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THE DEVELOPING HUMAN BRAIN

Hosted by
Silvia A. Bunge and Elizabeth D. O'Hare

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THE DEVELOPING HUMAN BRAIN

Hosted By
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Technological advances in brain imaging, genetics, and computational modeling have set the stage for novel insights into the cognitive neuroscience of human development during childhood and adolescence. As the field has expanded, research in this area increasingly incorporates highly interdisciplinary approaches utilizing sophisticated imaging, behavioral, and genetic methodologies to map brain, cognitive, and affective/social development. The articles in this Research Topic will highlight both the recent advances and future challenges inherent in this burgeoning interdisciplinary field. We invite both review articles and original research reports that consider any of the broad spectrum of topics within the field of developmental cognitive neuroscience.

Image credit/artist: Elizabeth Jameson.
It is a solar etching based on a 3-D reconstruction of a structural MRI scan of a boy, collected in my laboratory. A research scientist in my lab, Carter Wendelken, Ph.D., created the initial 3-D reconstruction, and Elizabeth made a solar etching from it.

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EDITORIAL
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The developing human brain: a frontiers research topic

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INTRODUCTION

The field of developmental cognitive neuroscience (DCN) examines how the human brain and behavior change over the lifespan, in particular over infancy, childhood, and adolescence. DCN researchers conduct basic research on typical brain development, and/or research on pediatric populations with neurodevelopmental disorders.

Developmental cognitive neuroscience is not child's play – it is a complex and challenging, but exciting, area of research. It is highly multidisciplinary, in that it builds on the fields of developmental, cognitive, affective/social, and clinical psychology and neuroscience. DCN research also relies on advances in computer science, physics, and mathematics. Increasingly, as we learn more about the developing human brain, findings from DCN have strong implications for medicine, education, law, public health, and social welfare.

The story of this compendium begins with a highly successful conference on neurocognitive development held at UC Berkeley in 2009 – a 3-day event with over 200 attendees and over 60 scientific presentations from around the United States and Europe. I thank Brian Meyer, a graduate student in Psychology at UC Berkeley, for helping me to organize this conference. Following on the heels of this conference, postdoctoral fellow Dr. Elizabeth O'Hare and I solicited submissions to a special issue of *Frontiers in Human Neuroscience* focused on the developing human brain. Dr. O'Hare deserves my gratitude for helping to handle the peer review of these submissions.

This Research Topic includes a selection of empirical and review papers from top DCN researchers, organized into six interrelated sections.

Section I examines how our visual and auditory systems develop to support the perception of complex stimuli. Recent DCN research highlights the point that, contrary to common belief, perceptual development is *not* complete after the first few years of life.

Face processing – both the recognition of individuals and of specific emotions – is a particularly challenging problem for the visual system, and continues to improve through adolescence. *Section II* extends the theme of Section I by exploring in more detail the perception of faces, which is critical for social functioning. This section ends with the exploration of deficits in social functioning after pediatric brain injury, which serves as a bridge from studies of perception to studies of action.

Section III examines two key neural changes that underlie shifts in behavior over development. The first is a change in the processing of rewards and feedback, which are powerful motivators of behavior. The second is a change in the ability to control behavioral responses, known as cognitive control or executive function, or more generally as self-regulation.

Developmental cognitive neuroscience researchers have begun to use sophisticated network analysis tools to examine developmental changes in the strength of interactions between brain regions in large-scale networks. *Section IV* explores how these brain networks are influenced by common genetic variation or by the presence of a disorder with a strong hereditary component.

Section V features a relatively new area of research within DCN: the exploration of societal influences on brain development. Two of the papers in this section compare neurocognitive function in children from North American and Chinese families. The other two papers review what is known currently about the influences of childhood adversity and socioeconomic status on brain development.

Finally, *Section VI* showcases several new ways of thinking about, and studying, the developing human brain. The first paper is an opinion piece highlighting the relevance to DCN of research at the level of neural circuits. The second paper shows how we can go beyond conventional fMRI imaging analyses to examine changes in brain function at a finer grain using multivariate pattern classification algorithms.

In conclusion, this compendium shows us both where the field of DCN is currently, and where it is headed. The advances over the last few years have been breathtaking – and the best is yet to come.

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Differential maturation of brain signal complexity in the human auditory and visual system

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Brain development carries with it a large number of structural changes at the local level which impact on the functional interactions of distributed neuronal networks for perceptual processing. Such changes enhance information processing capacity, which can be indexed by estimation of neural signal complexity. Here, we show that during development, EEG signal complexity increases from one month to 5 years of age in response to auditory and visual stimulation. However, the rates of change in complexity were not equivalent for the two responses. Infants' signal complexity for the visual condition was greater than auditory signal complexity, whereas adults showed the same level of complexity to both types of stimuli. The differential rates of complexity change may reflect a combination of innate and experiential factors on the structure and function of the two sensory systems.

Keywords: development, EEG, signal, entropy, complexity, vision, audition, infant

INTRODUCTION

Infancy is a time of enormous changes in brain structure and function. For example, glucose metabolism rises (Chugani, 1998) and brain volume shows a 4-fold increase during the first 4 years of life (Courchesne et al., 2000). Increases in synaptic density are followed by a declining slope within this period (Huttenlocher and Dabholkar, 1997), whereas myelination continues well into adolescence (Paus et al., 1999).

The functional consequence of these changes should be reflected in the increasing processing capacities of the developing child. More precisely, the changes in functional responses are thought to represent an increase in the integration within local neural ensembles together with more precise re-entrant interactions between distal ensembles (Thatcher et al., 2007; Fair et al., 2009). The balance between local integration and distal segregation is a characteristic architecture for a complex system that has an optimal capacity for information integration (Tononi et al., 1994). Thus, development can be conceived of as the movement of the brain towards a complex system. Measures of complexity may be a sensitive index of maturational changes in brain function. For example, a marked increase with age in complexity of the brain's electrical resting state activity has been shown by (Meyer-Lindenberg, 1996) using two measures of complexity, correlation dimensions and the Lyapunov coefficient. A recent study by McIntosh et al. (2008) showed an increase in EEG signal complexity with maturation in pre-adolescent children. Critically, they showed that this increase in complexity was strongly correlated with more stable and accurate behavioural performance.

There does appear to be a general increase in brain signal complexity with maturation. However, the maturational rates of different neural systems are not uniform. For example, rates of neuroanatomical and functional development for the visual and the auditory systems are distinct. The subcortical auditory pathways seem to mature earlier compared to the visual subcortical system. Brainstem auditory evoked potential of a few milliseconds post stimulus can be measured from birth (Jiang et al., 2001). Conversely, at the thalamocortical level, the tendency may be reversed. The geniculocalcarine tract (visual thalamocortical pathway) shows relative maturity at 5 months of age, whereas the maturation of the thalamic projections to the auditory cortex continues until 6 years of age (Yakovlev and Lecours, 1967; Moore and Linthicum, 2007). In addition, myelination begins earlier in the occipital lobe than in the temporal lobe (Yakovlev and Lecours, 1967). Synapse density shows a rapid increase in the occipital cortex until about 8 months of age that is followed by a decline (Huttenlocher and Dabholkar, 1997), whereas the synaptic density of the auditory cortex increases until 50 months before connections are pruned (Huttenlocher and Dabholkar, 1997). Even at later ages of childhood, the time course of grey matter thickness change differs across brain regions (Giedd et al., 2006). The differences in prenatal exposure of the sensory system to environmental stimulation may have an impact on the functional state. Human foetuses and newborns detect sound changes in frequencies (Draganova et al., 2005). Auditory abilities in the early post-natal months include discrimination of musical features (Leppanen and Lyytinen, 1997; Ceponiene et al., 2002; Morr et al., 2002), phonemic identity (Cheour et al., 1998), and gap detection (Trehub et al., 1995). By 8 months of age, infants already discriminate their native language better than any other languages (Kuhl, 2000). On the contrary, intrauterine visual competences of the infant have not been well explored. Prematurely born infants show a visual evoked potential response that is not advanced after term compared to infants born at term (Roy et al., 1995). The development of visual capacities is rapid in the first post-natal months. By 4 months of age, infants born at term show fixation/orientation abilities, saccades, binocular vision, and the visual fields are enlarged (Speeg-Schatz, 2007). Infants of 4–6 months of age and on can segment objects from texture orientations (Atkinson and Braddick, 1992; Burkhalter, 1993; Norcia et al., 2005), resulting in visuomotor behaviours development such as reaching and grasping capacities.

The neural response to visual and auditory stimuli evolve to show distinct ERP morphologies from infancy to adulthood (Lippe et al., 2007). The adult visual ERP is composed of the N1, P1, and N2 whereas the adult auditory ERP is composed of the P1, N1, P2, and N2 (Ellemberg et al., 2003). The maturation of visual and auditory evoked potentials have been widely studied using a black and white reversing stimuli and a broadband noise stimuli (Rotteveel et al., 1987; Taylor and McCulloch, 1992; Roy et al., 1995; Pasman et al., 1999; Ponton et al., 2000). Simple reversing checkerboard stimuli of low spatial resolution evokes a reliable P1 and N2 as early as 1-month old. The N1 appears within the first 4 months and latencies and amplitudes of all components vary until adulthood (Crognale, 2002; Lippe et al., 2007). Auditory evoked potentials triggered by broadband clicks are characterized by a large positive followed by a negative component in the first months of age (Rotteveel et al., 1987; Pasman et al., 1999; Ponton et al., 2000). Depending on the study, the auditory N1 has been reported to appear between 3 and 9 years of age (Tonnquist-Uhlen, 1996; Ceponiene et al., 1998; Pang and Taylor, 2000; Wunderlich and Cone-Wesson, 2006; Sussman and Steinschneider, 2009) and the early P1 appears thereafter (Lippe et al., 2009).

The developmental trajectories of the visual and the auditory systems cannot be compared directly through ERP responses because of morphological differences in the waveforms. Complexity measures, in this case, multiscale entropy (MSE) (Costa et al., 2002), do not depend on these specific features and thus could allow for direct comparison between sensory modalities. The MSE measure involves calculating sample entropy of the signal for different temporal scales. It has an advantage over other measures of complexity in that it can be applied to shorter time series (e.g 100–200 data points) than other measures of complexity that require much more data.

We measured complexity of the signal across groups of children from 1 month to 5 years of age and young adults. Furthermore, we compared, within the same individual, the complexity of the signal in response to a visual (achromatic checkerboard) and to an auditory (broad band noise) stimulation. Spectral power of the brain signal is known to evolve with age (Lippe et al., 2007). For comparison purposes, we calculated spectral power (SP) across age groups for the visual and the auditory conditions.

First, we hypothesised that the complexity of the signal increases with maturation. Second, we hypothesized that maximum complexity will be observed in the visual system as it undergoes more intensive maturational changes after birth compared to the auditory system.

MATERIALS AND METHODS

PARTICIPANTS

Forty infants and children aged from 27 days to 5 years 6 months. Data from five participants were excluded from the original sample because they did not succeed in attending to both the visual and auditory stimuli, or because of excessive movement artefacts. Infants were separated into four age groups [1–2 months ($n = 7$), 2–8 months ($n = 11$), 9–24 months ($n = 6$), 24–66 months ($n = 11$)]. While the results reported here are essentially the same if age was treated as continuous for children and infant, the groupings were chosen to examine carefully the first year of age, where the most rapid perceptual development occurs, and to enable the comparison of visual and auditory responses within group. Finally, 20 adults aged between 20 to 30 years of age were also tested.

Developmental information was gathered from interviews and a developmental questionnaire completed by the parents. All subjects were born at term; they had no history of psychiatric or neurological illnesses, and had normal or corrected vision. An intellectual developmental scale was administered to all children by one experimenter and one observer. All subjects 0–24 months of age scored within the normal range (within a standard deviation of 15 from 100) on the Mental Development Index of the Bayley Scale of Infant Development, II Ed (Bayley, 1993). All subjects from 2 years to 5 years 6 months obtained a global score within the normal range (within a standard deviation of 15 from 100) on the Stanford Binet Intelligence Scale, IV Ed (Thorndike et al., 1994). Parents and adult participants signed the consent form authorized by the ethics, administrative and scientific committees from the Ste-Justine's Hospital research centre and the University of Montreal.

APPARATUS AND STIMULI

Visual evoked potentials were measured in response to a black and white checkerboard stimulus with each square subtending a visual angle of 2°. Stimuli had a luminance of 40 cd/m^2. Pattern-reversals were presented binocularly for 500 ms at a distance of 70 cm from the subject's eyes subtended $38.5 \times 38.5°$ of visual angle and were presented at a reversal rate of 1 Hz. Auditory evoked potentials were measured in response to 50 ms broad band noise (BBNs) presented in free-field binaurally to the participants in a soundproof room. The intensity was 70 dB SPL and the resolution, 16 bits. BBNs were delivered binaurally (Optimus XTS 24) located laterally at a distance of 30 cm from each of the subject's ears. The inter-stimulus interval for all stimuli varied randomly between 1200 and 1400 ms. All stimuli were generated by a Dell GX 150 PC using E-Prime 2000 software (from Psychology Software Tools Inc. Pittsburgh).

Young infants were seated on their parent's lap. Their attention was drawn to the screen (plain screen for the auditory protocol) by small objects held in front of the lower middle part of the screen by the experimenter. Adults were instructed to look straight ahead for the auditory protocol and to look at the center of the screen for the visual protocol. Following a procedure widely used in developmental EEG experiments (Roy et al., 1995), the EEG was recorded only when the children were still and their gaze was focused on the screen centre. Recording was done with the 128 electrodes Electrical Geodesics System Inc. (Eugene, OR, USA); the reference was at the vertex and the impedances were maintained below 40 KΩ, as suggested by (Tucker, 1993). The EEG signal was amplified and analog bandpass filtered from 0.1 Hz to 100 Hz. The signal was digitized at 250 Hz in 1024 ms epochs. A G4 Macintosh computer controlled data acquisition.

OFF-LINE ANALYSES

Off-line data pre-processing was performed with EEGLAB (http://sccn.ucsd.edu/eeglab/) (Delorme and Makeig, 2004). Data were digitally filtered with a 0.5 to 50 Hz bandpass filter. Some electrodes were excluded from the data analysis because of excessive artefacts. 115 electrodes remained for all subjects and averaged reference was computed. The continuous EEG recording was segmented into the auditory and visual (−100 to 488 ms) stimuli events. In addition, artefact removal was performed using independent component analysis (ICA). Each component was examined using its topography, power spectrum, and activity over time and trials. ICA based artifact removal and correction consisted of the following steps: (1) trials contaminated with excessive amplitudes were removed first (2) an ICA decomposition was performed on the remaining concatenated trials and (3) components carrying residual ocular and muscle artifacts, identified based both on topography and frequency, were removed from the data set. The number of rejected ICA components was not statistically different between groups. Sixty good trials were kept for each condition and participant.

MSE was used to estimate sample entropy at different time scales. Full details of the MSE measure and its relevance for the analysis of signal complexity are given in Costa et al. (2002). The algorithm calculates sample entropy as a measure of regularity (predictability) of the signal at different scales. It involves two procedures: (1) coarse-graining of the time series and (2) calculating sample entropy for each coarse-grained time series. For scale *t*, the coarse-grained time series is constructed by averaging the data points within non-overlapping windows of length *t*. This procedure can be viewed as a smoother version of decimation. Sample entropy of each coarse-grained time series measures its regularity by evaluating the appearance of repetitive patterns. We first calculated single trial MSE using algorithm available at www.physionet.org/physiotools/mse/ with parameter values for pattern length (m) and similarity criteria (r) set to $m = 2$ and $r = 0.5$ respectively. The length of single trial time series was 125 time points. MSE was measured for four different time scales over 500 ms time windows. Subject and channel specific MSE estimates were obtained as mean across single trial MSE measures for scales 1–4, where scale 1 would be the raw time series and scale 4 the most temporally coarse (non-overlapping window of 4 data points, in other words 16 ms). MSE measures for scales >4 were not calculated because the corresponding coarse-grained time series were too short (<50 time points) for reliable sample entropy estimation. MSE curves were then averaged across all electrodes, as there were no strong spatial differences across the scalp in the MSE changes.

Spectral power distribution (SPD) across single trials was calculated using Fast Fourier Transform (FFT). Considering the known age-related differences in global signal power, the signal was first normalized (mean = 0, standard deviation = 1) in order to calculate relative contributions of different frequency bands to the total spectral power. The EEG power was calculated at 2, 4, 6, 8, 10, 12, 14–24, 26–40 Hz, for single trials within the visual and the auditory conditions and then averaged for each condition and for each subject.

Statistical assessment of maturational trends in MSE and SPD was performed using partial least squares (PLS) (Lobaugh et al., 2001). PLS is a multivariate technique similar to canonical correlation, except that is maximizes the covariance between two data sets rather than the correlation. PLS was performed on data matrices consisting of subject measures such that rows represented subjects within age groups. The columns of the data matrix were either the measures for MSE or SPD. The data matrices were averaged within group and grand mean centered by column across all five age groups. The mean-centered matrices were then decomposed with singular value decomposition to identify the strongest effects. From the decomposition comes three new matrices:

(1) weights for the rows, which indicate a contrast that characterizes the differences between groups and/or tasks,
(2) weights for the columns, which gives the linear combination of either MSE scale or SP distribution that maximally relates to the contrast, and
(3) the singular value, which is the covariance between the contrast and the MSE or SP weights.

The statistical significance of the effects was assessed using permutation tests for the singular values. The test involves random reassignment of subjects to age groups and conditions and then redoing the PLS with the permuted data. The probability value is then derived from the number of times out of 500 the singular value from each permuted data set is greater than or equal to that of the original data.

The reliability of SP weights was determined with bootstrap estimation of confidence intervals, using 500 bootstrap samples. The singular vector weights for each SP coefficient were divided by the bootstrap estimated standard error, giving a bootstrap ratio. This is similar to a z-score if the distribution of singular vector weights is Gaussian (McIntosh and Lobaugh, 2004). Bootstrap estimation was not necessary for the MSE scales as there was no variation in the reliability of differences at the different temporal scales.

RESULTS

DEVELOPMENTAL CHANGES

Figure 1 shows the ERPs and topographical maps of the group averaged VEP (**Figures 1A,B**) and AEP (**Figures 1C,D**). The ERPs show the expected developmental changes in morphology, amplitude, and latency previously found with typical means of filtering (e.g., ocular corrections, trial removals). From a large positive wave, the morphology of the ERPs evolves beyond 5 years of age in both modalities. Topographical maps at peak latencies show the expected location of brain activity (Lippe et al., 2007). The latency of the group average VEP decreased of 52 ms. Similarly, the latency of the group average AEP decreased of 56 ms. The dominant AEP positive component amplitude decreases from 2 to 5 years to adulthood, showing the developmental changes reported elsewhere (Lippe et al., 2009). VEP and AEP developmental descriptions are detailed in Lippe et al. (2007, 2009).

Complexity, as measured with MSE curves produced within each age group, are shown in **Figure 2**. As expected, the MSE curves showed the greatest entropy for adults and the least for the youngest infants. Results confirmed a linear increase of MSE with age, regardless of the condition and of the temporal scale ($p < 0.0001$) (**Figure 2**). The trend depicted in **Figure 2** is also present if age is treated as a continuous measure and if the adult group is excluded from the analysis (correlations of age and MSE visual: $r = 0.55, \pm 0.1$, 95% confidence interval; auditory, $r = 0.60, \pm 0.07$ 95% confidence interval).

