synaptic maturation in the cat striatum. *J. Neurosci.* 8, 3360–3375.
- Nishikawa, S., Goto, S., Hamasaki, T., Ogawa, M., and Ushio, Y. (1999). Transient and compartmental expression of the reeler gene product reelin in the developing rat striatum. *Brain Res.* 850, 244–248.
- Nishikawa, S., Goto, S., Yamada, K., Hamasaki, T., and Ushio, Y. (2003). Lack of Reelin causes malpositioning of nigral dopaminergic neurons: evidence from comparison of normal and Reelin (rl) mutant mice. *J. Comp. Neurol.* 461, 166–173.
- Oberlin, S. R., Konakova, M., Pulst, S., and Chesselet, M. F. (2004). Development and anatomic localization of torsinA. *Adv. Neurol.* 94, 61–65.
- Ohmori, T., Abekawa, T., and Koyama, T. (1995). Scopolamine prevents augmentation of stereotypy induced by chronic methamphetamine treatment. *Psychopharmacology (Berl.)* 121, 158–163.
- Olson, L., Seiger, A., and Fuxe, K. (1972). Heterogeneity of striatal and limbic dopamine innervation: highly fluorescent islands in developing and adult rats. *Brain Res.* 44, 283–288.
- Oo, T. F., Ries, V., Cho, J., Kholodilov, N., and Burke, R. E. (2005). Anatomical basis of glial cell line-derived neurotrophic factor expression in the striatum and related basal ganglia during postnatal development of the rat. *J. Comp. Neurol.* 484, 57–67.
- Ozelius, L. J., Hewett, J. W., Page, C. E., Bressman, S. B., Kramer, P. L., Shalish, C., De Leon, D., Brin, M. F., Raymond, D., Corey, D. P., Fahn, S., Risch, N. J., Buckler, A. J., Gusella, J. F., and Breakefield, X. O. (1997). The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. *Nat. Genet.* 17, 40–48.
- Parthasarathy, H. B., and Graybiel, A. M. (1997). Cortically driven immediate-early gene expression reflects modular influence of sensorimotor cortex on identified striatal neurons in the squirrel monkey. *J. Neurosci.* 17, 2477–2491.
- Passante, L., Gaspard, N., Degraeve, M., Frisen, J., Kullander, K., De Maertelaer, V., and Vanderhaeghen, P. (2008). Temporal regulation of ephrin/Eph signalling is required for the spatial patterning of the mammalian striatum. *Development* 135, 3281–3290.
- Perreault, M. L., Hasbi, A., Alijanian, M., Fan, T., Varghese, G., Fletcher, P. J., Seeman, P., O'dowd, B. F., and George, S. R. (2010). The dopamine D1-D2 receptor heteromer localizes in dynorphin/enkephalin neurons: increased high affinity state following amphetamine and in schizophrenia. *J. Biol. Chem.* 285, 36625–36634.
- Pert, C. B., Kuhar, M. J., and Snyder, S. H. (1976). Opiate receptor: autoradiographic localization in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* 73, 3729–3733.
- Peterson, D. A., Sejnowski, T. J., and Poizner, H. (2010). Convergent evidence for abnormal striatal synaptic plasticity in dystonia. *Neurobiol. Dis.* 37, 558–573.
- Pierret, P., Mechawar, N., Vallee, A., Patel, J., Priestley, J. V., Dunn, R. J., Dower, N. A., Stone, J. C., and Richardson, P. M. (2002). Presence of Ras guanyl nucleotide-releasing protein in striosomes of the mature and developing rat. *Neuroscience* 111, 83–94.
- Pisani, A., Bonsi, P., Centonze, D., Gubellini, P., Bernardi, G., and Calabresi, P. (2003). Targeting striatal cholinergic interneurons in Parkinson's disease: focus on metabotropic glutamate receptors. *Neuropharmacology* 45, 45–56.
- Playford, E. D., Fletcher, N. A., Sawle, G. V., Marsden, C. D., and Brooks, D. J. (1993). Striatal [18F]dopa uptake in familial idiopathic dystonia. *Brain* 116 (Pt 5), 1191–1199.

- Politis, M., Pavese, N., Tai, Y. F., Kiferle, L., Mason, S. L., Brooks, D. J., Tabrizi, S. J., Barker, R. A., and Piccini, P. (2011). Microglial activation in regions related to cognitive function predicts disease onset in Huntington's disease: a multimodal imaging study. *Hum. Brain Mapp.* 32, 258–270.
- Porrino, L. J., Smith, H. R., Nader, M. A., and Beveridge, T. J. (2007). The effects of cocaine: a shifting target over the course of addiction. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31, 1593–1600.
- Prensa, L., and Parent, A. (2001). The nigrostriatal pathway in the rat: a single-axon study of the relationship between dorsal and ventral tier nigral neurons and the striosome/matrix striatal compartments. *J. Neurosci.* 21, 7247–7260.
- Quartarone, A., Bagnato, S., Rizzo, V., Siebner, H. R., Dattola, V., Scalfari, A., Morgante, F., Battaglia, F., Romano, M., and Girlanda, P. (2003). Abnormal associative plasticity of the human motor cortex in writer's cramp. *Brain* 126, 2586–2596.
- Quartarone, A., Morgante, F., Sant'angelo, A., Rizzo, V., Bagnato, S., Terranova, C., Siebner, H. R., Berardelli, A., and Girlanda, P. (2008). Abnormal plasticity of sensorimotor circuits extends beyond the affected body part in focal dystonia. *J. Neurol. Neurosurg. Psychiatr.* 79, 985–990.
- Ragsdale, C. W. Jr., and Graybiel, A. M. (1988). Fibers from the basolateral nucleus of the amygdala selectively innervate striosomes in the caudate nucleus of the cat. *J. Comp. Neurol.* 269, 506–522.
- Ragsdale, C. W. Jr., and Graybiel, A. M. (1990). A simple ordering of neocortical areas established by the compartmental organization of their striatal projections. *Proc. Natl. Acad. Sci. U.S.A.* 87, 6196–6199.
- Ragsdale, C. W. Jr., and Graybiel, A. M. (1991). Compartmental organization of the thalamostriatal connection in the cat. *J. Comp. Neurol.* 311, 134–167.
- Rajakumar, N., Elisavich, K., and Flumerfelt, B. A. (1993). Compartmental origin of the striato-entopeduncular projection in the rat. *J. Comp. Neurol.* 331, 286–296.
- Raju, D. V., Shah, D. J., Wright, T. M., Hall, R. A., and Smith, Y. (2006). Differential synaptology of vGluT2-containing thalamostriatal afferents between the patch and matrix compartments in rats. *J. Comp. Neurol.* 499, 231–243.
- Redies, C., Kovjanic, D., Heyers, D., Medina, L., Hirano, S., Suzuki, S. T., and Puelles, L. (2002). Patch/matrix patterns of gray matter differentiation in the telencephalon of chicken and mouse. *Brain Res. Bull.* 57, 489–493.
- Reiner, A., Albin, R. L., Anderson, K. D., D'Amato, C. J., Penney, J. B., and Young, A. B. (1988). Differential loss of striatal projection neurons in Huntington disease. *Proc. Natl. Acad. Sci. U.S.A.* 85, 5733–5737.
- Reiner, A., Hart, N. M., Lei, W., and Deng, Y. (2010). Corticostriatal projection neurons – dichotomous types and dichotomous functions. *Front. Neuroanat.* 4:142. doi: 10.3389/fnana.2010.00142
- Reiner, A., Jiao, Y., Del Mar, N., Laverghetta, A. V., and Lei, W. L. (2003). Differential morphology of pyramidal tract-type and intratelencephalically projecting-type corticostriatal neurons and their intrastriatal terminals in rats. *J. Comp. Neurol.* 457, 420–440.
- Rivera, A., Cuellar, B., Giron, F. J., Grandy, D. K., De La Calle, A., and Moratalla, R. (2002). Dopamine D4 receptors are heterogeneously distributed in the striosomes/matrix compartments of the striatum. *J. Neurochem.* 80, 219–229.