Developmental changes were also addressed by spectral power distribution (SPD). The power distribution was computed over all electrodes. The PLS results for the group differences were

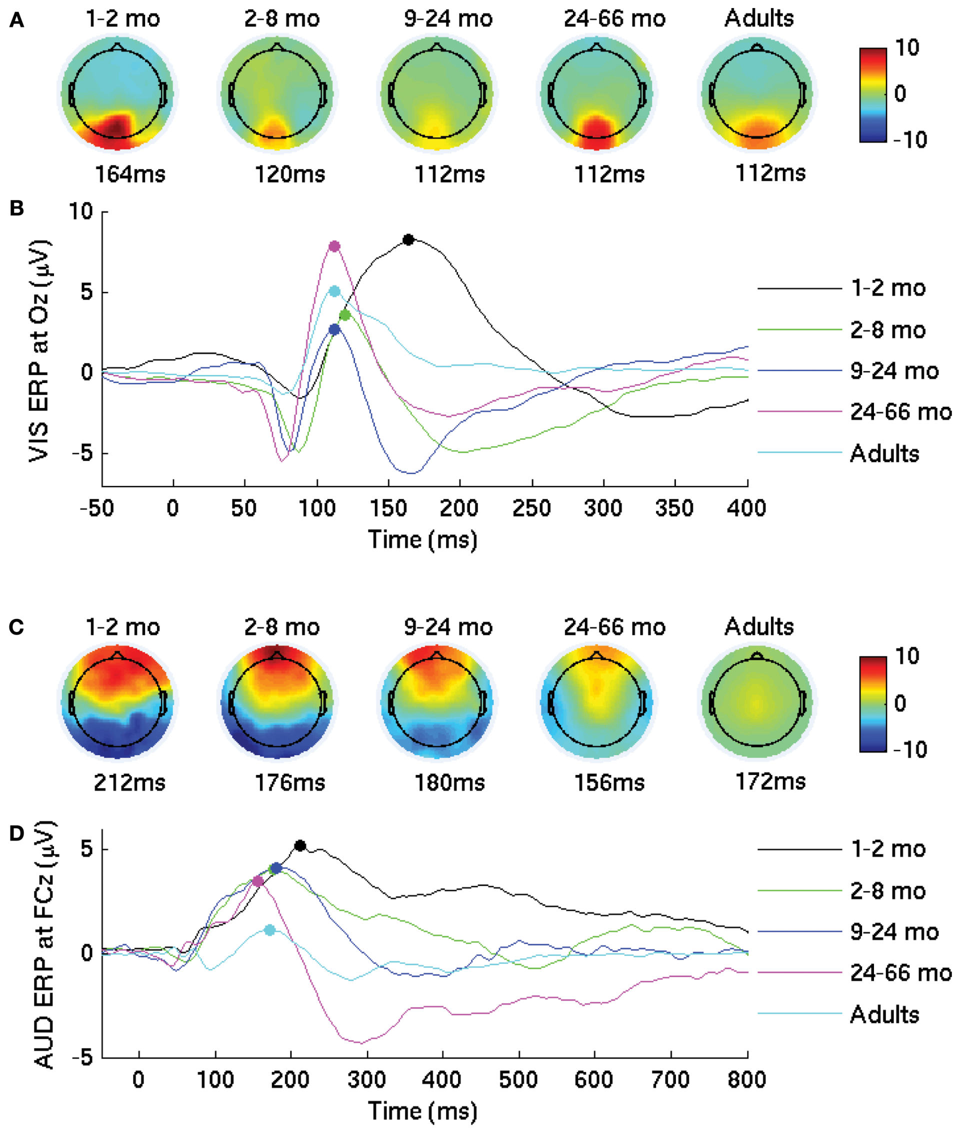

FIGURE 1 | Visual and auditory evoked potentials. (A,B) Group average of the visual evoked potentials. **(A)** Topographies at the latency of the highest amplitude peak for each group. **(B)** Group average waveforms at electrode Oz. **(C,D)** Group average of the auditory evoked potentials. **(C)** Topographies at the latency of the highest amplitude peak for each group. **(D)** Group average waveforms at electrode FCz.

significant for the visual and the auditory conditions ($p < 0.0001$). As expected, SPD results showed similar developmental trends in the visual and the auditory conditions (**Figure 3**). The bootstrap ratio figure on the right of **Figure 3** shows the differences in SP between groups. The trends for auditory and visual SP were similar. Compared to all infants and children groups, adults showed reduced power at lower frequencies and increased power at higher frequencies. We also observed the maturational change of dominant spectral activity from theta to alpha and a gradual shift to the pattern of power distribution seen in adults (Marshall et al., 2002). As with MSE, the trend for SPD is also present if age is treated as a continuous measure and if the adult group is excluded from the analysis (correlations of age and SPD visual: $r = 0.82, \pm 0.05$, 95% confidence interval; auditory, $r = 0.75, \pm 0.15$ 95% confidence interval).

COMPARISON OF AUDITORY AND VISUAL RESPONSES

Multiscale entropy

We tested whether complexity of auditory versus visual responses changes across age groups. The PLS analysis was done both to assess the difference between auditory versus visual complexity across groups, and to evaluate the difference within each group. The overall test of the interaction of MSE across groups showed significant differences between the visual and the auditory conditions ($p < 0.0001$) by age. In fact, MSE was significantly higher in the visual condition across all temporal scales compared to the auditory condition between 2–66 months of age (**Figure 4**). The differences between conditions were greatest at 2–8 months of age ($p = 0.0005$). In contrast, the complexity of auditory and visual responses could not be distinguished in adulthood ($p = 0.21$).

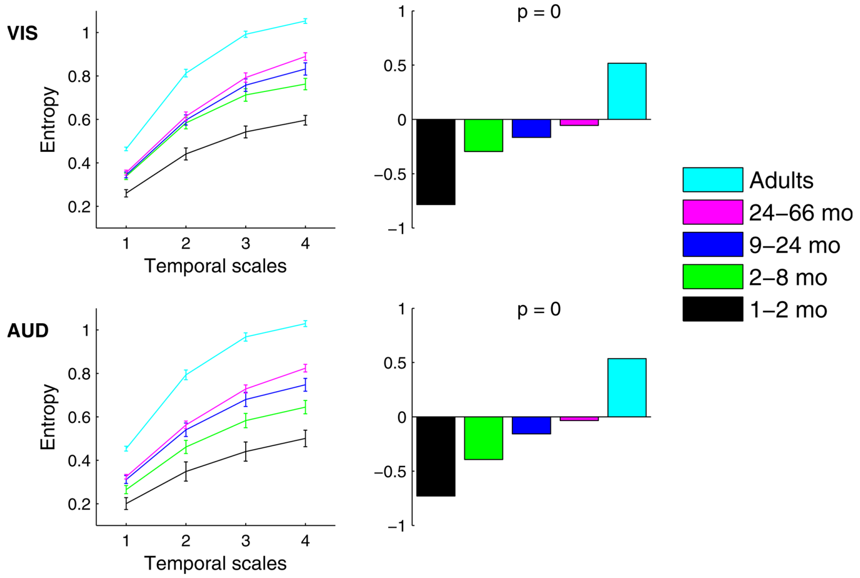

FIGURE 2 | Age-related differences in multiscale entropy estimated separately in VIS and AUD conditions. Left panels show group mean entropy with error bars representing group standard errors. Right panels show PLS contrasts representing age-related increase in entropy. Contrasts for both conditions were significant and stably expressed across all temporal scales.

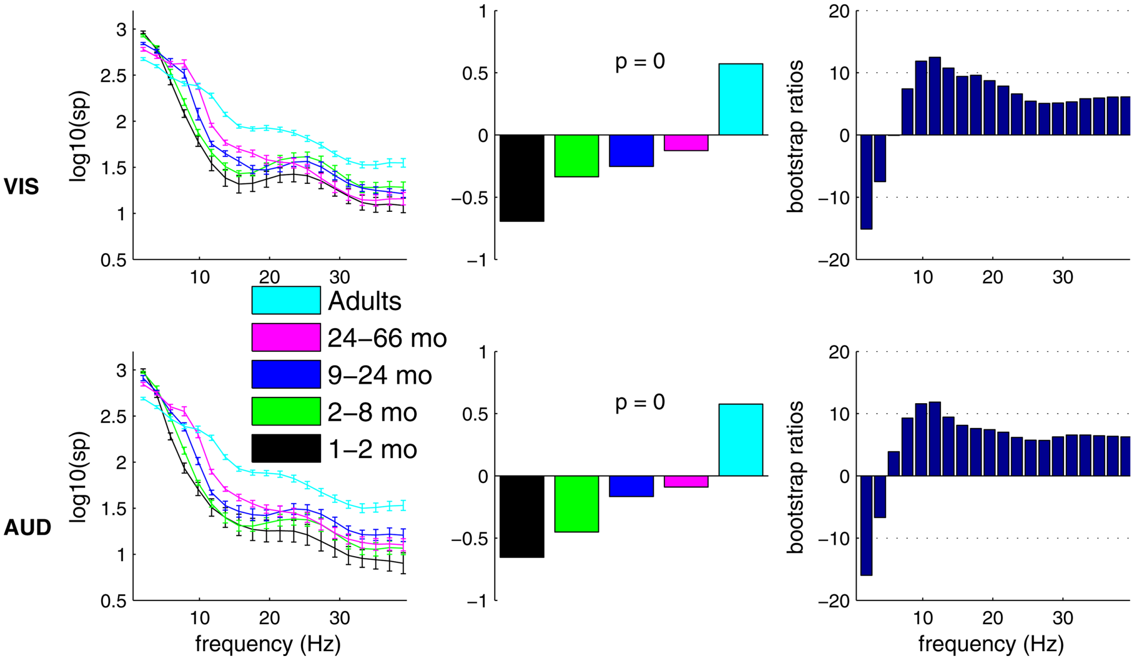

FIGURE 3 | Age-related differences in spectral power distribution estimated separately in VIS and AUD conditions. Left panels show group mean log power with error bars representing group standard errors. Middle panels show PLS contrasts representing age-related change of spectral power distribution. Contrasts for both conditions were significant and stably expressed across most frequencies as shown in right panels. Results from both conditions replicate a known shift towards higher frequencies during maturation. Loss of stability around 6 Hz is likely due to an interaction between development-related increase in alpha rhythm frequency (Marshall et al., 2002) and the overall redistribution of power towards higher frequencies.

Spectral power

PLS analyses were also conducted on SP between the visual and the auditory conditions (**Figure 5**). As with MSE, the interaction of group by auditory vs. visual response was significant ($p < 0.0001$). Differences between the visual and the auditory condition were found from 2 to 66 months, but were strongest within the 2- to 8-months group. Less low frequency SP and more high frequency SP were generally found in the visual condition.

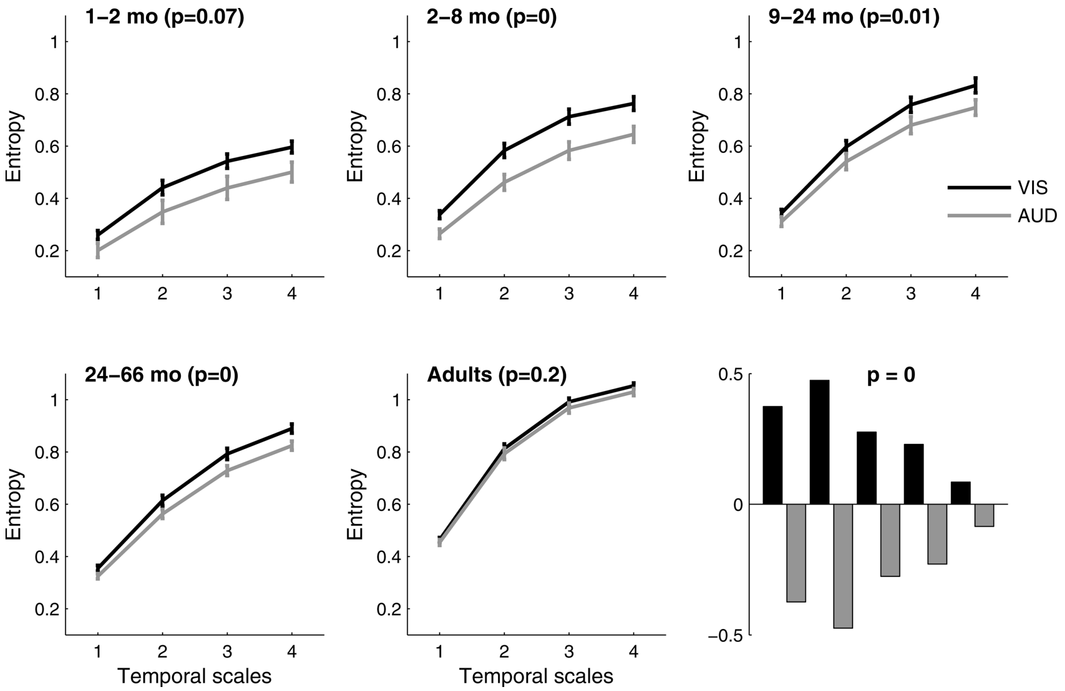

FIGURE 4 | Multi scale entropy in VIS vs. AUD conditions across five age groups. Group mean entropy is shown for VIS and AUD conditions, together with group standard errors. The outcome of the significance testing for differences in MSE between two conditions within each group are given as p values. The adult group showed no significant differences between the two conditions. Bottom right panel shows PLS contrast between VIS and AUD conditions across all five groups simultaneously, after correcting for overall group differences. The contrast was significant and stably expressed across all four temporal scales. VIS condition exhibits higher MSE compared to AUD condition across all groups and the difference is most strongly expressed in the second group (2–8 months) in relation to other groups.

In contrast, adults did not show differences between the two conditions. Although differences were found for almost all frequencies, the 2- to 8-months group showed enhancement of the beta and the gamma bands (~15–50 Hz) in the visual condition. From 24 to 66 months, differences were greatest for the alpha and beta band (~10–18 Hz).

We also performed all the analyses excluding the adults and found that the MSE and SPD differences between ages and conditions were the same as we report above. This indicates that the statistical effects are not biased by the larger differences between adults and children.

DISCUSSION

The two hypotheses put forth in the introduction were supported. Complexity, measured with MSE, of the visual and auditory brain responses increases with maturation. This was complemented by maturational changes in spectral power distribution (SPD), where there was a reduction in low frequency and an increase in high frequency responses. We also observed that these maturation changes were not equivalent for auditory versus visual responses. While both responses showed increased complexity with maturation, visual responses showed greater complexity for infants and children, but responses were indistinguishable in adults. Our study thus confirms a distinct rate of maturation between brain sensory systems in infants and children, which are likely caused by structural and functional changes.

The global increase in complexity may be related to structural modification with development. At the local level, it may correspond to the increase arborization of dendritic trees (Moore, 2002), and axons (Burkhalter, 1993), enhanced connections of interneurones (Ali et al., 2007), expression of receptors (Huang and Scheiffele, 2008; Rula et al., 2008) and synaptic stabilisation (Hua and Smith, 2004). The changes in cell firing characteristics and synaptic potential durations may result in enhanced oscillatory capacities in the higher frequency range. At a distal level, the increased complexity may correspond to the myelin development (Paus et al., 1999), brain region segregation (Gogtay et al., 2004), and network formation, which enhance binding and integration capacities (Yu et al., 2008).

The linear increase in complexity of the physiological signal contrasts with the nonlinear pattern of synaptic connection development. Complexity of the signal continues to increase during pruning of exuberant connections. The refinement of intra-areal connections may partly regulate the initial increases in complexity by enlarging the local repertoire. In the beta frequency band, long distance coherence has been found to diminish with age, whereas short distance coherence was found to increase (Thatcher et al., 2008). Refined connections from selective pruning of long distance connections establishes more precise reciprocal linkages between local and distributed systems (Thatcher et al., 2007), but also promotes local integration. In doing so, cortical development facilitates both optimized local and global computations.

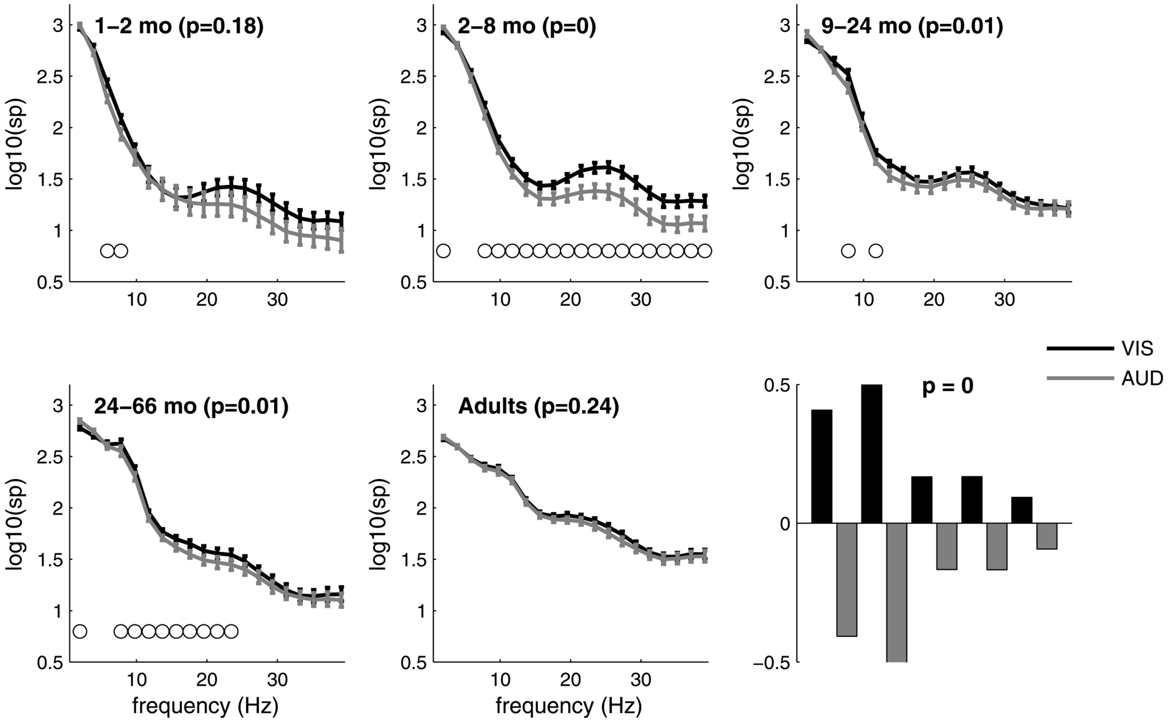

FIGURE 5 | Spectral power distribution in VIS vs. AUD conditions across five age groups. Group mean log power in VIS and AUD conditions is displayed, together with group standard errors. The outcome of significance testing for differences in spectral power distribution between two conditions within each group are given as p-values. The circles above the frequencies indicate portions of the spectral power curve that differ between conditions based on a 99% confidence interval derived from bootstrap estimation. The adult group showed no significant differences between the two conditions. The bottom right panel shows PLS contrast between VIS and AUD conditions across all five groups simultaneously, after correcting for group differences. The contrast was significant and stably expressed across almost all frequencies. VIS condition exhibits less low and more high frequencies compared to AUD condition across all groups and the difference is most strongly expressed in the second group (2–8 months).

Gradual increases in interburst interval variation and inter neuronal synchronisation accompany the formation of long-range connections between neuronal clusters. The organisation of the networks into functionally coherent and sparsely interconnected clusters supports high levels of complexity as collective network activity forms (Fuchs et al., 2007). Thus, both local and distal levels of development may be responsible for the increase in complexity with age.

Interestingly, differences in the complexity of the signal for the auditory and visual responses were found from 2 to 66 months only. At 1 month of age, complexity is similarly low for both the visual and the auditory conditions. One could posit that the intrauterine exposure to environmental sound and the advanced subcortical maturation of the auditory system could drive at the cortical level the full degree of complexity of neural activity, resulting in higher MSE in the first weeks of life.

From 2 to 66 months, complexity is greatest when in response to visual stimuli rather than auditory stimuli. Remarkably, this difference first increases to a maximum at 2–8 months, and then gradually seems to reduce, where in adults the complexity of response show no modality effect. The differential change in complexity across age may be related to the maturation of the network involved. From subcortical relay including the thalamus (lateral geniculate body), the visual information travels to the occipital cortex, surrounding extra striate areas, and the temporal and parietal lobes. The auditory information travels through multiple subcortical relays before it reaches the thalamus (median geniculate body), the temporal and ultimately the frontal lobe. In toddlers, functional responses to visual stimulation engage the occipital and parietal cortex, whereas non vocal auditory stimulation engage the temporal and the prefrontal cortex (Redcay et al., 2007). Temporal and prefrontal cortices have a prolonged maturation compared to occipital and parietal cortices (Sowell et al., 2004). Our results may thus reflect lobar maturation differences in children. The distinction in the complexity level from 2 to 66 months may result from the optic radiation myelination spurt, the higher degree of myelinated fibers in the occipital cortex, and the enhanced neuronal density. In that context, the greatest discrepancy in complexity between the visual and the auditory condition was found between 2–8 months of age. This period also corresponds to the marked beta and gamma SP increase in the visual conditions. Behaviourally, drastic improvements of visual competency such as perceptual acuity and sensitivity are also observed during this period (Maurer et al., 2007).

Anyone who has observed infants behaviours notices their changing interests towards the environment with age. Most studies agree on the influencial role of stimulus complexity on infants' fixation time. Infants seem to habituate faster to simple stimuli and their response to complex novel stimuli is enhanced (Kaplan and Werner, 1986). Here, we did not address their fixation time but neural response to a stimulus. Nevertheless, one may argue that relative stimulus complexity could account for the differences in brain activity complexity between the visual and auditory systems during childhood. Indeed, stimulus complexity can modulate the evoked potential response. For example, at all ages higher spatial resolution and low contrast of visual stimuli trigger or enhance the N1, a VEP component which slowly develops during the first months (Hammarrenger et al., 2003). To overcome the stimulus complexity issue, we used a low spatial resolution, high contrast and slow reversal rate visual stimulus, which is known to modulate mainly the P1, even in adults (Ellemberg et al., 2001). Similarly, the auditory stimulus was not language related. Instead, the broadband noise engages all frequency representations (Riquelme et al., 2006) at any age. In addition, the BBN was presented at a slow rate with a 200 ms random inter-stimulus interval variation to avoid habituation effect. It is thus unlikely that the increase complexity of the visual response over the auditory response found in infants and children was caused by relative differences in stimulus characteristics.

The enhanced complexity of the visual system during infancy has an important implication for the development of cognitive function. Visual information may particularly contribute to multisensory learning. For example, visual cues about speech articulation are known to enhance phoneme discrimination at 6 months of age (Teinonen et al., 2008). Auditory contribution to multisensory learning is less clear during this period (Perone and Oakes, 2006).

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Changes in complexity seem to parallel SPD. As expected, the low vs. high ratio of SP diminished with age and the dominant frequency was found to shift from the theta to the alpha band. Alteration in SP can partly explain our MSE results as stronger low frequency activity signals could show relatively less variability and lower relative complexity. McIntosh et al. (2008) increased the relative magnitude of the Fourier coefficients for low frequencies in the adult EEG data and found MSE estimates similar to children. However, jittering the phase of the Fourier coefficients while maintaining the relative magnitude changed the MSE estimates without altering the spectral density. Such results indicate that MSE captures the short-lived dependencies in neural signals that are not reflected in SP estimation. MSE and SP information are thus not redundant, but rather are complementary (Gudmundsson et al., 2007).

CONCLUSION

In conclusion, the complexity of the infant brain's signal increases in the context of structural changes such as synaptic exuberance and pruning. In addition, complexity distinguishes the maturation of auditory and visual sensory systems, which suggests such measure is sensitive to the maturational changes in infants, where behavioural responses cannot be easily recorded. Finally, the functional disparity we report between the two sensory systems may constrain the means by which cognitive developmental milestones are acquired.

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Dynamic object representations in infants with and without fragile X syndrome

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Our visual world is dynamic in nature. The ability to encode, mentally represent, and track an object's identity as it moves across time and space is critical for integrating and maintaining a complete and coherent view of the world. Here we investigated dynamic object processing in typically developing (TD) infants and infants with fragile X syndrome (FXS), a single-gene disorder associated with deficits in dorsal stream functioning. We used the violation of expectation method to assess infants' visual response to expected versus unexpected outcomes following a brief dynamic (dorsal stream) or static (ventral stream) occlusion event. Consistent with previous reports of deficits in dorsal stream-mediated functioning in individuals with this disorder, these results reveal that, compared to mental age-matched TD infants, infants with FXS could maintain the identity of static, but not dynamic, object information during occlusion. These findings are the first to experimentally evaluate visual object processing skills in infants with FXS, and further support the hypothesis of dorsal stream difficulties in infants with this developmental disorder.

Keywords: object tracking, occlusion, motion, dorsal/ventral visual streams, attention

INTRODUCTION

Our visual world is dynamic in nature; we move and objects around us move. For example, an object can go out of sight as our eye or head position changes, or if moving, it can go out of sight when obstructed by other objects. Despite incomplete perceptual information during an object's occlusion, the adult visual system is able to form, maintain, and if necessary, track a mental representation of an object such that a coherent and complete visual scene is perceived across time and space. A critical question remains unanswered, however: to what extent do infants possess the ability to preserve an accurate representation of an object's identity during a dynamic occlusion event?

Previous studies have typically used the preferential looking paradigm to show that from a young age infants look longer at the outcome of a static occlusion event when a feature of the hidden object is changed compared to an event in which features remain the same. Extensive research using this paradigm has also provided evidence that the specific features which infants use to discriminate the identity of an object change across development. Infants as young as 4 months primarily represent occluded objects based on spatial information such as location and size (Baillargeon, 1993; Spelke et al., 1994; Mareschal and Johnson, 2003), and by the end of the first year infants use surface properties including shape (7 months), texture (11 months) and color (12 months) (Wilcox, 1999; Leslie and Kaldy, 2001; Wilcox and Schweinle, 2002; Kaldy and Leslie, 2003). Aspects of infants' perception, such as encoding and binding of object properties to build and maintain a representation during a static occlusion event, have been well characterized. Less is known about the ability of infants to maintain an object representation during a dynamic occlusion event, namely, whether infants can track a mental representation across time and space (Richardson and Kirkham, 2004).

The developmental hierarchy in the type of information that infants use to represent occluded objects closely maps on to the two distinct visual processing streams in the brain; the ventral and dorsal pathways. The occipito-temporal ventral stream involves processing of object features such as form and color, and projects to the inferotemporal cortex, while processing of information involved in guiding actions, including spatial location and motion, are served by the occipito-parietal dorsal stream, extending to the posterior parietal cortex (Ungerleider and Mishkin, 1982; Milner and Goodale, 1995). One account of this hierarchy proposes that infants initially set up an object representation based on spatial information (Leslie et al., 1998; Tremoulet et al., 2000; Leslie and Kaldy, 2001; Wilcox and Schweinle, 2002; Kaldy and Leslie, 2003) because, though the ventral and dorsal pathways are each operative early in development, information that is carried by each pathway remains segregated until the end of the first year of life, with the dorsal stream developing first but also thought to have a longer maturational time course (Atkinson, 1993; Leslie et al., 1998; Johnson et al., 2001; Kaldy and Leslie, 2003; Mareschal and Johnson, 2003). It has therefore been proposed that during the early months of development, performance on tasks requiring predominantly single stream processing may be superior to performance on tasks requiring integration of information across the two streams. Once the connections between the two pathways become functionally mature, infants are able to keep track of both an object's location in space (dorsal) and its featural properties (ventral), in the service of identifying an object as changed or unchanged following an occlusion event. Indeed, most everyday activities are guided by extensive interaction between dorsal and ventral representations in order to integrate features required for planning and executing actions.

The functional, anatomical, and developmental dissociation between ventral and dorsal pathways has been influential for researchers investigating visual processing in various developmental disorders. Because of its protracted developmental time course, it has been suggested that the dorsal stream is particularly vulnerable to atypical development (Atkinson, 2000), thereby leading to a preponderance of dorsal stream deficits in developmental disorders, including fragile X syndrome (FXS).

FXS is the most is the most prevalent form of inherited mental retardation, with 1 in 3,800 males estimated to have the FXS full mutation and as many as 1 in 2,300 women estimated to carry the full mutation on at least one X chromosome (Crawford et al., 2001; Beckett et al., 2005). FXS is also the most common single-gene cause of autism (Reddy, 2005). The neurodevelopmental disorder is caused by the silencing of a single gene on the X chromosome, the Fragile X Mental Retardation 1 gene (Verkerk et al., 1991), which results in the reduction or absence of the fragile X mental retardation protein (FMRP) coded for by the gene. FMRP plays a key role in the post-synaptic development of dendritic spine morphology and acts as a repressor by regulating the translation of multiple dendritic mRNAs involved in synaptic development and function (Brown et al., 1982; Irwin et al., 2002). Dendritic abnormalities have been found in occipito-parietal areas of *FMR1* knock-out mice as well as visual cortices of autopsied tissue from patients with FXS (Comery et al., 1997; Irwin et al., 2002), suggesting that FMRP is an important protein in the development of neural networks involving visual areas of the brain. Because of its specific single gene etiology, FXS offers a unique opportunity to examine the functional role of a specific gene product on neurocognitive development, and more specifically on functions supported by the dorsal stream.

There is ample evidence showing that visual processing deficits observed in individuals with FXS are not global in nature; rather, deficits in this population are specific to tasks mediated by the dorsal stream. A finding that emerges consistently across neuropsychological studies in children and adults with FXS is that affected individuals perform worse than typically developing (TD) controls on tasks requiring visual-spatial and visual-motor coordination, such as replication of an abstract block design or copying a drawing from a model, while visual recognition and matching abilities are relatively unimpaired. Studies using visual psychophysics have reported that adolescents and adults with FXS show reduced contrast sensitivity for low spatial and high temporal frequency visual stimuli known to engage magnocellular pathway processing, but intact sensitivity for high spatial and low temporal frequency stimuli that elicit parvocellular pathway processing (Kogan et al., 2004a). Furthermore, impaired sensitivity for discriminating second-order static and first- and second-order moving visual stimuli, accompanied by near normal sensitivity for first-order static stimuli, has been found in adolescents and adults with FXS (Kogan et al., 2004b). These studies have been seminal in refining the picture of visual deficits in individuals with FXS, suggesting typical ventral stream processing accompanied by either atypical dorsal stream processing or a generalized impairment in higher-level neural mechanisms necessary for integrating dorsal and ventral visual input. These interpretations need not be mutually exclusive. Recent work investigating visual processing in infants with FXS has revealed that a selective deficit in sensitivity for detecting second-order motion stimuli can be identified early in development (Farzin et al., 2008). Because attentive tracking is believed to be necessary for the detection of dynamic second-order stimuli (Sperling, 1989; Cavanagh, 1992; Johnston et al., 1992; Nishida and Sato, 1995; Seiffert and Cavanagh, 1998), this finding was explained as a deficit in temporal or motion processing in infants with FXS, as either could result in poor attentive tracking and both types of processing are subserved by parietal areas of the brain. These results are consistent with and extend the work of Kogan et al. (2004b) by uncovering the developmental trajectory of the putative dorsal stream deficit in both male and female infants diagnosed with FXS. However, given that infants with FXS performed comparable to TD controls when detecting first-order motion stimuli, which are processed by a passive, velocity sensitive mechanism (Seiffert and Cavanagh, 1998), these results also call into question whether a low-level motion processing or strictly subcortical deficit exists in individuals with FXS. While we cannot directly compare levels of sensitivity obtained from infants in our study to sensitivity found in adults because of differences in stimuli (second-order stimuli are defined differently), tasks (detection versus discrimination), and age groups, we can speculate that FMRP may have a protracted time course in the early development of visual areas.

Other evidence of developmental visual abnormalities comes from studies with toddlers with FXS. First, Scerif et al. (2004) investigated selective visual attention in 2- and 3-year-olds with FXS using a visual search task, and found that the FXS group showed a perseverative error of selecting targets that had previously been found. Another study examined oculomotor control in toddlers with FXS (Scerif- et al., 2005) by presenting children with a task in which the goal was to inhibit saccades toward suddenly appearing peripheral stimuli (prosaccades) and direct them instead to contralateral locations (antisaccades). Consistent with the above finding of deficits in selective visual attention, they found that toddlers with FXS failed to suppress prosaccades toward the cue during the test trials, likely caused by atypical connectivity between parietal and frontal circuitry (McDowell et al., 2008). Taken together, these findings begin to converge on a picture of visual processing in young individuals with FXS in which there exists a disruption in the 'vision-for-action' pathway, or the dorsal stream (Milner and Goodale, 1995).

Here we conducted two experiments to assess how infants with and without FXS process changes in object properties during occlusion events which either involve a dynamic transformation across time and space, engaging dorsal stream processing, or which remain static, engaging ventral stream processing. The tasks were designed based on the hypothesis that dysfunction of parietal areas in processing basic spatial and temporal visual information is likely to be the underpinning source of cognitive impairments found in individuals with FXS. These findings presented here provide us with a better understanding of the development of dynamic object representations in typically and atypically developing infants, bridging the link between specific gene expression, brain development, and cognitive function.

EXPERIMENT 1

MATERIALS AND METHODS

Participants

Thirty-two infants diagnosed with the FXS full mutation (27 boys and 5 girls) and thirty-four mental age-matched full-term TD infants (27 boys and 7 girls) were included in the final sample. Mean chronological age for the FXS and TD groups was 26.37 months (±7.15, range = 14–45 months) and 18.09 months (±5.51, range = 11–31 months), respectively. Data from an additional three TD infants and four infants with FXS were not included in the final analysis because the infant did not provide gaze data on all four test trials. TD infants were recruited through letters to families, fliers, and word of mouth in Davis, California. Infants with FXS were recruited from, and clinically evaluated at, the UC Davis M.I.N.D. Institute Fragile X Research and Treatment Center (FXRTC), and molecular DNA testing was carried out to confirm their diagnosis. The Institutional Review Board at the University of California, Davis, approved the experimental protocol, and informed consent was obtained from a parent or caregiver of each infant.

Cognitive assessment. To control for differences in developmental level, all infants with FXS were assessed using the *Mullen Scales of Early Learning* (Mullen, 1995) to derive a mental age. The MSEL is a standardized developmental test for children ages 3 to 60 months, consisting of five subscales: gross motor, fine motor, visual reception, expressive language, and receptive language. Infants in the FXS group had a mean mental age of 17.39 months (±5.76, range = 11–36 months), which was matched to infants in the TD group (18.35 months ± 5.35, range = 11–31 months). An independent samples t-test confirmed that mental age did not differ significantly between the two final groups ($t(1, 64) = 0.702$, $p = 0.485$, 2-tailed).

Apparatus and stimuli

Stimuli were presented on a Tobii 17-inch LCD binocular eye tracker (1024 × 768 pixels resolution, 50-Hz capture rate, 60-Hz refresh rate) to record infants' fixations during the task. The calibration procedure was run using ClearView software (Tobii Technology, Sweden), which allows an optimal accuracy of 0.5°.

Stimulus creation and programming were done using Adobe Flash CS4 Professional software. Infants were shown two objects; a red sphere and a green cylinder, each fitting within a 5 deg by 5 deg square, presented 4 deg from the midline, against a black background. Each occluder was a white square that fully covered the object. During the task infants heard audio sequences of classical music through two standard computer speakers concealed behind the monitor.

Procedure

Infants were seated on a parent or caregiver's lab, 60 cm from the eye tracker monitor. The experiment began with a five-point calibration routine, followed by a single familiarization trial and four test trials. **Figure 1** presents a schematic of the experimental design.

Familiarization trial. A red sphere on the left and a green cylinder on the right were lowered from the top to the middle of the screen at a speed of 3 cm/s. The objects remained stationary for 5 s, after which they were concealed for 1 s by the occluders, also lowered from the top of the screen. The occluders were then raised to reveal the objects for 1 s and were lowered immediately after to re-occlude the shapes for 1 s. The occluders revealed the objects a final time and then dropped back down. The purpose of this trial was to provide infants with sufficient time to visually examine each object and its location, and to learn that the role of the occluders was to hide the objects without transforming them in any way.

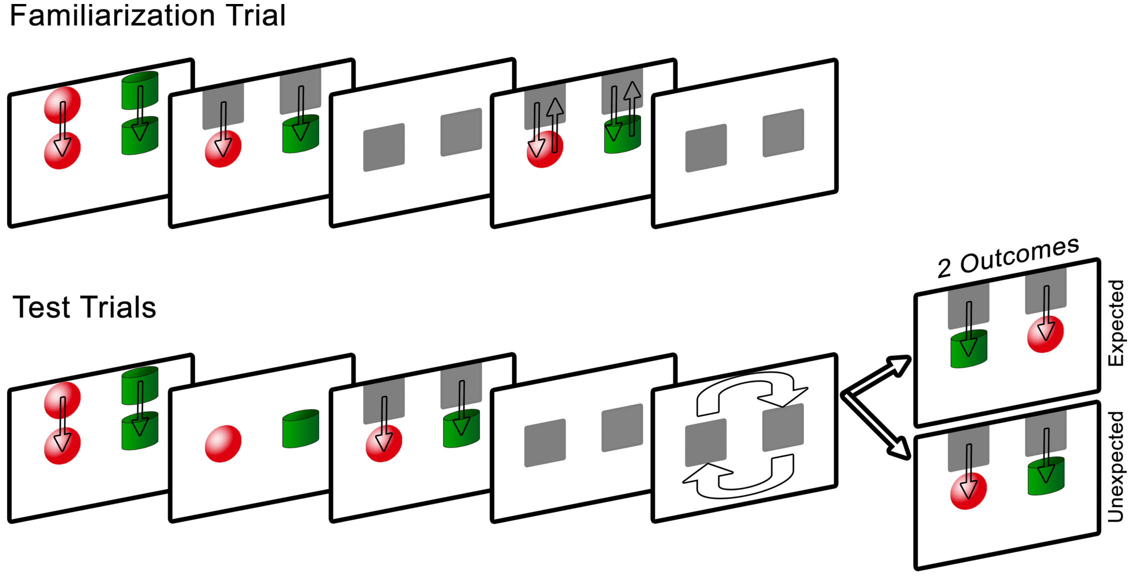

FIGURE 1 | Schematic of Experiment 1 design. Note that on the computer screen the background was black, the occluders were white, and the arrows were not present.

Test trials. Two pairs of test trials were presented in one of two orders: expected, unexpected, unexpected, expected or unexpected, expected, expected, unexpected, counterbalanced across subjects.

The test trials were similar to the familiarization trial in that following a 5-s viewing period of the objects, the occluders were lowered to cover the objects. During a 5-s occlusion, the occluders rotated 180° in a clockwise direction (local orientation maintained), after which they were raised to reveal either the objects in their newly rotated positions whereby the cylinder was now on the left (expected outcome) or the objects in their original positions (unexpected outcome). Infants were given 10 s to view the outcome, after which the occluders were lowered and rotated counterclockwise back to their starting positions, and the screen faded to black. After 2 s the subsequent test trial began and the sequence repeated until all four test trials were completed. The total task lasted 2 min and 24 s. It was predicted that if infants successfully encoded and bound the individual object identities to specific spatial locations, and tracked this information during the dynamic occlusion period, they should respond to the violation of object location by looking longer at the unexpected outcome compared to the expected outcome.

Coding

Eye tracking data was coded using the Area-of-Interest definition tool within ClearView. AOIs were defined by creating a 6 deg by 6 deg square around each object. The primary measure of interest included duration of fixations to each AOI region, where a fixation was defined as a period of looking in which the position of the eyes did not shift more than 30 pixels for a minimum of 200 ms. Fixations outside of the two AOIs were coded as either "away" or off-screen. Coding began once the occluders were raised to reveal half of the objects and ended when the objects were fully re-concealed. Mean looking time during each test trial was then calculated by summing the fixation duration to the two AOIs.

RESULTS AND DISCUSSION

Preliminary analyses of mean looking time differences between the expected and unexpected outcomes using a repeated measures analysis of variance (ANOVA) showed no effects involving mental age, gender, or trial order; therefore these factors were excluded from the following analyses. Mean looking time during each test trial was entered into a repeated measures ANOVA with two within-subject factors: outcome (expected or unexpected) and trial pair (first or second), and one between-group factor: group (typically developing or FXS). The analysis revealed a significant main effect of diagnosis ($F(1,64) = 14.03, p = 0.0001, \eta^2 = 0.180$) whereby TD infants looked significantly longer overall ($M = 6.31$ s, $SD = 2.38$ s) compared to infants with FXS ($M = 4.29$ s, $SD = 2.57$ s). A significant interaction between outcome and diagnosis ($F(1, 64) = 7.502, p = 0.008, \eta^2 = 0.105$) was also found, driven by TD infants' longer looking times during the unexpected ($M = 6.86$ s, $SD = 2.11$ s) than the expected ($M = 5.76$ s, $SD = 2.55$ s) outcome test trials ($t(32) = 2.556, p = 0.015$, SEM = 0.43). Infants with FXS trended toward longer looking during the expected ($M = 4.52$ s, $SD = 2.67$ s) than the unexpected ($M = 4.05$ s, $SD = 2.47$ s) outcome test trials ($t(31) = -1.507, p = 0.142$, SEM = 0.35). A binomial sign confirmed that while 24 out of 34 (71%, $p = 0.024$) TD infants looked longer at the unexpected compared to expected outcome trials, only 16 out of 32 (50%, $p = 1.14$) infants with FXS looked longer at unexpected outcome trials. No other significant effects or interactions were found. **Figure 2** shows mean looking times during the expected and unexpected test trial outcomes for the two groups.

Perhaps infants with FXS neglected to track the movement of the occluders during the rotation period, thereby leaving them unable to respond to the unexpected object location. To answer this question, a third AOI was created which encompassed the area through which the occluders moved, and looking time was calculated for each infant and each test trial. The result of an independent samples t-test confirmed that mean looking time to the occluders during the rotation periods did not differ significantly between the two groups ($t(1, 64) = 2.255, p = 0.136$, 2-tailed), thus the failure of infants with FXS to look longer at the unexpected outcome cannot be attributed to differences in looking time to the rotation of the occluders. There was also no difference in looking time during the familiarization period between infants with and without FXS ($t(1,64) = 2.920, p = 0.092$, 2-tailed), which could have influenced perceptual novelty preferences.

These results demonstrate that TD infants successfully maintained the identity of an occluded object across a spatiotemporal transformation, and infants with FXS did not. Instead, infants with FXS showed a tendency to look longer during the expected test trial outcome, suggesting that the object representation was not maintained throughout the rotation of the occluders. Thus, objects in their new positions may have violated the expectation that object features would remain bound to their original locations. This looking pattern was not dependent on age, sex, test trial order, looking time during the familiarization trial, or time spent looking at the occluders during the transformation period.