- Roberts, R. C., Roche, J. K., and Conley, R. R. (2005). Synaptic differences in the patch matrix compartments of subjects with schizophrenia: a postmortem ultrastructural study of the striatum. *Neurobiol. Dis.* 20, 324–335.
- Robinson, T. E., and Berridge, K. C. (2008). Review. The incentive sensitization theory of addiction: some current issues. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 3137–3146.
- Rosas, H. D., Salat, D. H., Lee, S. Y., Zaleta, A. K., Hevelone, N., and Hersch, S. M. (2008). Complexity and heterogeneity: what drives the ever-changing brain in Huntington's disease? *Ann. N. Y. Acad. Sci.* 1147, 196–205.
- Roze, E., Betuing, S., Deyts, C., Marcon, E., Bami-Cherrier, K., Pages, C., Humbert, S., Merienne, K., and Caboche, J. (2008). Mitogen- and stress-activated protein kinase-1 deficiency is involved in expanded-huntingtin-induced transcriptional dysregulation and striatal death. *FASEB J.* 22, 1083–1093.
- Roze, E., Soumare, A., Pironneau, I., Sangla, S., De Cock, V. C., Teixeira, A., Astorquiza, A., Bonnet, C., Bleton, J. P., Vidailhet, M., and Elbaz, A. (2009). Case-control study of writer's cramp. *Brain* 132, 756–764.
- Russo, S. J., Dietz, D. M., Dumitriu, D., Morrison, J. H., Malenka, R. C., and Nestler, E. J. (2010). The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci.* 33, 267–276.
- Sadikot, A. F., Parent, A., Smith, Y., and Bolam, J. P. (1992). Efferent connections of the centromedian and parafascicular thalamic nuclei in the squirrel monkey: a light and electron microscopic study of the thalamostriatal projection in relation to striatal heterogeneity. *J. Comp. Neurol.* 320, 228–242.
- Saka, E., Elibol, B., Erdem, S., and Dalkara, T. (1999). Compartmental changes in expression of c-Fos and FosB proteins in intact and dopamine-depleted striatum after chronic apomorphine treatment. *Brain Res.* 825, 104–114.
- Saka, E., Goodrich, C., Harlan, P., Madras, B. K., and Graybiel, A. M. (2004). Repetitive behaviors in monkeys are linked to specific striatal activation patterns. *J. Neurosci.* 24, 7557–7565.
- Saka, E., Iadarola, M., Fitzgerald, D. J., and Graybiel, A. M. (2002). Local circuit neurons in the striatum regulate neural and behavioral responses to dopaminergic stimulation. *Proc. Natl. Acad. Sci. U.S.A.* 99, 9004–9009.
- Sako, W., Morigaki, R., Kaji, R., Tooyama, I., Okita, S., Kitazato, K., Nagahiro, S., Graybiel, A. M., and Goto, S. (2011). Identification and localization of neuron-specific isoform of TAF1 in rat brain: implications for neuropathology of DYT3 dystonia. *Neuroscience* 189, 100–107.
- Sako, W., Morigaki, R., Nagahiro, S., Kaji, R., and Goto, S. (2010). Olfactory type G-protein alpha subunit in striosome-matrix dopamine systems in adult mice. *Neuroscience* 170, 497–502.
- Sandell, J. H., Graybiel, A. M., and Chase, M. F. (1986). A new enzyme marker for striatal compartmentalization: NADPH diaphorase activity in the caudate nucleus and putamen of the cat. *J. Comp. Neurol.* 243, 326–334.
- Santini, E., Alcacer, C., Cacciatore, S., Heiman, M., Herve, D., Greengard, P., Girault, J. A., Valjent, E., and Fisone, G. (2009). L-DOPA activates ERK signaling and phosphorylates histone H3 in the striatonigral medium spiny neurons of hemiparkinsonian mice. *J. Neurochem.* 108, 621–633.