Attentive tracking of occluded objects is believed to involve correspondence matching of features across changing spatial positions over time, a function shown to be mediated by parietal and frontal areas of the brain (Cavanagh, 1992; Yantis, 1992; Culham et al., 1998, 2001). Therefore, these results support the hypothesis that infants with FXS are impaired on an object occlusion task

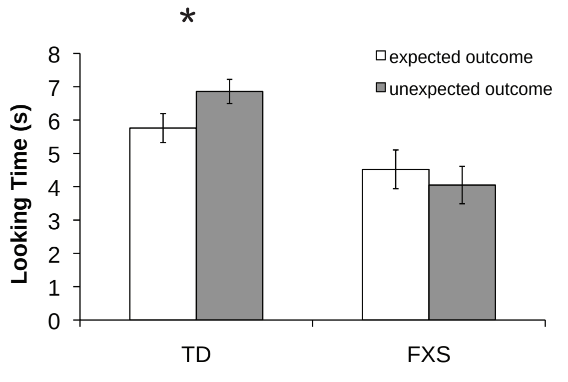

FIGURE 2 | Results from Experiment 1. Mean looking time in seconds to the expected (white bars) and unexpected (gray bars) test trial outcomes for each group. The effect of outcome was significant only in TD infants, as indicated by an asterisk ($p < 0.05$). Error bars represent ±SEM.

requiring dorsal stream function, either as a result of a selective spatial or temporal processing deficit or a deficit in the processing of integrated spatiotemporal information.

EXPERIMENT 2

To rule out the possibility that infants with FXS were unable to maintain an object representation during a period of static occlusion, a control experiment was performed using the same experimental procedure but removing the dynamic quality of the occlusion. Namely, in this experiment, there was no spatiotemporal transformation during the occlusion period; rather, the occluders remained static and the object property that changed during the occlusion period was color. Color was selected as the feature violation because it is known to differentially activate the ventral pathway. The question was whether infants with FXS would be able to form accurate expectations about the identity of the occluded objects in the absence of the occluders' motion.

MATERIALS AND METHODS

The method was the same as in Experiment 1 unless otherwise noted.

Participants

Twenty-four infants diagnosed with the FXS full mutation (19 boys and 4 girls) and thirty-three mental age-matched full-term TD infants (25 boys and 8 girls) were included in the final sample. Mean chronological age for the FXS and TD groups was 27.52 months (±8.36, range = 11–46 months) and 16.52 months (±5.15, range = 11–32 months), respectively. Infants in the FXS group had a mean mental age of 16.54 months (±5.74, range = 11–28 months), matched to infants in the TD group (16.88 months ± 4.96, range = 11–32 months). There was no significant difference between the two groups on developmental level ($t(1, 55) = 0.244$, $p = 0.809$, 2-tailed).

Twelve TD infants were not included as a result of poor calibration (1), parent verbal interference (1), or incomplete gaze data for all four test trials (10). Data from an additional eight infants with FXS were not included in the final analysis because of poor calibration (2) or incomplete gaze data (6).

Apparatus and stimuli

Infants were shown two objects; an orange triangle and a blue circle, each fitting within a 5 deg by 5 deg square region, presented 4 deg from the midline, against a black background. Each occluder was a white square that fully covered the object.

Procedure

Figure 3 presents a schematic of the experimental design.

Familiarization trial. Infants were presented with a blue triangle on the left and an orange circle on the right, lowered from the top to the middle of the screen at a speed of 3 cm/s. The objects remained stationary for 5 s, after which the occluders were lowered to conceal the objects for 1 s. The occluders were then raised to reveal the objects for 1 s and were immediately lowered to re-occlude the shapes for 1 s. This process was repeated a second time.

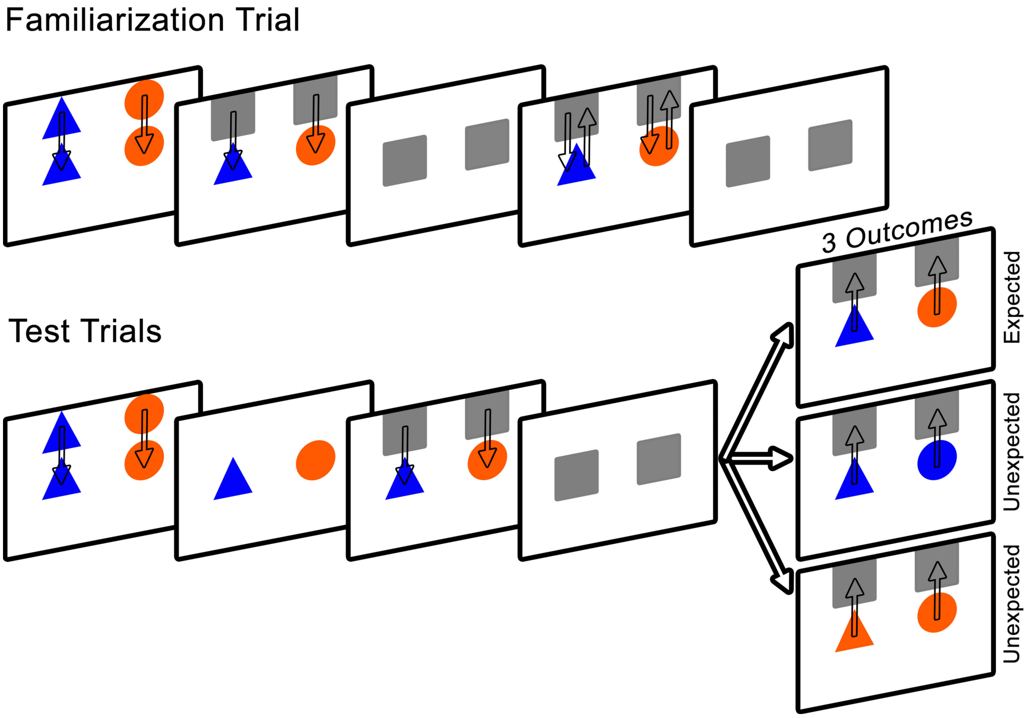

FIGURE 3 | Schematic of Experiment 2 design. Note that on the computer screen the background was black, the occluders were white, and the arrows were not present.

Test trials. Two pairs of test trials were presented in one of two orders: expected, unexpected, unexpected, expected or unexpected, expected, expected, unexpected, counterbalanced across infants.

The test trials were similar to the familiarization trial in that the objects were presented and remained stationary for 5 s. The occluders were lowered to cover the objects for a period of 5 s, during which they remained stationary.

The occluders were then raised to reveal either the unchanged objects (expected outcome) or one object that had changed color (unexpected outcome). To avoid presenting infants with a novel color, the object was changed to match the color of the second object (orange to blue or blue to orange; color of changed object alternated on unexpected trials). Infants were given 10 s to view the trial outcome, after which the occluders were lowered and the screen faded to black. After 2 s, the subsequent test trial began. The total task lasted 2 min and 24 s. We expected that if infants encoded and bound the features of each object to their appropriate spatial location, and retained this information during the static occlusion, they should respond to the violation of color by demonstrating increased looking time to the unexpected outcome compared to the expected outcome trials. More specifically, infants should look longer at the spatial location of the object whose identity changed.

Coding

Coding was the same as in Experiment 1. In addition to mean looking time during each test trial, a Visual Preference (VP) score, indexing the proportion of looking time to the object that changed color, was calculated using the following formula: (looking time to changed object)/(looking time to both objects). VP scores could be between 0 and 1, with 0.5 considered equal preference for both objects. For each infant, a VP score was calculated for each unexpected outcome test trial[1].

RESULTS AND DISCUSSION

Preliminary analyses of mean looking time differences between the expected and unexpected outcomes using a repeated measures ANOVA showed no significant effects involving mental age, gender, or trial order; therefore these factors were excluded from further analyses. Mean looking time during each test trial was entered into a repeated measures ANOVA with two within-subject factors: outcome (expected or unexpected) and trial pair (first or second), and one between-group factor: group (typically developing or FXS). The analysis revealed a significant main effect of outcome ($F(1, 55) = 13.39$, $p = 0.001$, $\eta^2 = 0.196$) in which infants looked longer at the unexpected ($M = 5.32$ s, $SD = 2.98$ s) compared to the expected ($M = 3.19$, $SD = 2.12$ s) outcome test trials. A significant main effect of trial pair ($F(1,55) = 6.44$, $p = 0.014$, $\eta^2 = 0.105$) was also found, resulting from longer looking times during the first trial pair ($M = 5.22$ s, $SD = 3.33$ s) than the second ($M = 4.34$ s, $SD = 3.24$ s), most likely an effect of boredom or fatigue toward the end of the task. No other significant effects or interactions were found. Critically, there was not a significant interaction between outcome and group ($F(1, 55) = 0.572$, $p = 0.453$). **Figure 4** shows mean looking times to the expected and unexpected test trial outcomes for the two groups.

These findings were further explored by conducting a series of planned comparisons (paired t-tests) showing that TD infants looked significantly longer to the unexpected ($M = 6.44$ s, $SD = 3.15$ s) than the expected ($M = 5.55$ s, $SD = 3.04$ s) outcome test trial for the first pair of test trials ($t(32) = 2.441$, $p = 0.019$, SEM = 0.59) and significantly longer to the unexpected ($M = 5.69$ s, $SD = 3.28$ s) than the expected ($M = 4.06$ s, $SD = 2.56$ s) outcome test trial for the second pair of test trials ($t(32) = 3.216$, $p = 0.003$, SEM = 0.50). Infants with FXS looked significantly longer to the unexpected ($M = 4.65$ s, $SD = 3.11$ s) than the expected ($M = 3.54$ s, $SD = 3.55$ s) outcome test trial for the first pair of test trials ($t(23) = 2.514$, $p = 0.020$, SEM = 0.43) and trended toward longer looking to the unexpected ($M = 3.88$ s, $SD = 3.47$ s) compared to expected outcome ($M = 3.28$ s, $SD = 3.39$ s) test trial for the second pair

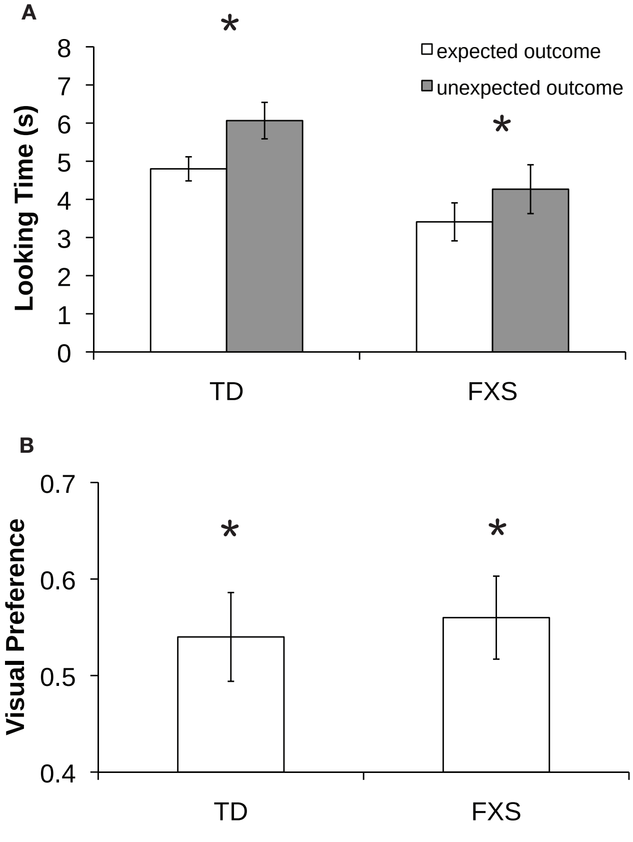

FIGURE 4 | Results from Experiment 2. (A) Mean looking time in seconds to the expected (white bars) and unexpected (gray bars) test trial outcomes for each group. The effect of outcome was significant in both groups as indicated by an asterisk ($p < 0.05$). **(B)** Mean visual preference for the changed object for each group. Asterisks indicate significant difference from chance ($p < 0.05$). Error bars represent ±SEM.

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[1] Note that a VP score calculation was not possible for Experiment 1 because the unexpected outcome consisted of *both* objects in their original positions and there was therefore no reason to believe that infants would choose to look at the location of one object longer than the other.

of test trials ($t(23) = 0.956$, $p = 0.349$, SEM = 0.62). A binomial sign test was carried out separately for each group of infants, and confirmed that 18 out of 24 (75%, $p = 0.023$) infants with FXS looked longer during unexpected outcome test trials, while 27 out of 33 (82%, $p = 0.0001$) TD infants looked longer during unexpected compared to expected outcome test trials.

No group difference was identified for VP for the changed object during the unexpected outcome trials [$F(1,56) = 1.698$, $p = 0.199$], and overall scores were significantly greater than the chance level of 0.5 ($t(55) = 2.375$, $p = 0.021$, SEM = 0.035), indicating reliable discrimination of the changed object identity (**Figure 4**).

The results from Experiment 2 indicate that during an occlusion event in which objects remained static, thereby minimizing dorsal stream involvement in motion tracking, TD infants and infants with FXS were able to maintain object representations such that a change in color of one of the objects elicited a violation of the infant's visual expectation. Further, during unexpected outcome test trials, infants in both groups spent a significantly greater proportion of time looking specifically at the location of the object that underwent the color change. Most importantly, this looking pattern was found independent of diagnosis, ruling out the possibility that infants with FXS have difficulty in encoding and maintaining the identity-location binding of an occluded object or exhibit a generalized working memory deficit. While we acknowledge that object properties such as color or motion are unlikely to elicit *purely* ventral or dorsal representations, because color is mostly restricted to ventral-stream processing, the results of Experiment 2 lead to the conclusion that infants with FXS have relatively spared ventral-stream object processing.

Results from this experiment replicate those of previous work showing that TD infants are capable of mentally representing the featural identity of a static object during a period of occlusion, and that there is differential processing of stimulus features such that by 12-months infants can use color information when reasoning about objects.

GENERAL DISCUSSION

The experiments reported here were designed to investigate object processing of dynamic and static occlusion events in infants with and without FXS as a means of explicitly testing dorsal and ventral visual abilities. This is the first study to experimentally examine object processing skills in infants with FXS. Our findings reveal that, in contrast to mental age-matched TD infants, infants with FXS were unable to mentally represent *and* track the identity of occluded objects during a dynamic event. However, infants with FXS performed comparable to controls when representing the identity of objects during a static occlusion event. Thus, infants with FXS appear to have a selective deficit in the visual processing required for object tracking, supporting the hypothesis of a deficit of dorsal stream functioning. While these results are consistent with a selective dorsal stream deficit, an equally plausible explanation is that infants with FXS may have difficulty in maintaining integrated ventral and dorsal information during a dynamic occlusion event. Both interpretations involve the reduced ability to represent the spatial position of a moving object, which is subserved by higher stages of visual processing including but not limited to parietal cortical areas of the brain (Culham et al., 1998; Battelli et al., 2001, 2007).

The mechanisms involved in maintaining the representation of an occluded object across changes in time and space are thought to primarily involve selective visual attention (Awh and Jonides, 1999; Scholl et al., 2001) and visual working memory (Pasternak and Greenlee, 2005; Xu and Chun, 2006). Several functional neuroimaging studies have shown that increased response of posterior parietal cortex is associated with maintaining spatiotemporal information about an object in working memory (Olson et al., 2003; Todd and Marois, 2004; Shuwairi et al., 2007). This is thought to be part of a larger cortical network involved in perceptual completion, motion perception, and timing estimation. Further, in adults, the prefrontal cortex has been found to play a critical role in integrating dorsal and ventral stream information required for action planning and execution by the dorsal stream as well as for maintaining visual information in working memory (Rao et al., 1997; Rossetti and Pisella, 2002; Mohr and Linden, 2005).

These data add to the growing body of research suggesting that a dorsal stream deficit is present in individuals with FXS, and extend the existing findings by showing that this deficit is present during infancy. These results are supported by evidence from children and adults with FXS demonstrating lower performance on other tasks mediated by the dorsal stream such as coherent motion processing, visual-motor coordination, and basic numerical computation (Cornish et al., 1999; Rivera et al., 2002; Kogan et al., 2004). Additional studies that disentangle the spatial from the temporal aspects of visual processing are required in order to fully understand the observed object tracking impairment in infants with FXS.

CONCLUSION

Our visual system encounters the constant challenge of encoding, maintaining, and tracking object information in our dynamic environment. Here we show that TD infants can reliably represent and track an occluded object across changes in time and space, while infants diagnosed with FXS cannot. These findings provide important baseline data from which to understand the developmental trajectory of visual and cognitive processing in both groups of infants. We conclude that infants with FXS experience disrupted dorsal stream processing, known to be directly involved in spatiotemporal tracking of objects. It is not clear how early in development this visual processing impairment may arise or if there is a critical period for the involvement of FMRP in the early development this network of areas. Future experiments are needed to further examine the nature of this spatial and/or temporal visual processing deficit, particularly as it relates to downstream cognitive abilities characteristic of the disorder.

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Scene complexity: influence on perception, memory, and development in the medial temporal lobe

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Regions in the medial temporal lobe (MTL) and prefrontal cortex (PFC) are involved in memory formation for scenes in both children and adults. The development in children and adolescents of successful memory encoding for scenes has been associated with increased activation in PFC, but not MTL, regions. However, evidence suggests that a functional subregion of the MTL that supports scene perception, located in the parahippocampal gyrus (PHG), goes through a prolonged maturation process. Here we tested the hypothesis that maturation of scene perception supports the development of memory for complex scenes. Scenes were characterized by their levels of complexity defined by the number of unique object categories depicted in the scene. Recognition memory improved with age, in participants ages 8–24, for high-, but not low-, complexity scenes. High-complexity compared to low-complexity scenes activated a network of regions including the posterior PHG. The difference in activations for high- versus low-complexity scenes increased with age in the right posterior PHG. Finally, activations in right posterior PHG were associated with age-related increases in successful memory formation for high-, but not low-, complexity scenes. These results suggest that functional maturation of the right posterior PHG plays a critical role in the development of enduring long-term recollection for high-complexity scenes.

Keywords: declarative memory, perception, cognitive development, visual scenes, fMRI, parahippocampal gyrus

INTRODUCTION

The neural systems mediating declarative or explicit memory for facts and events in adults have been identified through convergent lesion and functional neuroimaging evidence. Medial temporal lobe (MTL) structures, including the hippocampus and surrounding perirhinal and parahippocampal cortices, are essential for the formation of new declarative memories. Bilateral MTL injury results in global amnesia, defined by an inability to form new declarative memories (Scoville and Milner, 1957; Zola-Morgan et al., 1986; Squire, 1992). Prefrontal cortex (PFC) is not essential for memory formation, but PFC lesions impair declarative memory for contextual details of an experience (source memory) (Schacter et al., 1984; Janowsky et al., 1989). Functional neuroimaging studies in healthy adults have found that greater magnitudes of PFC and MTL activation during encoding correlate, on a stimulus-by-stimulus or event-related basis, with successful memory formation as evidenced by accurate subsequent memory for scenes, words, and faces (Brewer et al., 1998; Wagner et al., 1998; Buckner et al., 1999; Davachi et al., 2003; Nichols et al., 2006).

Compared to the large knowledge about the neural correlates of memory in adults, little is known about the normal development of declarative memory systems from childhood through adulthood (Menon et al., 2005; Chiu et al., 2006; Ofen et al., 2007; Paz-Alonso et al., 2008). Behavioral evidence suggests that declarative memory develops from childhood through adulthood, and that development is most protracted for memory tasks that demand greater detail in recollection, such as remembering specific details of the context in which information was presented, relative to the information itself (Dirks and Neisser, 1977; Mandler and Robinson, 1978; Cycowicz et al., 2001; Billingsley et al., 2002; Ghetti and Angelini, 2008). The developmental distinction between different aspects of recognition memory may reflect two dissociable mnemonic processes; recollection, that includes specific knowledge of the details of the event, and familiarity, that may include less contextual details (Jacoby et al., 1993; Yonelinas, 1999). In a functional neuroimaging study of memory encoding for scenes in 8 to 24-year olds, development of successful memory encoding was associated with increased activation in PFC regions that were specifically associated with recollection (but not familiarity) for scenes (Ofen et al., 2007). In contrast, MTL regions were similarly activated across age. These findings suggested that the protracted development of recollection relative to familiarity could reflect protracted maturation of PFC relative to MTL functions supporting memory formation.

The lack of developmental changes for activations in the MTL in our previous study suggested that memory processes served by MTL are mature by the age of 8 years. This conclusion, however, contradicts other evidence that implicates prolonged maturation of MTL subregions. First, memory for high-level visual stimuli such as natural scenes and faces grows from childhood through adolescence into young adulthood (Diamond and Carey, 1977; Mandler and Robinson, 1978), and this development of memory ability has been associated with the development of cortical areas that are specialized for visual perception of scenes and faces (Golarai et al., 2007). Specifically, functionally-defined scene-selective parahippocampal cortex known as the 'parahippocampal place area' or PPA (Epstein and Kanwisher, 1998) grows in size from childhood through adulthood, and this growth in size correlates with recognition memory for scenes. These findings suggest that prolonged maturation of at least parts of the MTL ought to be associated with prolonged maturation of some aspects of memory for scenes. Second, post-mortem and structural imaging evidence indicates that PFC maturation appears to continue into late adolescence (Huttenlocher, 1979; Giedd et al., 1999; Gogtay et al., 2004; Sowell et al., 2004), but it also suggests continued development of MTL regions in the human brain (Giedd et al., 1996; Sowell and Jernigan, 1998; Sowell et al., 2002; Gogtay et al., 2006). These anatomical findings support the idea that PFC memory functions develop more slowly than MTL memory functions in the human brain, but also raise the possibility that functional developmental may occur in the MTL in the 8 to 24-year-old range.

Here, we examined the effect of visual scene complexity on three related issues. First, we tested whether scene complexity modulates the magnitude of brain activation in scene-perception regions. Second, we tested whether scene complexity effects undergo prolonged maturation. Third, we tested whether scene complexity influences the development of memory, and MTL functions associated with memory formation. Complexity of real-world scenes is represented by multiple dimensions including the quantity of objects in the scene (Oliva et al., 2004). When regularities are present in a scene, e.g., identical objects, the visual pattern is simplified by perceptual grouping (Feldman, 1997). Thus, a scene picturing a forest may depict many trees, but perceptual grouping allows the trees to be perceived as a single group. A scene picturing a typical living room or kitchen, however, may depict a variety of distinct objects. To account for the effect of grouping, we characterized scene complexity as the number of unique object categories in the scene. We hypothesized that processing more complex scenes will likely result in greater activations in scene-selective regions in the parahippocampal gyrus (PHG). We further hypothesized that processing of more complex scenes will have protracted maturation. These effects in turn may be associated with improvement in memory ability with age. Thus the third hypothesis tested here is that developmental effects of MTL functions associated with memory formation will be more pronounced for more complex scenes. Accurate recognition memory for more complex scenes may be limited in children who have an immature functionally-defined scene-selective PHG (Golarai et al., 2007). Children may not have the mature representational capacities needed for the encoding of detailed specificity found in more complex scenes. This less than optimal representation at encoding will then limit successful retrieval at test. Such an effect of complexity may have been obscured in the prior study (Ofen et al., 2007) because half the scenes were outdoor pictures with low visual complexity (trees, lakes, mountains, etc.).

We also examined whether a low-level visual property of the scenes, spatial frequency, influenced our findings because high-complexity scenes have higher spatial frequencies than low-complexity scenes. Neurophysiological (De Valois et al., 1982; Foster et al., 1985) and fMRI studies (Vuilleumier et al., 2003; Iidaka et al., 2004; Peyrin et al., 2004) suggest that information associated with different spatial frequencies is represented in different brain regions. We therefore measured the mean spatial frequency in each scene and examined whether spatial frequency influenced the effects of scene complexity on activations in regions involved in scene perception, the maturation effects in these regions and the influence of scene complexity on measures of memory formation.

We present the results of a reanalysis of previously published data (Ofen et al., 2007) that examined the normal development of activations related to successful memory formation, as measured by functional magnetic resonance imaging (fMRI), in healthy children, adolescents and adults from age 8 to 24. Participants viewed indoor and outdoor scenes during a scanned study phase. Afterwards, they received a recognition memory test with the previously studied scenes and new (foil) scenes. For scenes judged as having been seen at study, participants rated their memories as "remembered" (R responses which indicated a vivid memory accompanied by details of the experience) or "familiar" (K responses which indicated that participants *know* they have studied the scene, but that the memory was not accompanied by details of the experience). We coded all scenes for complexity, and reanalyzed the behavioral and imaging data to examine the effects of complexity. We tested the effects scene complexity on (1) activations in regions involved in scene perception (2) functional maturation in scene-perception regions, and (3) the development of memory formation via behavior and MTL activation.

MATERIALS AND METHODS

MATERIALS

A total of 500 full-color photographs of natural scenes (250 indoor, 250 outdoor) were used, and randomly divided into ten lists of 50 pictures. Lists were counterbalanced across participants, such that all lists were presented equally often as study and test items across participants. The number of unique object categories for each scene was calculated as an index for scene complexity using the LabelMe image toolbox (Russell et al., 2008) (**Figures 1A,B**). Scenes that included three or fewer object categories were classified as low-complexity scenes, and scenes that included five or more object categories were classified as high-complexity scenes. Out of the 250 studied scenes, 103.7 ± 1.9 (mean ± SD) were low-complexity scenes, and 113.9 ± 4.4 were high-complexity scenes. Out of the 250 foils used in the unscanned recognition test, 103.3 ± 1.9 were low-complexity scenes, 113.1 ± 4.4 were high-complexity scenes. Scenes with four object categories were classified as mid-level complexity scenes (32.4 ± 6.0 studied scenes, and 33.6 ± 6.0 foils). Results are presented for the high- and low-complexity scenes.

To characterize the spatial frequency for each scene, the power spectrum of the scene was calculated by performing a 2D discrete Fourier transform on the image. The power spectrum can be modeled as a function of frequency f and orientation θ. The frequency distribution at different orientations in the image varies for different types of scenes (Torralba and Oliva, 2003). Here we considered eight different orientations: horizontal, vertical, and six oblique orientations. The power spectrum for each scene was fitted to a linear model at the frequency interval $f = [0.01\ 0.45]$ cycles/pixel for each of the eight orientations $\theta = [0\ \pi]$. The average slope and intercept of the fitted line for the 8 orientations were used to characterize the frequency distribution of the scene. High-complexity scenes had significantly higher spatial frequencies than low-complexity scenes $[t(432) = 3.43, p < 0.001]$. We therefore included spatial frequency for each scene as a parametric modulator in the imaging data analysis to account for effects associated with spatial frequency (*fMRI data analysis* section).

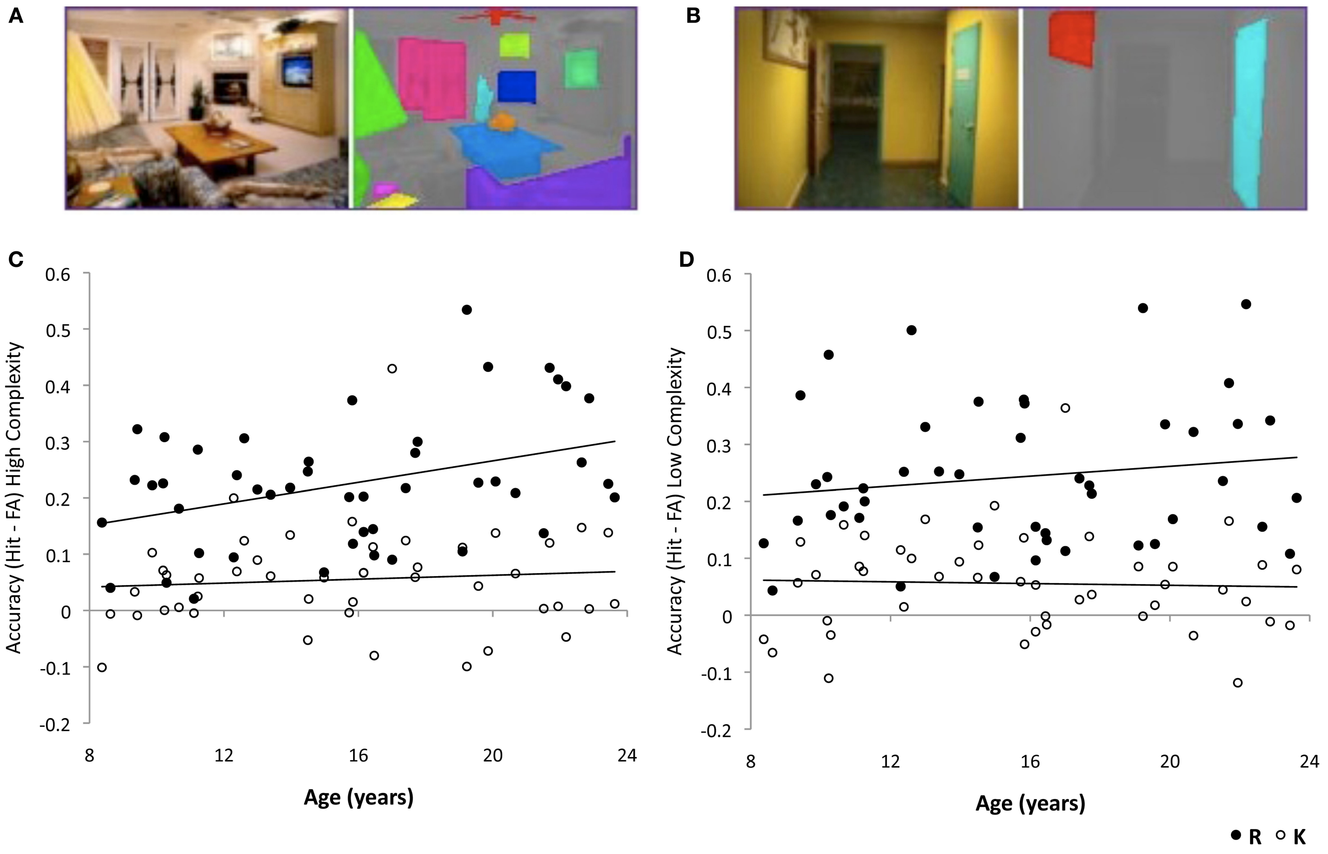

FIGURE 1 | Recollection memory for high-complexity, but not low-complexity, scenes improved significantly with age. Examples of original scenes (left) and items in those scenes identified for complexity calculation (right) for **(A)** high-complexity scene and **(B)** low-complexity scene. Adjusted recollection rates ($R_{HIT} - R_{FA}$) (filled circles) and adjusted familiarity rates ($K_{HIT} - K_{FA}$) (open circles) for **(C)** high-complexity scenes and **(D)** low-complexity scenes plotted against the participants' age. Recollection for low-complexity scenes and familiarity for high- and low-complexity scenes did not change reliably with age.

PARTICIPANTS

Fifty-two volunteers, ages 8–24 years, were recruited from the Stanford University community and provided informed consent as indicated by a Stanford University IRB-approved protocol. All participants were right-handed, had normal or corrected-to-normal vision, with no history of psychiatric or neurological disorder. Data are presented for 46 participants (24 females). Two participants were excluded as a result of sustained image artifacts (male, age 12; female, age 10). In addition, four participants (males, ages 8, 11, 13; female, age 15) were excluded due to incomplete behavioral data.

PROCEDURE

Participants viewed 125 indoor and 125 outdoor scenes during a scanned study phase that was followed by a recognition memory test. During scanning, each picture was presented for 3 s with 1 s of inter-trial interval. Participants made "indoor" or "outdoor" judgments to each scene by pressing a button on the button box. Trials with incorrect or no responses were excluded from the analyses (error trials). The study phase was divided into five sessions, each with 50 scenes. After the scanning session, participants were given a self-paced recognition test of the 250 scenes studied during the scanning session and 250 new scenes. If the participant responded "old" to a scene, they were further asked to indicate if they "actually remembered" the scene (R) or if the scene "just looks familiar" (K). Adjusted memory accuracy was calculated by subtracting the false alarm rate ("old" responses to new pictures) from the hit rate ("old" responses to studied pictures). If a "new" response was given to a studied scene, the trial was classified as a "forgotten" trial (F).

MRI DATA ACQUISITION

MRI data were acquired in a 1.5 T GE scanner. Functional images were acquired in 24 slices parallel to the line connecting anterior and posterior commissures, with 2 s repetition time, 60° flip angle, 64 × 64 voxels, and 3.75 mm in-plane resolution.

fMRI DATA ANALYSIS

Functional imaging data were analyzed in SPM5 (Department of Imaging Neuroscience, London, UK). Images were slice timing corrected, motion corrected, spatially normalized to the T2 Montreal Neurological Institute (MNI) template, and smoothed with a 6-mm Gaussian kernel. Data were inspected for artifacts and motion using custom software (http://web.mit.edu/swg/software/). First-level analysis was performed with a general linear model (GLM) with regressors for six conditions of interest (R, K, and F trial types for low- and high-complexity scenes) and two conditions of no interest (trials from mid-level complexity scenes, and error trials, including trials when no response was recorded). Additional regressors accounted for head movement (three translation, three rotation parameters) and outlier scans (images in which average intensity deviated more than 3 SD from the mean intensity in the session or in which movement exceeded 0.5 mm or 0.01° from the previous image). For each participant, contrasts of different trial types were created from linear combination of the GLM regressors. These contrasts included (a) High > Low complexity (for each level of complexity, activation for R, F, K conditions were combined) (b) R > F high complexity and (c) R > F low complexity. These contrasts were entered into a second-level random effects analysis to identify activations on a group level. In order to investigate age differences we conducted a whole brain regression analysis with age as a regressor of interest. Region of interest (ROI) in the posterior PHG was defined functionally as a region where activations increased with age in the contrast High > Low complexity. We extracted contrast estimates of subsequent memory performance from the contrasts R > F and K > F for both high- and low-complexity scenes from this ROI. These extracted measures from all participants were tested for correlation with age. Unless otherwise mentioned, we report activations that survived a threshold of $p < 0.001$ at the voxel-level, corrected at the cluster-level of $p < 0.05$. In a second GLM model, spatial frequency of each scene was included as a parametric modulator for each condition to control for the effects of spatial frequency. The same conditions and regressors as described above were computed at the first-level analyses.

RESULTS

BEHAVIOR

Across all participants, 50% ± 13 of studied high-complexity scene were later recognized as "old" (hit rate), and 52% ± 18 of studied low-complexity scenes were later recognized as "old". For new scenes presented at test (foils), 22% ± 16 of high- complexity scenes were falsely categorized as "old" (false alarm rate), and 22% ± 14 of low-complexity scenes were falsely categorized as "old". Across all participants, adjusted hit rate (hit rate – false alarm rate) for high-complexity scenes (28% ± 13) did not differ from that for low-complexity scenes (30% ± 14) ($t_{(45)} = 1.02, p = 0.31$). Critically, the adjusted hit rate increased with age for high-complexity ($r = 0.40$, $p = 0.006$), but not for low-complexity ($r = 0.11, p = 0.46$) scenes. Thus, overall participants had similar memory accuracy for high- and low-complexity scenes, but memory accuracy improved with age only for high-complexity scenes.

Participants categorized as "remembered" (R_{HIT}), 28% ± 12 of the studied high- complexity scenes, and 29% ± 15 of the studied low-complexity scenes. Participants had similar rates of falsely categorizing as "remembered" (R_{FA}) high (5% ± 6) and low (5% ± 6) complexity foils. Across all participants, adjusted R rates (R_{HIT}–R_{FA}) for high-complexity scenes did not differ from that for low-complexity scenes ($t_{(45)} = 1.41$, $p = 0.17$). Adjusted R rates increased with age for high-complexity ($r = 0.38$, $p = 0.009$), but not for low-complexity ($r = 0.16$, $p = 0.30$), scenes (**Figures 1C,D**). The difference between the adjusted R rates of high- and low- complexity scenes increased with age ($r = 0.27, p = 0.03$; 1-tailed), suggesting that the age-related improvements in memory performance were more robust for recollected high-complexity scenes. Thus, overall, participants had similar memory accuracy for recollected high- and low-complexity scenes, but accuracy for recollected high-complexity scenes improved with age, whereas accuracy for recollected low-complexity scenes did not change with age.

Participants categorized as "familiar" (K_{HIT}), 22% ± 9 of studied high-complexity scenes, and 22% ± 10 of studied low-complexity scenes. Participants falsely categorized as "familiar" (K_{FA}) 17% ± 11 of high-complexity, and 17% ± 12 of low-complexity foils. Across all participants, adjusted K rates (K_{HIT}–K_{FA}) for high- complexity scenes did not differ from that for low-complexity scenes ($t_{(45)} = 0.06, p = 0.95$). Adjusted K rates did not change with age for either high-complexity or low-complexity scenes ($|r|s < 0.09, ps > 0.56$) (**Figures 1C,D**).

During the encoding task, participants were overall faster to respond to low-complexity scenes (1072 ± 275 ms) than to high-complexity scenes (1093 ± 287 ms) ($t_{(45)} = 2.31, p = 0.03$). Response times decreased with age for low- ($r = -0.37$, $p = 0.01$) and high- ($r = -0.33, p = 0.03$) complexity scenes. The difference between the response times for high- and low-complexity scenes, however, did not change with age ($r = 0.14, p = 0.35$). Response times decreased with age for R, K and F trial types for both high- and low-complexity scenes ($-0.36 < r < -0.30$, $ps < 0.04$). The difference between response times for R and F trial types, however, did not change with age for either high ($r = 0.12$, $p = 0.43$) or low complexity scenes ($r = 0.08, p = 0.60$).

IMAGING

Scene complexity effects

Across all participants, there were greater activations for high- than low-complexity scenes in occipital and posterior medial temporal lobe regions (**Table 1**). To examine the effects of low-level visual properties of the scenes, we constructed a second GLM model in which the spatial frequency was included as a parametric modulator to account for the effects of spatial frequency of the scenes. The same clusters remained significant after the effects of spatial frequencies were accounted for.

Development of scene complexity effects

A whole-brain regression analysis for High > Low complexity with age as the regressor revealed age-related increases in activations in right posterior PHG and frontal cortex (BA 6) (**Table 1**). Activations in both of these regions remained significant after the effects of spatial frequency were accounted for.

Development of scene complexity effects that are related to subsequent memory – region of interest analysis

We further examined subsequent memory effects in the PHG region (26, −54, −4) in which activations for High > Low complexity scenes increased with age (**Figure 2**). This analysis allowed us to relate developmental changes in processing of high versus low complexity scenes to developmental changes in subsequent memory.

Development of subsequent memory effects for high-complexity scenes. Age-related increases in subsequent memory activations were found only for the contrast R > F for high-complexity

Table 1 | Activations for high- versus low-complexity scenes.

		BA	*x*	*y*	*Z*	*T*	*# voxels*
HIGH > LOW							
Right	Posterior cingulate/precuneus	31	22	–60	20	9.64	3344
	Parahippocampal gyrus	19	30	–42	–6	8.19	
Left	Posterior cingulate/precuneus	31	–18	–60	24	6.98	
Left	Parahippocampal gyrus	19/37	–30	–42	–4	8.96	873
	Parahippocampal gyrus	35	–24	–28	–16	3.65	
Left	Middle occipital gyrus	18/19	–26	–90	0	6.73	1347
	Lingual gyrus	19	–26	–82	0	4.94	
Right	Middle occipital gyrus	18	22	–88	–4	6.61	950
		18/19	34	–90	12	6.22	
Right	Medial frontal gyrus	11/10	6	52	–10	4.72	68
Right	Middle/Inferior temporal gyrus	21	52	–6	–18	4.41	106
Left	Precuneus/Superior parietal lobule	19	–32	–80	40	4.36	62
HIGH > LOW, INCREASED WITH AGE							
Right	Parahippocampal gyrus	19	26	–54	–4	5.15	215
Right	Middle frontal gyrus	6	36	–2	42	4.73	78

Coordinates (x y z) are based on MNI brain (Montreal Neurologic Institute). BA, Brodmann area; t, t value at peak voxel.

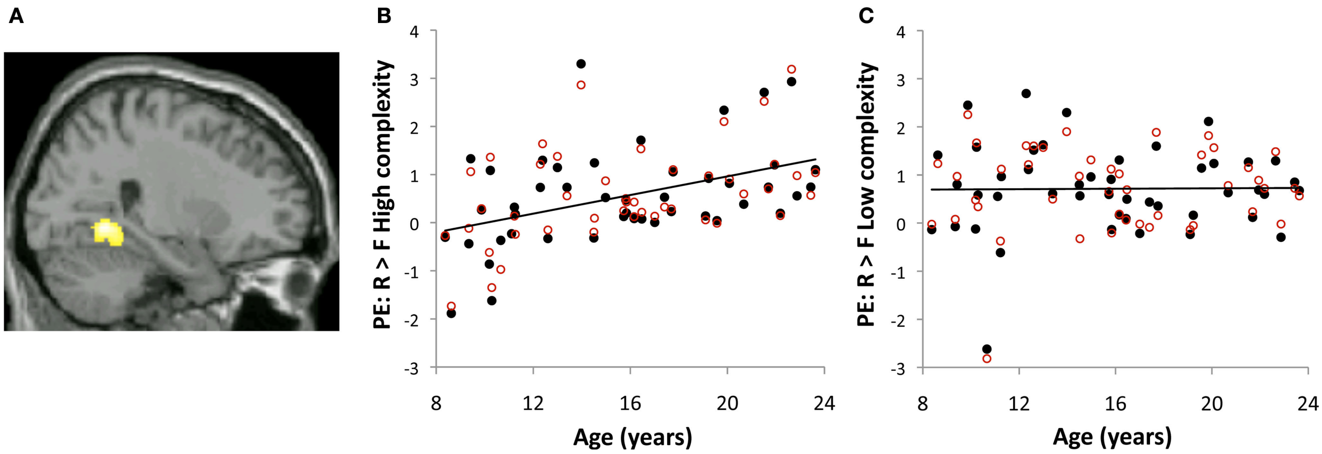

FIGURE 2 | Activations associated with successful memory formation for high- and low- complexity scenes in posterior parahippocampal gyrus. **(A)** Axial brain image depicting right posterior parahippocampal ROI defined by increase in activation with age for high > low complexity scenes. Image corresponds to MNI × coordinate 26. (B, C) Plots of correlations of the individual parameter estimates (PE) in the contrast R > F for high- **(B)** and low- **(C)** complexity scenes (black, filled circles). Overlaid in red (open circles) are the individual parameter estimates extracted from contrasts R > F for high- **(B)** and low- **(C)** complexity scenes after correcting for the scene's spatial frequency.

scenes ($r = 0.44$, $p = 0.002$). There were no age-related changes in subsequent memory activations for the contrast R > F for low complexity scenes ($r = 0.01$, $p = 0.94$). Furthermore, there were no age-related changes for subsequent memory activations in the contrast K > F for either high- ($r = 0.02$, $p = 0.89$) or low- ($r = 0.04$, $p = 0.78$) complexity scenes. The behavioral improvement in memory with age implied that with age, participants remembered more high-complexity scenes. We thus tested whether age-related increases in subsequent memory activations for high-complexity scenes merely reflect less statistical power to detect subsequent memory activation in younger participants. Age-related increases in subsequent memory activations for the contrast R > F for high-complexity scenes remained significant when recognition accuracy (adjusted R rates) was accounted for ($r = 0.35$, $p = 0.02$), and when the number of remembered high-complexity scenes was accounted for ($r = 0.40$, $p = 0.007$). These results suggest that the age-related increase in subsequent memory activations in the right posterior PHG for high-complexity scenes is not the result of reduced statistical power to detect activations in the younger participants, but rather that with age, increased activations for high-complexity scenes, compared to low-complexity scenes, contributed to improvement in memory for high-complexity scenes.