- Santini, E., Sgambato-Faure, V., Li, Q., Savasta, M., Dovero, S., Fisone, G., and Bezard, E. (2010). Distinct changes in cAMP and extracellular signal-regulated protein kinase signalling in L-DOPA-induced dyskinesia. *PLoS ONE* 5, e12322. doi: 10.1371/journal.pone.0012322
- Santini, E., Valjent, E., Usiello, A., Carta, M., Borgkvist, A., Girault, J. A., Herve, D., Greengard, P., and Fisone, G. (2007). Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signalling in L-DOPA-induced dyskinesia. *J. Neurosci.* 27, 6995–7005.
- Sato, K., Kaji, R., Matsumoto, S., Nagahiro, S., and Goto, S. (2007). Compartmental loss of striatal medium spiny neurons in multiple system atrophy of parkinsonian type. *Mov. Disord.* 22, 2365–2370.
- Sato, K., Sumi-Ichinose, C., Kaji, R., Ikemoto, K., Nomura, T., Nagatsu, I., Ichinose, H., Ito, M., Sako, W., Nagahiro, S., Graybiel, A. M., and Goto, S. (2008). Differential involvement of striosome and matrix dopamine systems in a transgenic model of dopa-responsive dystonia. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12551–12556.
- Schiffmann, S. N., and Vanderhaeghen, J. J. (1992). Ontogeny of gene expression of adenosine A2 receptor in the striatum: early localization in the patch compartment. *J. Comp. Neurol.* 317, 117–128.
- Schmidt, A., Jabusch, H. C., Altmüller, E., Hagenah, J., Brügemann, N., Lohmann, K., Enders, L., Kramer, P. L., Saunders-Pullman, R., Bressman, S. B., Munchau, A., and Klein, C. (2009). Etiology of musician's dystonia: familial or environmental? *Neurology* 72, 1248–1254.
- Schoen, S. W., and Graybiel, A. M. (1992). 5'-nucleotidase: a new marker for striosomal organization in the rat caudoputamen. *J. Comp. Neurol.* 322, 566–576.
- Segawa, M. (2011). Hereditary progressive dystonia with marked diurnal fluctuation. *Brain Dev.* 33, 195–201.
- Seto-Oshima, A., Emson, P. C., Lawson, E., Mountjoy, C. Q., and Carrasco, L. H. (1988). Loss of matrix calcium-binding protein-containing neurons in Huntington's disease. *Lancet* 1, 1252–1255.
- Shashidharan, P., Kramer, B. C., Walker, R. H., Olanow, C. W., and Brin, M. F. (2000). Immunohistochemical localization and distribution of torsinA in normal human and rat brain. *Brain Res.* 853, 197–206.

- Shi, X., and Mcginty, J. F. (2006). Extracellular signal-regulated mitogen-activated protein kinase inhibitors decrease amphetamine-induced behavior and neuroepitide gene expression in the striatum. *Neuroscience* 138, 1289–1298.
- Shulman, J. M., De Jager, P. L., and Feany, M. B. (2011). Parkinson's disease: genetics and pathogenesis. *Annu. Rev. Pathol.* 6, 193–222.
- Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M. R., Muentner, M., Baptista, M., Miller, D., Blancato, J., Hardy, J., and Gwinn-Hardy, K. (2003). alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 302, 841.
- Sinha, R. (2008). Chronic stress, drug use, and vulnerability to addiction. *Ann. N. Y. Acad. Sci.* 1141, 105–130.
- Somogyi, P., Bolam, J. P., Totterdell, S., and Smith, A. D. (1981). Monosynaptic input from the nucleus accumbens – ventral striatum region to retrogradely labelled nigrostriatal neurones. *Brain Res.* 217, 245–263.
- Stack, E. C., Dedeoglu, A., Smith, K. M., Cormier, K., Kubilus, J. K., Bogdanov, M., Matson, W. R., Yang, L., Jenkins, B. G., Luthi-Carter, R., Kowall, N. W., Hersch, S. M., Beal, M. F., and Ferrante, R. J. (2007). Neuroprotective effects of synaptic modulation in Huntington's disease R6/2 mice. *J. Neurosci.* 27, 12908–12915.
- Sun, Z., Wang, H. B., Deng, Y. P., Lei, W. L., Xie, J. P., Meade, C. A., Del Mar, N., Goldowitz, D., and Reiner, A. (2005). Increased calbindin-D28k immunoreactivity in striatal projection neurons of R6/2 Huntington's disease transgenic mice. *Neurobiol. Dis.* 20, 907–917.
- Surmeier, D. J., Song, W. J., and Yan, Z. (1996). Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. *J. Neurosci.* 16, 6579–6591.
- Takahashi, K., Liu, F. C., Hirokawa, K., and Takahashi, H. (2003). Expression of Foxp2, a gene involved in speech and language, in the developing and adult striatum. *J. Neurosci. Res.* 73, 61–72.
- Takahashi, K., Liu, F. C., Oishi, T., Mori, T., Higo, N., Hayashi, M., Hirokawa, K., and Takahashi, H. (2008). Expression of FOXP2 in the developing monkey forebrain: comparison with the expression of the genes FOXP1, PBX3, and MEIS2. *J. Comp. Neurol.* 509, 180–189.
- Tamura, Y., Ueki, Y., Lin, P., Vorbach, S., Mima, T., Kakigi, R., and Hallett, M. (2009). Disordered plasticity in the primary somatosensory cortex in focal hand dystonia. *Brain* 132, 749–755.
- Tan, A., Moratalla, R., Lyford, G. L., Worley, P., and Graybiel, A. M. (2000). The activity-regulated cytoskeletal-associated protein arc is expressed in different striosome-matrix patterns following exposure to amphetamine and cocaine. *J. Neurochem.* 74, 2074–2078.
- Tanabe, L. M., Kim, C. E., Alagem, N., and Dauer, W. T. (2009). Primary dystonia: molecules and mechanisms. *Nat. Rev. Neurol.* 5, 598–609.
- Tang, T. S., Chen, X., Liu, J., and Bezprozvanny, I. (2007). Dopaminergic signaling and striatal neurodegeneration in Huntington's disease. *J. Neurosci.* 27, 7899–7910.
- Tanimura, Y., King, M. A., Williams, D. K., and Lewis, M. H. (2011). Development of repetitive behavior in a mouse model: roles of indirect and striosomal basal ganglia pathways. *Int. J. Dev. Neurosci.* 29, 461–467.
- Tappe, A., and Kuner, R. (2006). Regulation of motor performance and striatal function by synaptic scaffolding proteins of the Homer1 family. *Proc. Natl. Acad. Sci. U.S.A.* 103, 774–779.
- The Huntington's Disease Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* 72, 971–983.
- Thony, B., and Blau, N. (1997). Mutations in the GTP cyclohydrolase I and 6-pyruvoyl-tetrahydropterin synthase genes. *Hum. Mutat.* 10, 11–20.
- Thony, B., and Blau, N. (2006). Mutations in the BH4-metabolizing genes GTP cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase, sepiapterin reductase, carbinolamine-4a-dehydratase, and dihydropteridine reductase. *Hum. Mutat.* 27, 870–878.
- Thorn, C. A., Atallah, H., Howe, M., and Graybiel, A. M. (2010). Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. *Neuron* 66, 781–795.
- Threlfell, S., and Cragg, S. J. (2011). Dopamine signaling in dorsal versus ventral striatum: the dynamic role of cholinergic interneurons. *Front. Syst. Neurosci.* 5:11. doi: 10.3389/fnsys.2011.00011
- Thu, D. C., Oorschot, D. E., Tippet, L. J., Nana, A. L., Hogg, V. M., Synek, B. J., Luthi-Carter, R., Waldvogel, H. J., and Faull, R. L. (2010). Cell loss in the motor and cingulate cortex correlates with symptomatology in Huntington's disease. *Brain* 133, 1094–1110.
- Tippet, L. J., Waldvogel, H. J., Thomas, S. J., Hogg, V. M., Van Roon-Mom, W., Synek, B. J., Graybiel, A. M., and Faull, R. L. (2007). Striosomes and mood dysfunction in Huntington's disease. *Brain* 130, 206–221.