Development of subsequent memory effects for high-complexity scenes is not affected by low-level visual properties of scenes. We extracted subsequent memory activations in the same PHG ROI described above (**Figure 2**, red open circles) from results obtained with a second GLM model that took into account the effect of a low-level visual property of the scene, spatial frequency. Similar to results presented above, age-related increases in subsequent memory activations in the PHG ROI were only found for the contrast R > F of high-complexity scenes ($r = 0.44$, $p = 0.002$). There were no age-related changes in subsequent memory activations for the contrast R > F of low-complexity scenes ($r = 0.04$, $p = 0.79$). Thus, controlling for spatial frequency did not change the pattern of result in age-related changes in subsequent memory activation, suggesting that these effects were driven by the high-level scene property (image complexity) rather than a low-level visual property (spatial frequency).

Subsequent memory effects as a function of scene complexity

Subsequent memory for remembered scenes were investigated separately for high- and low-complexity scenes by comparing activations for remembered and forgotten scenes (contrast R > F) (**Table 2**). Across all participants, activations for subsequent memory of

Table 2 | Subsequent memory activations for remembered scenes.

		BA	*x*	*y*	*z*	*T*	*# voxels*
R > F (HIGH)							
Right	Precuneus	7	24	–76	40	6.95	3398
	Middle temporal gyrus	37	42	–56	–8	5.81	
	Precuneus	19	34	–88	24	5.45	
Left	Parahippocampal gyrus	19	–34	–56	–6	6.12	2755
	Middle occipital gyrus	19	–36	–80	2	6.06	
	Hippocampus		–30	–30	–14	5.60	
Right	Posterior cingulate	29	20	–56	20	5.19	316
Left	Precuneus	7	–22	–72	54	4.01	84
	Superior parietal lobule	7	–22	–66	46	3.84	
Right	Inferior frontal gyrus	9	44	12	28	3.92	100
	Middle frontal gyrus	9	58	16	34	3.41	
R > F (HIGH), CORRELATION WITH AGE							
Right	Cerebellum		2	–52	–26	4.08	96
Left	Cerebellum		–12	–50	–28	3.97	
Right	Cuneus	18	28	–88	24	4.03	108
Right	Parahippocampal gyrus*	19	24	–52	–6	4.00	60
R > F (LOW)							
Right	Fusiform gyrus	37	38	–44	–14	11.07	8654
	Precuneus	19	28	–76	38	9.70	
	Superior occipital gyrus	19	36	–84	28	9.01	
Left	Middle occipital gyrus	18	–28	–92	10	8.72	7350
	Middle occipital gyrus	19	–32	–80	16	7.99	
	Middle occipital gyrus	19	–38	–82	8	7.60	
Right	Inferior frontal gyrus	9	52	10	32	7.32	660
	Inferior frontal gyrus	9	44	10	26	6.82	
	Middle frontal gyrus	46	54	24	28	3.99	
Left	Inferior frontal gyrus	9	–44	6	32	5.93	642
	Middle frontal gyrus	9	–52	8	40	4.65	
	Precentral gyrus	6	–50	0	52	3.51	
Left	Middle frontal gyrus	11	–38	36	–14	4.89	172
	Inferior frontal gyrus	47	–50	38	–6	3.95	
	Inferior frontal gyrus	47	–48	20	–8	3.89	
Left	Inferior frontal gyrus	46	–46	32	14	4.83	172
Right	Inferior frontal gyrus	46	42	36	10	4.64	125
Left	Superior frontal gyrus	6	–6	16	60	4.59	173
Right	Postcentral gyrus	8	6	12	64	3.36	
R > F (LOW), CORRELATION WITH AGE							
	None						

**p = 0.06 (cluster-level).*

high-complexity remembered scenes were located in large bilateral posterior clusters spanning the middle occipital cortex, extending ventrally into the middle temporal, fusiform and parahippocampal gyri, and dorsally into the precuneus and superior parietal lobule, and in several PFC regions. There were age-related increases associated with subsequent memory for high-complexity scenes in several regions, including right posterior PHG (**Table 2**).

Subsequent memory activation for low-complexity remembered scenes across all participants were found in locations similar to those found for remembered high- complexity scenes, (**Table 2**). These activations, however, did not change with age. Thus, activation in right posterior PHG, an MTL subregion, increased with age for subsequently remembered high-complexity, but not low-complexity, scenes (**Figure 3**).

We also examined subsequent memory for correctly recognized old scenes that were given "familiar" responses (contrast K > F). Across all participants, the K > F contrast was associated with activations in posterior regions. For high-complexity scenes, K > F contrast identified activations in the PHG, middle occipital gyrus, and fusiform gyrus. For low-complexity scenes, K > F contrast identified activations in the middle temporal gyrus and middle occipital gyrus. Activations in regions responsive to K > F did not change with age for either high- or low-complexity scenes.

DISCUSSION

We found three major influences of scene complexity on human brain function. First, activations in a posterior network including the posterior PHG were greater for high-complexity than low-complexity scenes. Second, the difference in activations for high-complexity versus low-complexity scenes in right posterior PHG grew in magnitude with age. Third, activations in the same right PHG region that were associated with successful memory formation grew with age for high-complexity but not low-complexity scenes. Therefore, functional maturation of right posterior PHG may play a critical role in the development of high-level perception and long-term memory for high-complexity scenes.

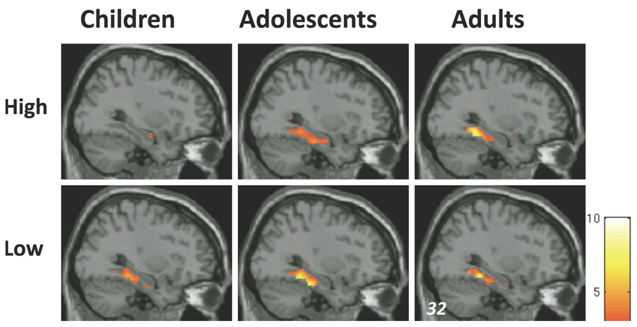

FIGURE 3 | MTL activations associated with successful memory formation (R > F) for children, adolescents, and young adults, for high-complexity scenes (top), and low- complexity scenes (bottom), scenes. Activation maps are overlaid on standard T1 image (for display purposes, $p < 0.005$, uncorrected, T value scale presented on the right). Children, $n = 16$, 8 male, mean age 10.7, range 8–12 years of age; adolescents, $n = 16$, 8 male, mean age 15.9, range 13–17 years of age; adults, $n = 14$, 7 male, mean age 21.3, range 19–24 years of age. This three-group stratification is illustrative, but statistical analyses were conducted with age as a continuum.

IMAGE COMPLEXITY DRIVES POSTERIOR PHG RESPONSE

The finding that posterior PHG activation was greater for high-complexity than low-complexity scenes can be related to ideas about how scenes are represented in posterior PHG. In the present study, scenes were categorized by complexity, but there was a strong correlation between the indoor/outdoor and high-complexity/low-complexity characteristics of the scenes (i.e., if we had analyzed our data with indoor/outdoor factor, we would have found developmental relations between PHG activation and recollection for indoor scenes). Posterior PHG bilaterally is more active when viewing indoor than when viewing outdoor scenes (Henderson et al., 2007), suggesting it may prefer certain types of scene stimuli to others. One explanation for greater posterior PHG activations for indoor scenes is that this region encodes spatial layout information that is primarily defined by the geometric structure in the scene (Epstein and Kanwisher, 1998; Epstein and Ward, 2010). The geometric structure of indoor scenes is more prominent than that of outdoor scene because they include fixed background elements such as walls and windows. However, posterior PHG is also more activated when participants view indoor scenes that contain more rather than fewer objects (Bar et al., 2008), suggesting that the posterior PHG activations observed in our study are not fully explained by the indoor characteristics of the high-complexity scenes. Furthermore, in our and most other studies, the outdoor scenes are nature scenes. If urban or other scenes had been included, then many outdoor scenes would have been high in complexity. Further studies are needed to determine which of these scene dimensions is more relevant for activations observed in posterior PHG.

Since measures of high-level visual properties, such as our measure of scene complexity, tend to correlate with low-level visual properties, such as spatial frequency, we tested if the effects of scene complexity were confounded by low-level visual properties of the scenes, spatial frequency. The activations in PHG remained higher for high-complexity scenes compared to low-complexity sense after spatial frequency was statistically controlled, which indicates that the PHG response was driven by a high-level visual property of complexity (number of unique item categories) rather than a low-level visual property (spatial frequency) of the scenes.

FUNCTIONAL MATURATION OF SCENE-COMPLEXITY PERCEPTION

We found a major developmental influence on the neural correlates of scene-perception that was dependent on scene complexity: scene complexity did not influence the level of activation in posterior PHG in children, but became more pronounced in older participants, suggesting that the specific manner in which posterior PHG supports scene representation undergoes prolonged maturation through young adulthood. There is evidence of maturation in scene-selective regions in the left posterior PHG region (parahippocampal place area, PPA), as evident by larger extent of these regions in adults (Golarai et al., 2007). Scene-selective regions were defined in that study as those that were more active for scenes than for other visual categories (such as objects and faces). In our study, we identified regions in the posterior PHG that were more active for high-complexity scenes than low-complexity scenes. These different approaches of identifying posterior

PHG activations that relate to scene processing may account for the different lateralization in the developmental effects that are found across the studies.

Scene complexity may be an important dimension in the maturation of scene processing in the brain. High-complexity scenes include more geometric configurations than low-complexity scenes, suggesting that maturational effects may be specific to the processing of geometric configurations. Developmental evidence suggest that although processing of some aspects of geometric information, such as the shape of a room develops early in life (Hermer and Spelke, 1994; Lee and Spelke, 2008), processing of other aspects, such as geometric configuration of objects in space have protracted maturation (Gouteux and Spelke, 2001). It is possible that the processing of these geometric configurations in high-complexity scenes is limited in children.

An alternative explanation for the maturation of the scene complexity perception is that since high-complexity scenes contained greater numbers of objects, the increased experience with these objects in adults compared to children facilitates the processing of the high-complexity scenes. It is also possible that since high-complexity scenes contained mostly indoor scenes with man-made objects, whereas low-complexity scenes contained mostly outdoor scenes natural objects, the maturation effect of scene complexity perception may reflect our more innate ability to process natural objects/scenes versus man-made objects/scenes.

DEVELOPMENTAL CHANGES ASSOCIATED WITH FORMING MEMORIES FOR COMPLEX SCENES

The development of neural correlates of recognition memory for scenes depended on the visual complexity of the scenes. Recollection of high-, but not low-, complexity scenes improved with age. These improvements in the formation of detailed memory for scenes were associated with the functional maturation of right posterior PHG. Activations in right posterior PHG region were greater for subsequently remembered than forgotten scenes across all participants, indicating a role for this region in the formation of memory for scenes. Critically, however, activations in this region increased with age for subsequent memory of high-complexity but not low-complexity scenes.

A central question is what aspect of memory encoding changes with age with respect to scene complexity. In the present study, age was specifically associated with the formation of memories that supported subjectively vivid and detailed recollection of more complex visual scenes. Age was not associated with recollection of low-complexity scenes, or familiarity-based recognition of either high- or low-complexity scenes. The specific behavioral relation between age and recollection for high-complexity scenes was paralleled by activations in right posterior PHG that increased with age only for recollection of high-complexity scenes. Furthermore, these age effects in PHG, remained significant after spatial frequency was statistically controlled, which indicates that the age-related changes in MTL activations is driven by a high-level visual property of complexity (number of unique item categories) rather than a low-level visual property (spatial frequency) of the scenes. The convergence of behavioral and imaging findings indicates that the right posterior PHG plays a specific role in the formation of episodic memories that are contextually rich for complex materials.

Interpretation of the selectivity of the developmental influence on MTL-memory relations for high-, but not low-, complexity scenes is constrained by the specific manner in which we defined complexity in our analysis. We defined complexity by counting the number of unique object categories in the scene. The difference between high- and low-complexity scenes could be considered with respect to two perceptual dimensions that may differentially relate to proposed roles of MTL in mnemonic processes. On the one hand, more complex scenes may simply have more object categories and this increase in the sheer number of object categories results in increased demand for detailed representation. Alternatively, it is possible that complex scenes include more spatial relations among the objects and thus may result in increased demand to represent the relations among constituent elements of the scene. Encoding and retrieving detailed contextual information is thought to be supported by the posterior PHG subregions of the MTL, while the representation of relations among the constituent elements of scenes are thought to be specifically subserved by the hippocampus (Cohen et al., 1999; Diana et al., 2007; Eichenbaum et al., 2007). Although these two aspects of mnemonic processes may be complementary, here we show that the age-related effects are unique to the posterior PHG, suggesting that it is the ability to process more contextual details that changes with age. We did not find age-related changes in the hippocampus. The interpretation of the lack of developmental effects in the hippocampus is limited by the nature of the task and the specific stimuli we used. Further studies that manipulate relational memory construction can reveal whether hippocampal activations vary with age under more specific demands for relational memory processes.

The interpretation that immature MTL structure and function limit the episodic specificity of memory formation for complex visual material through development is consistent with several related lines of evidence. First, lesion evidence in the monkey suggests that the posterior PHG play a critical role in memory for relatively demanding associations (Malkova and Mishkin, 2003). In line with its role in supporting the representation of demanding associations, activations in posterior PHG are related to recollection and the encoding and retrieval of contextual information (Diana et al., 2007). Second, maturation in brain regions that support processing of scenes was related to memory performance (Golarai et al., 2007). Here, we were able to document a functional maturation in scene-selective regions and to show that this maturation is directly linked to improvement in recognition memory. This extends the previous findings by Golarai et al. (2007) who correlated measures of the size of scene-selective regions with recognition measures that were taken in an independent session. Third, other evidence for functional changes in MTL contribution have been demonstrated during memory encoding and retrieval. One study of memory encoding showed that connectivity between MTL and PFC in the context of memory encoding changes with age (Menon et al., 2005). Two other studies found developmental changes in MTL activations for recognition memory of verbal material. Activations in anterior left MTL were associated differently with recognition of sentences in older versus younger children (Chiu et al., 2006), and with verbal memory retrieval in adults versus chidren (Paz-Alonso et al., 2008). Our analysis focused on the way in which processing of complex visual material may lead to better recognition. Our results are thus complementary to these previous findings and demonstrate that activations in posterior regions in the PHG increase with age when processing complex scenes and that these reduced activations in children may serve as a limiting factor for memory formation in children. Taken together, these findings suggest that protracted maturation in different subregions within the MTL may be evident depending on specific task demands. Future research is needed to determine the developmental trajectory of different subregions within the MTL.

SUMMARY

Scene complexity modulated activation in a network of regions that are typically involved in scene perception, including a subregion of the MTL, the posterior PHG. Activations in these regions were greater for high- compared to low-complexity scenes. Further, these scene complexity effects increased with age in the right posterior PHG, suggesting that this region undergoes functional maturation with respect to processing of high-complexity scenes. Finally, recollection for high-, but not low-complexity scenes improved with age; recollection for low-complexity scenes and familiarity for high- and low-complexity scenes did not change with age. Activations associated with subsequent memory in the right posterior PHG increased with age for high-, but not low-, complexity scenes. Thus, functional maturation of the PHG appears to be important both for the high-level perception of high-complexity scenes and for the formation of detailed recollection for those scenes. The prolonged functional maturation of this PHG region extends from childhood into young adulthood, and its functional immaturity may limit children and adolescents from forming the detailed and enduring memories for high-complexity scenes that are formed by adults with mature PHG function.

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Developmental continuity and change in responses to social and nonsocial categories in human extrastriate visual cortex

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It is well known that adult human extrastriate visual cortex contains areas that respond in a selective fashion to specific categories of visual stimuli. Three regions have been identified with particular regularity: the fusiform face area (FFA), which responds to faces more than to other objects; the parahippocampal place area (PPA), which responds selectively to images of houses, places, and visual scenes; and the extrastriate body area (EBA), which responds specifically to images of bodies and body parts. While the presence of these regions in the mature human brain is well-established, the degree to which children possess these areas and the degree of functional specialization of these areas in children of various ages has thus far remained unclear. This functional magnetic resonance imaging study examined the development of the FFA, EBA, and PPA in healthy, typically developing 7- to 11-year-old children and adults. Our results revealed a right FFA and a bilateral EBA and PPA in the children that were localized in a way consistent with these same regions in adults. In addition, the response profiles of these regions were very similar in adults and children with comparable levels of functional specificity at all of the ages tested. We discuss the implications of this research for understanding abnormal regional specialization for social and nonsocial object categories in individuals with autism spectrum disorders.

Keywords: fMRI, category selectivity, faces, places, bodies

INTRODUCTION

Prior functional magnetic resonance imaging (fMRI) studies in adult humans have documented the response properties of a small set of brain regions in the extrastriate visual cortex that are remarkably selective in their response to specific categories of social (defined here as containing a representation of another person) or nonsocial (not containing people) visual stimuli. To date, three regions have been identified with striking regularity. One is a face-selective region that has been dubbed the "fusiform face area" (FFA) because it responds more strongly to faces than to any other object category that has been tested. The FFA is localized to the lateral fusiform gyri in the human ventral temporal cortex (e.g., Allison et al., 1994; Puce et al., 1995; Kanwisher et al., 1997; McCarthy et al., 1997). Another region, termed the "extrastriate body area" (EBA), is most responsive to images of bodies and body parts (e.g., Downing et al., 2001). While these first two regions are exquisitely selective for socially relevant stimuli, a third visual processing region, the "parahippocampal place area" (PPA), exhibits selectivity for a class of nonsocial stimuli, images of houses, places, and visual scenes (e.g., Epstein and Kanwisher, 1998; Epstein et al., 1999). While much is known about the localization, organization, and response properties of these category-selective visual processing regions in adults, relatively little is known about the emergence and development of these regions in children. To date, a handful of functional neuroimaging studies have been reported concerning the FFA and PPA in children, but no studies have addressed the EBA.

The earliest developmental studies to investigate the functional organization of the human extrastriate cortex in children examined the role of the lateral fusiform gyri in face processing. A positron emission tomography study reported areas in the ventral occipito-temporal cortices that were more responsive to faces than to shapes in 2-month-old infants (Tzourio-Mazoyer et al., 2002), suggesting the possibility of very early specialization for faces. However, this study was flawed in its methods, particularly the choice of control stimuli, and thus cannot clearly demonstrate the presence of early face specificity. The results of studies of older children using fMRI and more adequate control stimuli have been much more equivocal. An initial fMRI study of 10- to 12-year olds found bilateral, face-selective activity in the fusiform gyri (Passarotti et al., 2003). In another fMRI study, 5- to 8-year olds failed to exhibit an FFA, but 9- to 11-year olds showed activity in an area of the fusiform gyri similar to the adult FFA (Gathers et al., 2004). Aylward et al. (2005) found that 12- to 14-year-old children but not 8- to 10-year-old children exhibited face-selective activity in a region of the fusiform gyri that was consistent with the expected location of the FFA.

More recently, fMRI studies have continued to examine the development of face-selective activity in the fusiform gyrus in children of various ages. Scherf et al. (2007) compared the activity evoked by pictures of places, faces, and objects in 6- to 8-year-old children, 11- to 14-year-old adolescents, and adults. Replicating some prior findings, they were unable to identify adult-like face-selective regions of activity in the younger children, but they were able to localize the FFA in the adolescents and the adults. In contrast, the localization of brain activity evoked by pictures of places and objects was equivalent across the observed age range. Subsequently, Golarai et al. (2007) reported that children as young as 7 years had face-selective activity localized to the fusiform gyri, but the volume of category-selective activation in the FFA and parahippocampal gyrus to faces and places, respectively, increased across the 7- to 11-year period and correlated with improved recognition memory for the respective stimulus class.