- Toki, S., Kawasaki, H., Tashiro, N., Housman, D. E., and Graybiel, A. M. (2001). Guanine nucleotide exchange factors CalDAG-GEFI and CalDAG-GEFII are colocalized in striatal projection neurons. *J. Comp. Neurol.* 437, 398–407.
- Tokuno, H., Chiken, S., Kametani, K., and Morizumi, T. (2002). Efferent projections from the striatal patch compartment: anterograde degeneration after selective ablation of neurons expressing mu-opioid receptor in rats. *Neurosci. Lett.* 332, 5–8.
- Trytek, E. S., White, I. M., Schroeder, D. M., Heidenreich, B. A., and Rebec, G. V. (1996). Localization of motor- and nonmotor-related neurons within the matrix-striosome organization of rat striatum. *Brain Res.* 707, 221–227.
- Turner, B. H., Wilson, J. S., McKenzie, J. C., and Richtand, N. (1988). MPTP produces a pattern of nigrostriatal degeneration which coincides with the mosaic organization of the caudate nucleus. *Brain Res.* 473, 60–64.
- Valjent, E., Bertran-Gonzalez, J., Herve, D., Fisone, G., and Girault, J. A. (2009). Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci.* 32, 538–547.
- Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., Stipanovich, A., Caboche, J., Lombroso, P. J., Nairn, A. C., Greengard, P., Herve, D., and Girault, J. A. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc. Natl. Acad. Sci. U.S.A.* 102, 491–496.
- Van Der Kooy, D., and Fishell, G. (1992). Embryonic lesions of the substantia nigra prevent the patchy expression of opiate receptors, but not the segregation of patch and matrix compartment neurons, in the developing rat striatum. *Brain Res. Dev. Brain Res.* 66, 141–145.
- Vanderschuren, L. J., Schoffelmeier, A. N., Van Leeuwen, S. D., Hof, L., Jonker, A. J., and Voorn, P. (2002). Compartment-specific changes in striatal neuronal activity during expression of amphetamine sensitization are the result of drug hypersensitivity. *Eur. J. Neurosci.* 16, 2462–2468.
- Vezina, P., and Leyton, M. (2009). Conditioned cues and the expression of stimulant sensitization in animals and humans. *Neuropharmacology* 56(Suppl. 1), 160–168.
- Villares, J. (2007). Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. *Neuroscience* 145, 323–334.
- Vitek, J. L., Chockkan, V., Zhang, J. Y., Kaneoke, Y., Evatt, M., Delong, M. R., Triche, S., Mewes, K., Hashimoto, T., and Bakay, R. A. (1999). Neuronal activity in the basal ganglia in patients with generalized dystonia and hemiballismus. *Ann. Neurol.* 46, 22–35.
- Volkow, N. D., Wang, G. J., Telang, F., Fowler, J. S., Logan, J., Childress, A. R., Jayne, M., Ma, Y., and Wong, C. (2006). Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *J. Neurosci.* 26, 6583–6588.
- Voon, V., Fernagut, P. O., Wickens, J., Baunez, C., Rodriguez, M., Papon, N., Juncos, J. L., Obeso, J. A., and Bezdud, E. (2009). Chronic dopaminergic stimulation in Parkinson's disease: from dyskinesias to impulse control disorders. *Lancet Neurol.* 8, 1140–1149.
- Walker, R. H., Arbutnot, G. W., Baughman, R. W., and Graybiel, A. M. (1993). Dendritic domains of medium spiny neurons in the primate striatum: relationships to striosomal borders. *J. Comp. Neurol.* 337, 614–628.
- Wang, H., and Pickel, V. M. (1998). Dendritic spines containing mu-opioid receptors in rat striatal patches receive asymmetric synapses from prefrontal corticostriatal afferents. *J. Comp. Neurol.* 396, 223–237.
- Wang, H. B., Deng, Y. P., and Reiner, A. (2007). In situ hybridization histochemical and immunohistochemical evidence that striatal projection neurons co-containing substance P and enkephalin are overrepresented in the striosomal compartment of striatum in rats. *Neurosci. Lett.* 425, 195–199.
- Wang, H. B., Laverghetta, A. V., Foehring, R., Deng, Y. P., Sun, Z., Yamamoto, K., Lei, W. L., Jiao, Y., and Reiner, A. (2006a). Single-cell RT-PCR, in situ hybridization histochemical, and immunohistochemical studies of substance P and enkephalin co-occurrence in striatal projection neurons in rats. *J. Chem. Neuroanat.* 31, 178–199.

- Wang, Z., Kai, L., Day, M., Ronesi, J., Yin, H. H., Ding, J., Tkatch, T., Lovinger, D. M., and Surmeier, D. J. (2006b). Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. *Neuron* 50, 443–452.
- Wang, J. Q., Daunais, J. B., and McGinty, J. F. (1994). NMDA receptors mediate amphetamine-induced upregulation of zif/268 and preprodynorphin mRNA expression in rat striatum. *Synapse* 18, 343–353.
- Ward, R. P., and Dorsa, D. M. (1996). Colocalization of serotonin receptor subtypes 5-HT2A, 5-HT2C, and 5-HT6 with neuropeptides in rat striatum. *J. Comp. Neurol.* 370, 405–414.
- Westin, J. E., Vercammen, L., Strome, E. M., Konradi, C., and Cenci, M. A. (2007). Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D1 receptors. *Biol. Psychiatry* 62, 800–810.
- White, N. M., and Hiroi, N. (1998). Preferential localization of self-stimulation sites in striosomes/patches in the rat striatum. *Proc. Natl. Acad. Sci. U.S.A.* 95, 6486–6491.
- Wilson, C. J. (1987). Morphology and synaptic connections of crossed corticostriatal neurons in the rat. *J. Comp. Neurol.* 263, 567–580.
- Xu, M., Moratalla, R., Gold, L. H., Hiroi, N., Koob, G. F., Graybiel, A. M., and Tonegawa, S. (1994). Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. *Cell* 79, 729–742.
- Yamada, T., McGeer, P. L., Baimbridge, K. G., and McGeer, E. G. (1990). Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D28K. *Brain Res.* 526, 303–307.
- Yin, H. H., Knowlton, B. J., and Balleine, B. W. (2004). Lesions of dorso-lateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *Eur. J. Neurosci.* 19, 181–189.
- Yin, H. H., Ostlund, S. B., and Balleine, B. W. (2008). Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks. *Eur. J. Neurosci.* 28, 1437–1448.
- Yung, K. K., Smith, A. D., Levey, A. I., and Bolam, J. P. (1996). Synaptic connections between spiny neurons of the direct and indirect pathways in the neostriatum of the rat: evidence from dopamine receptor and neuropeptide immunostaining. *Eur. J. Neurosci.* 8, 861–869.
- Zachariou, V., Sgambato-Faure, V., Sasaki, T., Svenningsson, P., Berton, O., Fienberg, A. A., Nairn, A. C., Greengard, P., and Nestler, E. J. (2006). Phosphorylation of DARPP-32 at Threonine-34 is required for cocaine action. *Neuropsychopharmacology* 31, 555–562.
- Zahm, D. S. (1991). Compartments in rat dorsal and ventral striatum revealed following injection of 6-hydroxydopamine into the ventral mesencephalon. *Brain Res.* 552, 164–169.
- Zhang, P., Anglade, P., Hirsch, E. C., Javoy-Agid, F., and Agid, Y. (1994). Distribution of manganese-dependent superoxide dismutase in the human brain. *Neuroscience* 61, 317–330.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 June 2011; paper pending published: 11 July 2011; accepted: 18 August 2011; published online: 07 September 2011.

Citation: Crittenden JR and Graybiel AM (2011) Basal ganglia disorders associated with imbalances in the striatal striosome and matrix compartments. *Front. Neuroanat.* 5:59. doi: 10.3389/fnana.2011.00059

Copyright © 2011 Crittenden and Graybiel. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.