In comparison to the studies of the FFA, studies of children and adolescents have consistently reported the presence of a PPA as early as middle childhood (Golarai et al., 2007; Scherf et al., 2007). However, there are some inconsistencies in the findings regarding the nature of the developmental trajectory thereafter. Scherf et al. (2007) reported bilateral, adult-like PPA activation in 6- to 8-year olds, followed by more right-lateralized (but still bilateral) activation in 11- to 14-year olds, and a return to more evenly bilateral activation in adults. Overall, no difference in the extent of activation among the three age groups was found (Scherf et al., 2007). In contrast, although no age effects on size were found in the right PPA, Golarai et al. (2007) found that the left PPA was significantly smaller in children (ages 7–11) than in adults. Adolescents (ages 12–16 years) also showed a trend towards smaller size than adults. The three-fold increase in size of the left PPA across the observed age range was suggested to reflect the increase in selectivity reported (Golarai et al., 2007).

As the summaries above illustrate, prior studies have generated initial, often conflicting findings. Consequently, it is difficult to arrive at a clear conclusion regarding the development of the PPA and FFA, and there are no available developmental findings for the EBA. Thus, the prior work serves to highlight the need for additional developmental data. In the present study, we wished to further assess the development of the FFA and PPA in children in order to potentially clarify the controversies over their presence or absence in children. We also sought to conduct an initial fMRI study of the EBA in children. To this end, we employed a stimulus set that included faces, bodies, and places, along with flowers and sporting goods, in a blocked-design fMRI study of adults and children ages 7–11 years.

This kind of developmental study is critical for translational efforts. Studies of people with autism spectrum disorders have indicated abnormal development of face-selective responses in the FFA (for a review, see Schultz, 2005). However, in the absence of a better understanding of the normative developmental trajectory in this region, it is difficult to evaluate whether this abnormality plays a causal role in the emergence of the disorder or is an effect of the lack of interest in faces that is a characteristic of autism spectrum disorders.

MATERIALS AND METHODS

PARTICIPANTS

Ten adults (age range = 18.9–29.2 years; $M = 22.5$, SD = 3.5 years; seven females) and 22 children (age range = 7.2–11.9 years; $M = 9.7$ years, SD = 1.3 years; 14 females) participated in this study. Participants had no history of neurological or psychiatric disorders as measured by self (adults) or parent (for the children) report and had normal or corrected-to-normal vision. All participants were right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1970). Participants (adults) or their parents (for the children) provided written consent for this study, which was approved by the local Human Investigations Committee.

PROCEDURE

Stimuli and design

Figure 1 shows sample stimuli for each of the five categories of visual stimuli used in this study. In a block design, stimulus types were shown in 12-s stimulation blocks alternating with 12-s fixation blocks. No single type of stimulation block was shown twice in a row. Within each stimulus block, 24 images from that category were displayed for 500 ms each. In order to ensure that participants were attending, they were instructed to push a button when the same image was presented twice in immediate succession, which occurred approximately three times per run. Identical stimuli were used for child and adult participants. The study involved two scanning runs. The first run lasted 6.23 min and included five blocks each of photographs of faces, flowers, and bodies. The second run was 4.23 min long and consisted of five blocks each of photographs of places and sporting goods.

Imaging

Scanning was performed on a 3-Tesla Signa Excite HD scanner system with 50-mT/m gradients from General Electric Health Technologies (General Electric, Waukesha, WI). Parallel imaging was performed using an eight-channel head coil. Participants' heads were immobilized using tape and a vacuum cushion. Sixty-eight high-resolution anatomical images were acquired using a 3D fast SPGR pulse sequence (TR = 500 ms; TE = 20 ms; FOV = 24 cm; image matrix = 256^2; voxel size = $0.9375 \times 0.9375 \times 1.9$ mm) and used for coregistration with the functional data. These structural images were aligned in the near-axial plane defined by the anterior and posterior commissures. Whole-brain functional images were acquired using an echoplanar imaging sequence sensitive to blood oxygenation level dependent contrast (TR = 2000 ms; TE = 35 ms; FOV = 24 cm; image matrix = 64^2; voxel size 3.75 mm × 3.75 mm × 3.8 mm; 34 axial slices). Functional and structural images were coplanar. Global field homogeneity was ensured through use of a semi-automated high-order shimming program, and the first four radio frequency excitations of each run were discarded in order to have steady state equilibrium.

Analyses

Image preprocessing was performed with custom programs and SPM modules (Wellcome Department of Cognitive Neurology, UK). Head motion was detected by center-of-mass measurements. None of the 22 children and 10 adults included in the data set described in this paper exhibited greater than a 3-mm deviation in the center of mass in any dimension. However, we did exclude two additional adults and seven additional children because they exhibited excessive head motion. Images were time-adjusted to compensate for the interleaved slice acquisition and then motion-corrected to compensate for small (<3 mm) head movements. Our analyses employed a voxel-based analytical approach. The realigned and motion-corrected images were first normalized to the Montréal Neurological Institute (MNI) template found in SPM 99. These normalized functional data were then high-pass filtered and spatially smoothed with a 6-mm isotropic Gaussian kernel prior to statistical analysis. These realigned, motion-corrected, normalized, and smoothed data were used in the random-effects analyses described below. By normalizing the imaging data from adults and children

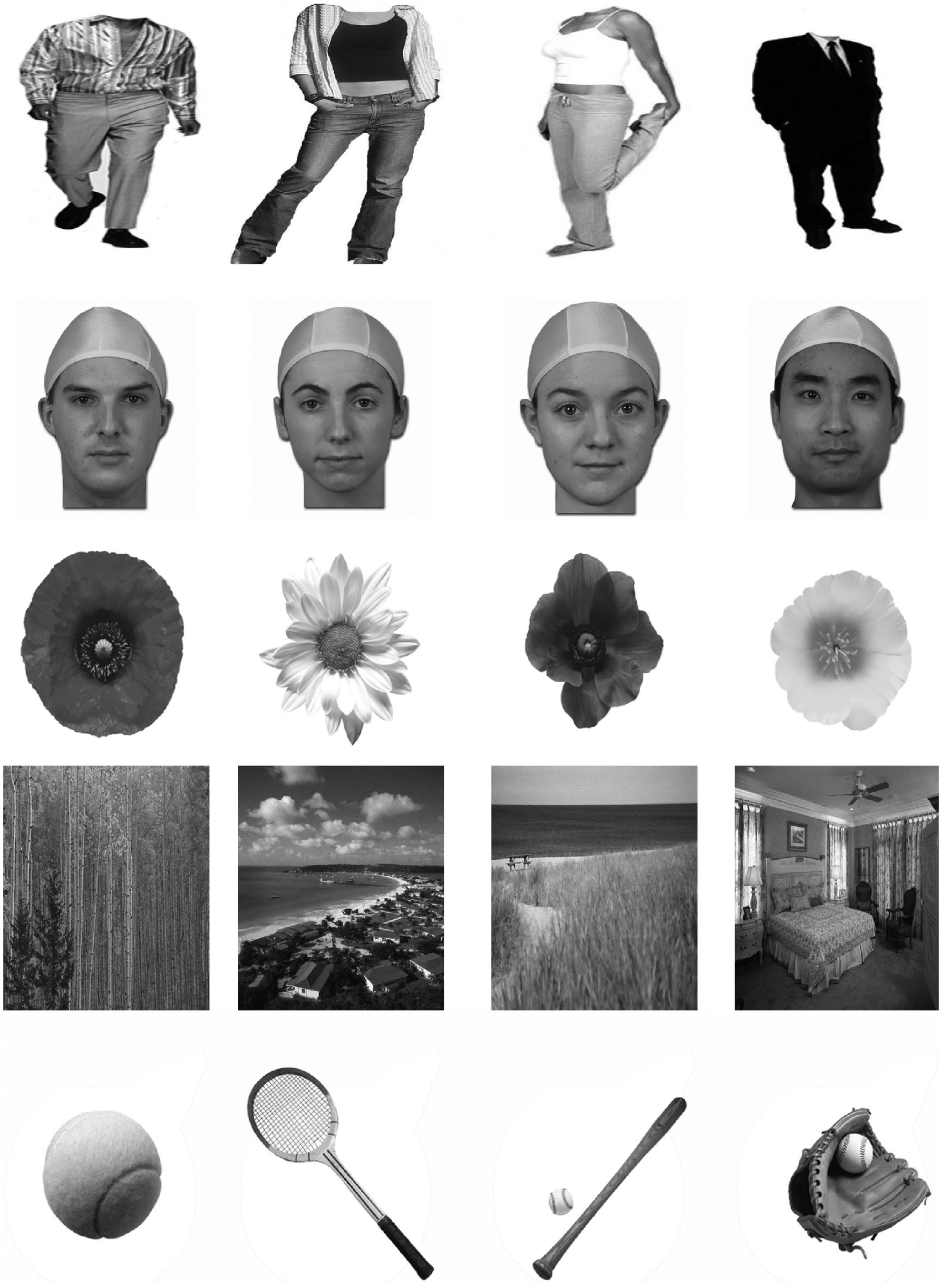

FIGURE 1 | Sample stimuli. The stimuli for this study included black-and-white images of bodies (Row 1), faces (Row 2), flowers (Row 3), places (Row 4), and sporting goods (Row 5).

to the MNI space, we were able to compare functional activation foci from both groups of participants within a common template. Kang et al. (2002) provided an empirical validation of normalization for analysis of fMRI data from children. They found very small differences (relative to the resolution of fMRI data) in the spatial correspondence among several brain loci between young children and adults after warping child and adult fMRI data into a common space. These and other similar findings (Burgund et al., 2002) gave us confidence in the feasibility of directly comparing data from adults and children in a common, adult space.

In order to localize the three functionally defined regions that were of primary interest in this study (the FFA, PPA, and EBA), random-effects assessments of the differences between key pairs of conditions at the expected peak of the hemodynamic response (HDR) were performed. These analyses consisted of the following steps: (1) The epochs of image volumes beginning one image before (–2.0 s) and

11 images after (22 s) the onset of each block containing pictures of a particular stimulus category of interest were extracted from the continuous time series of volumes and averaged together by stimulus type. (2) The average intensity of the HDR within its expected peak (6–16-s post stimulus onset) was computed. (3) A t-statistic was then computed at each voxel within the brain to quantify the HDR differences between sets of two conditions of interest (i.e., FFA = faces versus flowers; EBA = bodies versus flowers; PPA = places versus sporting goods). This process was performed separately for each subject. (4) The individual t-maps created in the preceding step were then subjected to a random-effects analysis that assessed the significance of stimulus-category response differences across subjects. This procedure was preformed separately for children and adults.

RESULTS

We organize our results first by brain region (the FFA, PPA, and EBA). Then, within each brain region, the findings are organized around a set of key questions. Specifically, by comparing the locations of activations, we evaluated whether children exhibit adult-like FFAs, PPAs, and EBAs. Then, by examining the response properties of the localized FFAs, EBAs, and PPAs, we evaluated whether these regions differed in children of various ages and in children as compared to adults with regard to their selectivity for the defining stimulus categories.

FUSIFORM FACE AREA

Do children exhibit an adult-like FFA by middle childhood? Consistent with a prior fMRI study of adults (McCarthy et al., 1997), the FFA was defined as a cluster of contiguous voxels in and around the fusiform gyri that exhibited a significantly greater response to faces than to flowers ($p < 0.05$). Flowers were chosen for this comparison because they are a category of object with a moderate degree of visual complexity, and individual species of flowers can be identified at the subordinate level by comparison of different blossoms. The locations of the adult and child FFAs are illustrated in the leftmost panel of **Figure 2**. As can be seen, in adults (red color map), a region of face-related activation was localized to the lateral fusiform gyrus in both the right (47x, –51y, –14z) and left hemispheres (–37x, –53y, –17z). In adults, the FFA was larger in the right hemisphere (175 voxels) than in the left hemisphere (45 voxels). In children (blue color map), an FFA was identified only in the right hemisphere fusiform gyrus (MNI coordinates: 41x, –44y. –17z; voxel count = 108). The centroid of the adult right-hemisphere FFA localized in this study was localized slightly more lateral (6 mm), posterior (7 mm), and inferior (3 mm) compared to the child FFA. The stereotaxic coordinates reported for the adult and child FFAs observed in this study are consistent with those from many other published studies of face perception using a blocked stimulus design (e.g., Clark et al., 1996: 37x, –55y, –10z; Haxby et al., 1994: 38x, –58y, 0z; Kanwisher et al., 1997: 40x, –55y, –10z; Clark et al., 1998: 39x, –56y, –22z; Gauthier et al., 1999: 41x, –51y, –12z; McCarthy et al., 1997: 40x, –59y, –22z; Sergent et al., 1992: 37x, –55y, –11z; average of these seven studies: 39x, –56y, –13z). Note the coordinates from the average of these seven prior studies are within the spatial extent of the current study's activations. The waveforms from the right–hemisphere child and adult FFAs are illustrated in the leftmost panels of **Figure 3**. As expected, in both groups of subjects, the response to faces was significantly greater than the response to flowers. Also note that inspection of these plots reveals that the absolute magnitude of responses to faces and flowers was quite similar for children and adults in the FFA. Likewise, the magnitude of the difference between the responses to faces and flowers was similar.

Although the FFAs identified here in adults and children exhibited a greater response to faces than to flowers [(children: $t = 2.80$, $p = 0.01$); (adults: $t = 2.88$, $p = 0.01$)], we found no significant difference between the responses to faces and bodies (presented in the same run) in these same regions in children ($t = 0.11$, $p = 0.92$) and adults ($t = 0.40$, $p = 0.70$). This is likely indicative of the presence of the putative "fusiform body area" (Peelen and Downing, 2005). This region is distinct from but adjacent to the FFA (Schwarzlose et al., 2005); however, we were not able to make this fine–grained distinction here in the absence of high-resolution fMRI data. These results do suggest the presence of a fusiform body area in children, an issue that we intend to address in future research.

Do the response properties of the FFA change over middle childhood or between middle childhood and adulthood? To evaluate this question, we calculated a face selectivity index for each participant using the following formula: $\mathrm{SI}_{\mathrm{faces}} = (\text{mean response}_{\mathrm{faces}} - \text{mean response}_{\mathrm{places}}) \times 100$. Children (mean $\mathrm{SI}_{\mathrm{faces}} = 0.14\%$; SE = 0.05%) and adults (mean $\mathrm{SI}_{\mathrm{faces}} = 0.20\%$; SE = 0.07%) did not significantly differ in the average selectivity of their right-hemisphere FFAs ($F = 0.43$, $p = 0.52$). Furthermore, no correlation was found between the selectivity of the right FFA for faces and age in children ($r = 0.10$, $p = 0.65$). While we defined the FFA in children and adults by comparing responses to faces and flowers, we chose a different category (places) to calculate the selectivity index for this region. This helped us to circumvent issues of non-independence (e.g., Vul and Kanwisher, in press) in our analyses. That is, by employing a third object category that was not used to define the FFA, we were more conservative in allowing for the possibility that the response could be equivalent to faces and places. We also examined the question of differences in selectivity by calculating this index using faces and flowers. The results were essentially identical. This same strategy was used for the analyses of the EBA and PPA.

EXTRASTRIATE BODY AREA

Do children exhibit an adult-like EBA by middle childhood? The EBA was defined as a contiguous cluster of body-selective voxels (relative to pictures of faces) in the lateral occipitotemporal cortex (Downing et al., 2001). As illustrated in the middle panel of **Figure 2**, bilateral EBA activation was identified through random-effects analyses in both children and adults ($p < 0.01$). In children, the EBA was larger in the right hemisphere (45x, –66y, 9z, 712 voxels than in the left hemisphere (–39x, –67y, 9z, 251 voxels). The opposite pattern was observed in adults, wherein the left EBA (–39x, –79y, 17z, 480 voxels) was larger than the right-hemisphere EBA (39x, –83y, 15z, 240 voxels). The coordinates for the adult and child EBAs were very similar to each other and consistent with the coordinates originally reported by Downing et al. (2001): 51x, –71y, 1z and –51x, –72y, 8z, as well as other published descriptions of the EBA (e.g., Astafiev et al., 2004; –40x, –58y, 7z; –48x, –69y,

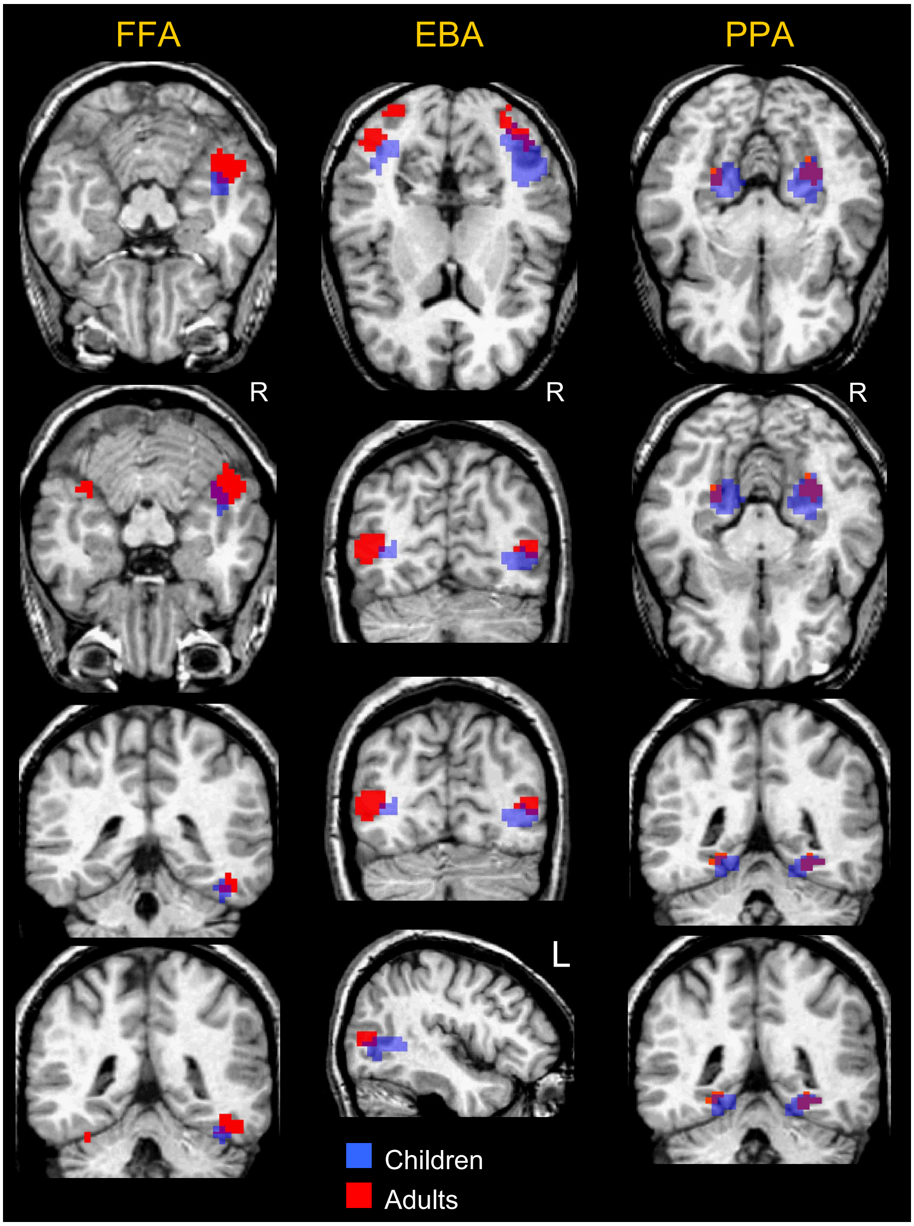

FIGURE 2 | Activation maps. The results of statistical comparisons used to identify category-selective brain regions are presented for children (in blue) and adults (in red). The first column contains images of the identified FFA. The second column includes activation maps for the EBA. The third column shows the PPA.

$-6z$; Taylor et al., 2007: $-47x$, $-71y$ and $0z$; $49x$, $-65y$, $4z$). The coordinates from these papers are well within the spatial extent of the current activations. The waveforms from the right and left child and adult EBAs are illustrated in the rightmost panels of **Figure 3**. In both groups of subjects, the response to pictures of bodies was significantly greater than the response to faces or flowers.

Do the response properties of the EBA change over middle childhood or between middle childhood and adulthood? We calculated a body selectivity index for each participant using the following formula: $\mathrm{SI}_{\mathrm{bodies}} = (\text{mean response}_{\mathrm{bodies}} - \text{mean response}_{\mathrm{flowers}}) \times 100$. Children (mean $\mathrm{SI}_{\mathrm{bodies}} = 0.30\%$; $\mathrm{SE} = 0.06\%$) and adults (mean $\mathrm{SI}_{\mathrm{bodies}} = 0.31\%$; $\mathrm{SE} = 0.11\%$) did not differ in the average selectivity of their right-hemisphere EBAs ($F = 0.04$, $p = 0.85$). Likewise, children (mean $\mathrm{SI}_{\mathrm{bodies}} = 0.24\%$; $\mathrm{SE} = 0.06\%$) and adults (mean $\mathrm{SI}_{\mathrm{bodies}} = 0.28\%$; $\mathrm{SE} = 0.05\%$) did not differ in the average selectivity of their left-hemisphere EBAs ($F = 0.23$, $p = 0.64$). Furthermore, no correlation was found between the selectivity of the right ($r = -0.21$, $p = 0.35$) or left ($r = -0.24$, $p = 0.29$) EBA for bodies and age in children.

PARAHIPPOCAMPAL PLACE AREA

Do children exhibit an adult-like PPA by middle childhood? The PPA was defined as a cluster of contiguous voxels in and around the parahippocampal gyri that exhibited a significantly greater response to pictures of places than to pictures of sporting goods

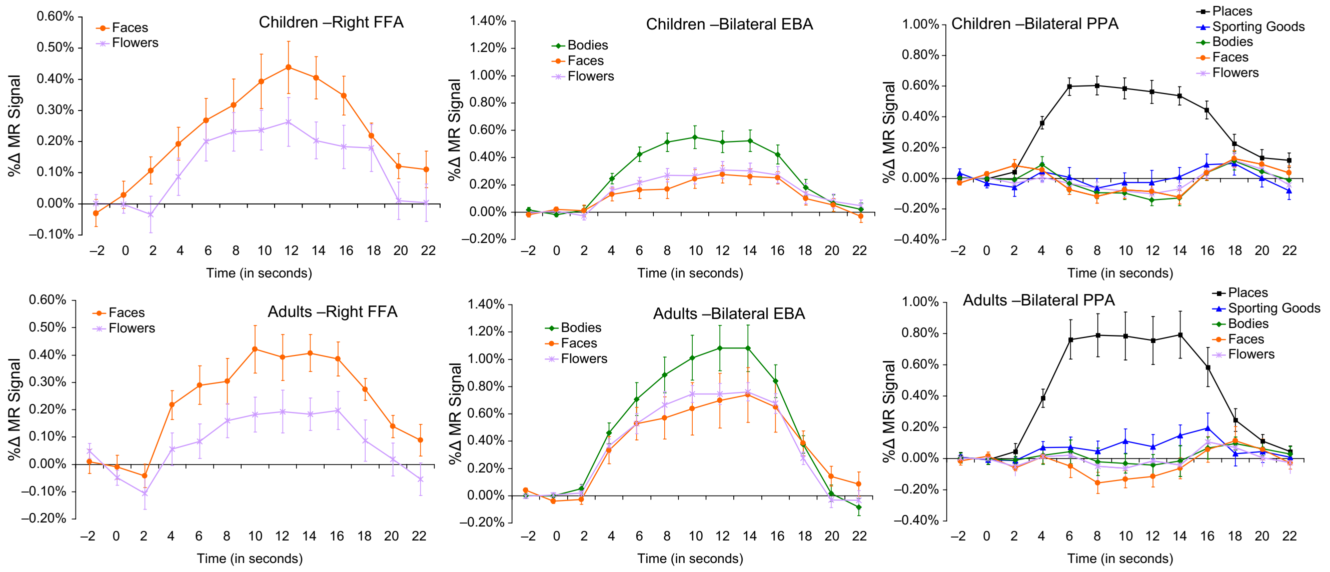

FIGURE 3 | Waveforms. In the first column are the HDR waveforms from the identified FFA in children (top) and adults (bottom) for faces (orange squares) versus flowers (purple Xs). The second column contains waveforms from the EBA for children (top) and adults (bottom) for bodies (green diamonds) relative to faces (orange squares) and flowers (purple Xs). Waveforms from the PPA in children (top) and adults (bottom) are presented in the third column, with data from places (black rectangles) presented relative to sporting goods (blue triangles), bodies (green diamonds), faces (orange squares), and flowers (purple Xs).

($p < 0.0001$). Our analyses revealed a place-selective region in the bilateral parahippocampal gyri of adults and children. The locations of the adult and child PPAs are illustrated in the rightmost panel of **Figure 2**. As can be seen, in children and adults, a region of place-selective activation was localized to the parahippocampal gyrus in both the right and left hemispheres. In both children and adults, the PPA was larger in the right hemisphere (children: 25x, –43y, –10z, 516 voxels; adults: 28x, –47y, –3z, 104 voxels) than in the left hemisphere (children: –19x, –43y, –5z, 427 voxels; adults: –25x, –49y, –4z, 40 voxels). The coordinates for the adult and child PPAs were very similar to each other and to previously reported coordinates from studies of adults (e.g., Epstein et al., 2003: experiment 1 = 28x, –46y, –10z; –26x, –48y, –9z; experiment 2 = 29x, –43y, –11z; –28x, –47y, –10z). The waveforms from the right and left child and adult PPAs are illustrated in the rightmost panels of **Figure 3**. In both groups of subjects, the response to pictures of places was significantly greater than the response to any of the other categories of stimuli viewed by the participants.

Do the response properties of the PPA change over middle childhood or between middle childhood and adulthood? Here, we calculated a place selectivity index for each participant using the following formula: $\mathrm{SI}_{\mathrm{places}} = (\text{mean response}_{\mathrm{places}} - \text{mean response}_{\text{sporting goods}}) \times 100$. Children (mean $\mathrm{SI}_{\mathrm{places}} = 0.57\%$; SE = 0.06%) and adults (mean $\mathrm{SI}_{\mathrm{places}} = 0.67\%$; SE = 0.07%) did not differ in the average selectivity of their right-hemisphere PPAs ($F = 1.00$, $p = 0.33$). Likewise, children (mean $\mathrm{SI}_{\mathrm{places}} = 0.54\%$; SE = 0.06%) and adults (mean $\mathrm{SI}_{\mathrm{places}} = 0.59\%$; SE = 0.10%) did not differ in the average selectivity of their left-hemisphere PPAs ($F = 0.30$, $p = 0.59$). Furthermore, no correlation was found between the selectivity for places of the right ($r = –0.17$, $p = 0.44$) or left ($r = –0.28$, $p = 0.21$) PPA and age in children.

DISCUSSION

In this study, we sought to clarify prior, sometimes conflicting, findings regarding the presence or absence of an FFA and a PPA in school-aged children. Additionally, we sought to provide a first investigation of the EBA in children. To do this, we conducted an fMRI study to examine the developmental trajectories of these three social and nonsocial category-selective extrastriate visual regions in terms of their localization and specificity of responses in 7- to 11-year-old children. We also studied a sample of adults in order to provide an anchoring assessment of the mature state of these category-selective regions.

We identified a face-selective region of the right fusiform gyrus in 7- to 11-year-old children and adults. The right FFA was localized similarly in adults and children and the selectivity of this region did not vary as a function of age. These findings contrast with two prior fMRI studies that failed to identify the FFA in a group of children within this age range (Aylward et al., 2005; Scherf et al., 2007), but are consistent with three other studies that have demonstrated the existence of an FFA in school-age children (e.g., Passarotti et al., 2003; Gathers et al., 2004; Golarai et al., 2007). A prior report described a potential shift in the localization of the FFA in children versus adolescents and adults. Scherf et al. (2007) reported that children exhibited a more posterior and lateral face-sensitive region of the right fusiform gyrus; however, with a more generous contrast, they instead reported two different regions that were more dorsal and medial to the adult FFA. In contrast, we found that the FFA in children was slightly more anterior and medial to the adult FFA. It is difficult to evaluate the importance of the reported shifts in localization. In both fMRI studies, normalization of the fMRI data was used to allow direct comparisons of participants of various ages. However, different normalization algorithms were employed between the two studies. Within either study, the differences in localization could be attributable to the normalization process. Further research is necessary to determine whether these reported shifts are indeed meaningful.

It does appear potentially important that the child FFA was only found in the right hemisphere, whereas the adult participants displayed an FFA in both hemispheres. Similar right-lateralization of the FFA in children followed by bilateral adult activity has been reported by other researchers (Gathers et al., 2004; Aylward et al., 2005). Future research should address the behavioral consequences of the emergence of a left FFA during childhood. Behavioral studies have reported a variety of developmental changes in different aspects of face processing across the 7- to 11-year period, including a shift from more feature-based to configuration-based strategies (e.g., Carey and Diamond, 1977; Diamond and Carey, 1977; Carey, 1992), improvements in emotion discrimination (Camras and Allison, 1985; Gnepp, 1989; Felleman et al., 1993), the emergence of an inversion effect (e.g., Carey and Diamond, 1977; Carey, 1992), and improvements in recognition abilities (Baenninger, 1994; Chung and Thomson, 1995; Brace et al., 2001; Taylor et al., 2004; for review, see Passarotti et al., 2007). It could be that the neurofunctional change observed here would be related to these behavioral changes.

Notably, we did not observe any change in the response properties (defined here as the specificity of the response for the preferred stimulus class compared to another category of stimuli) of the right FFA across this age range. This finding supports the conclusion that the right FFA is already “adult-like” by age 7 years in terms of differentiating faces and objects. This finding is consistent with the results of comparisons of functional characteristics of the FFA in 9- to 11-year-old children reported by Gathers et al. (2004). However, this finding differs from the findings of Aylward et al. (2005) that suggested that children lack a face-selective region in the fusiform gyrus.

This study is the first to localize the EBA in children. This region was identified bilaterally with very similar coordinates in children and adults. In both groups, the coordinates were similar to those reported in prior studies of adults (Downing et al., 2001; Astafiev et al., 2004; Taylor et al., 2007). The selectivity for images of bodies did not differ between children and adults, and there was no correlation between age and specificity in the children. These findings demonstrate, for the first time, the presence of the EBA in children. Furthermore, they illustrate that the EBA is already adult-like in terms of localization and specificity by 7 years of age.

The PPA was localized in both children and adults in the bilateral parahippocampal gyri, consistent with previous reports (Passarotti et al., 2003; Golarai et al., 2007; Scherf et al., 2007). Moreover, the coordinates for the regions identified in the two groups were similar to each other and to previous reports (e.g., Epstein and Kanwisher, 1998). Notably, we found that adults and children showed more place-sensitive voxels in the right hemisphere than in the left hemisphere, in accord with previous adult findings (Epstein and Kanwisher, 1998; Passarotti et al., 2003). This similarity contrasts with the findings reported by Scherf et al. (2007) that suggested that young children showed bilateral activity, older children illustrated right-lateralized activity, and adults demonstrated more evenly bilateral activation patterns again, although they noted that there was a significantly larger proportion of active voxels in the right than the left hemisphere in all age groups. As with the EBA, no age differences were found in selectivity of this area, in contrast with other findings (Golarai et al., 2007) that suggested that children had lower place selectivity than adults in the left hemisphere.

While we believe that this study provides a number of important findings to the growing literature on the development of category-selective regions in the human brain, the study suffers from a number of limitations that should be addressed in future work. For example, scanning younger children would likely be more informative with regard to informing competing theories of neurofunctional brain development. Moreover, our design was cross-sectional. A longitudinal design would have allowed us to examine the shape of developmental trajectories of brain functioning in the different, category-selective regions, and this would have provided more detail on the nature of differences in development pathways among these brain regions than did comparisons of different children at various ages and comparisons between children and adults. Lastly, future work should include more categories of images. In particular, the addition of classes of stimuli such as letters and numbers potentially could result in longer and/or later developmental trajectories owing to later learning and familiarity with such stimuli during childhood.

Along with these potentially later-developing categories, it would be interesting to explore in greater detail some visual categories that draw upon multiple brain regions for their processing. For example, examinations of complex facial stimuli, possibly displaying emotions, motion, or different forms of eye contact, could draw upon the FFA, the posterior superior temporal sulcus region (STS), and the amygdala. Currently, it is known that the STS has relatively mature functioning for some tasks during middle childhood, such as assessing the intentionality of eye movements (Mosconi et al., 2005), but it is still developing for other processes, including the perception of biological motion (Carter and Pelphrey, 2006). This could signify that different networks of regions that include the posterior STS are developing at different rates owing to different interactions, resulting in varying degrees of tuning for stimuli.

Our findings raise important questions about differences in the developmental trajectories of the PPA, EBA, and FFA. In particular, we and others have found that the PPA is the most adult-like at a young age out of the three regions examined. Why should this be so? We hypothesize that this difference in developmental trajectories can be explained by the possibility of fewer regions involved in place processing more generally, resulting in fewer interactions that need to develop and relatively complete development early in ontogeny, along with less potential for complexity inherent in these stimuli relative to faces and bodies. Faces and bodies are social entities that not only can be viewed from many different angles, but can convey identity through simple features, motion patterns, and feature configurations. They can also illustrate various emotions and other conditions without changing their identities. Moreover, all of these factors can and do change rapidly, and small nuances are important in social interactions. Also, whereas a viewer can change his or her location relative to a place and therefore change his or her viewpoint, it is highly unlikely that the location will change relative to him while he stays completely still. Individuals do not move quickly relative to places that they are viewing, resulting in lesser demands on processing speed as compared to watching other individuals who move unexpectedly and with great speed. Viewers are not in control of their perspectives on the faces and bodies of others, as other people are separate agents. All of these factors might increase the perceptual complexity of social stimuli like faces and bodies and the amount of attention that needs to be allocated to these categories of stimuli for proper processing.

Finally, our findings might offer important implications for understanding the etiology of social deficits in autism spectrum disorders. Previous reports have identified abnormalities in the localization and specificity of the FFA in adults with autism spectrum disorders (e.g., Pierce et al., 2001; Hubl et al., 2003; Schultz et al., 2003; Ashwin et al., 2007; Pelphrey et al., 2007). Understanding the normative developmental pathway and mechanisms by which this region typically develops could provide invaluable data for understanding the causes of FFA dysfunction in individuals with autism spectrum disorders. This information could also inform our understanding of treatments for social perception deficits in autism with the goal of normalizing developmental pathways or supporting alternative, compensatory mechanisms.

In conclusion, we have identified face-, body-, and place-sensitive areas of the cortex in school-aged children that are somewhat, but not completely, similar to those of adults. Importantly, it further elucidates the developmental trajectories for these category-specific regions. This work also has import for research on autism spectrum disorders. Future work should study these regions longitudinally, along with other category-selective regions identified in adults.

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Differential development of the ventral visual cortex extends through adolescence

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The ventral temporal cortex (VTC) in humans includes functionally defined regions that preferentially respond to objects, faces, and places. Recent developmental studies suggest that the face selective region in the fusiform gyrus ('fusiform face area', FFA) undergoes a prolonged development involving substantial increases in its volume after 7 years of age. However, the endpoint of this development is not known. Here we used functional magnetic resonance imaging (fMRI) to examine the development of face-, object- and place selective regions in the VTC of adolescents (12–16 year olds) and adults (18–40 year olds). We found that the volume of face selective activations in the right fusiform gyrus was substantially larger in adults than in adolescents, and was positively correlated with age. This development was associated with higher response amplitudes and selectivity for faces in face selective regions of VTC and increased differentiation of the distributed response patterns to faces versus non-face stimuli across the entire VTC. Furthermore, right FFA size was positively correlated with face recognition memory performance, but not with recognition memory of objects or places. In contrast, the volume of object- and place selective cortical regions or their response amplitudes did not change across these age groups. Thus, we found a striking and prolonged development of face selectivity across the VTC during adolescence that was specifically associated with proficiency in face recognition memory. These findings have important implications for theories of development and functional specialization in VTC.

Keywords: fusiform face area, parahippocampal place area, ventral temporal cortex, development, adolescent, gender, face processing

INTRODUCTION

Functional magnetic resonance imaging (fMRI) studies of the ventral temporal cortex (VTC) have revealed a consistent functional organization in adult humans. One aspect of this organization is characterized by regions that respond more strongly to some types of visual stimuli than to others. These include face selective regions which respond more to faces than to other objects or scenes, including a region in the fusiform gyrus referred to as the fusiform face area (FFA, Kanwisher et al., 1997) which is implicated in face recognition (Tong et al., 1998; Golby et al., 2001; Grill-Spector et al., 2004; Ranganath et al., 2004; Nichols et al., 2006). Other regions respond more strongly to objects than to scrambled images of objects (Malach et al., 1995), including an object selective region along the posterior fusiform gyrus and occipito-temporal sulcus (pFus/OTS, Vinberg and Grill-Spector, 2008). Place selective regions respond more strongly to scenes and buildings than objects and faces, including a region along collateral sulcus and the parahippocampal gyrus known as the parahippocampal place area (PPA, Epstein and Kanwisher, 1998). Category-selectivity also manifests as distinctly distributed responses to specific categories across the entire VTC (Haxby et al., 2001), including voxels with weak or no selectivity. Indeed, faces, objects and places each evoke reproducible and distinct patterns of distributed responses across the VTC (Haxby et al., 2001; Cox and Savoy, 2003; Grill-Spector et al., 2006). These observations in adults have generated much interest in the development of the functional organization of VTC and the role of experience in shaping it (Kanwisher, 2000; Tarr and Gauthier, 2000).

Recent studies indicate that some category selective regions in the VTC undergo a prolonged development that continues at least through the first decade of life (Gathers et al., 2004; Aylward et al., 2005; Golarai et al., 2007; Scherf et al., 2007; Peelen et al., 2009). Accumulating evidence suggests that the volume of the right FFA (rFFA) is substantially larger in adults compared to children, but not the overlapping object selective (lateral occipital complex) region (Golarai et al., 2007; Scherf et al., 2007) or the body-part selective extra-striate region (Peelen et al., 2009; Pelphrey et al., 2009)]. rFFA volume also correlates with behavioral improvements in recognition memory for faces (Golarai et al., 2007). However, the endpoint of this development is not known, as previous fMRI studies report inconclusive results. For example, one study reported no difference between adolescents' (ages 11–14 years) and adults' FFA volume based on group analyses (Scherf et al., 2007). Another study reported larger FFA size in adults compared to the combined data from both children and adolescents (Peelen et al., 2009). A third study found that adolescents' FFA size was at an intermediate level between children and adults, but these between group differences did not reach statistical significance (Golarai et al., 2007). Nevertheless, behavioral studies suggest a prolonged development of face recognition memory proficiency, consistently reporting substantial improvements in face recognition memory during adolescence (Diamond and Carey, 1977, 1986; Carey et al., 1980; Golarai et al., 2007). Thus, one hypothesis is that VTC's activation to faces undergoes a particularly prolonged development that continues into adolescence, which may have been previously undetected, perhaps due to methodological limitations. Alternatively, the entire VTC may undergo a prolonged development during adolescence, involving developmental changes in activations to faces, objects and places. A third hypothesis is that the VTC reaches an adult-like state before adolescence, and the face selective improvements in face recognition memory performance are due to general mnemonic and/or other cognitive developments that occur outside the VTC. To test these alternative hypotheses, we used fMRI in 14 adolescents (ages 12–16 years) and 11 adults (18–40 years) and examined each subject's VTC responses to faces, places and object stimuli, as well as their recognition memory for images from these categories.

We first asked how developmental changes during adolescence might manifest in the fMRI measurements. We considered three possibilities that are not mutually exclusive. First, adolescent development may involve age-related increases in the volume of face selective activations in the VTC, analogous to previous findings of larger FFA in adults compared to children (Aylward et al., 2005; Golarai et al., 2007; Scherf et al., 2007; Peelen et al., 2009). Second, development might manifest as age-related changes in the magnitude or selectivity of responses to specific stimuli in functionally defined regions, as previously reported in the developing FFA (Scherf et al., 2007; Peelen et al., 2009). Third, development may change the distributed response patterns to stimulus categories across the entire VTC, affecting the within-category reproducibility of distributed responses and/or the between-category distinctness. To our knowledge no previous study has examined the development of these distributed response patterns in the VTC of children or adolescents. Finding any one or a combination of these changes only in the response profile to faces would support the first hypothesis, positing a particularly prolonged development of the neural mechanisms of face processing in the VTC. In contrast, finding uniform developmental changes across the entire VTC to faces, places and objects would be consistent with the alternative hypothesis of a general development of the VTC during adolescence. Finally, finding no age-related changes in VTC would support the third hypothesis that the face selective behavioral improvements in face recognition memory in adolescence are mediated by the development of brain regions outside the VTC.

We also asked if developmental changes in VTC responses to faces depend on the age of face stimuli. Some behavioral studies suggest better face recognition memory performance when observers view faces of their own- versus other-age groups (Anastasi and Rhodes, 2005; Perfect and Moon, 2005), leading to the possibility that a similar own-age advantage may be evident in brain responses. Thus, VTC responses to faces may be stronger in adults when viewing adult faces than when viewing faces of children and adolescents, while the opposite may be found in adolescents. Such findings would also suggest that previous reports of smaller FFA volume during development may be explained by the presentation of adult faces to subjects in those studies, predicting relatively similar face responses in the VTC of adolescents and adults if the age of observer and face stimuli were similar.

Finally, we asked if developmental changes in face selective responses during adolescence specifically relate to improvements in face recognition memory performance or instead reflect general improvements in recognition memory for non-face stimuli or other cognitive abilities. Therefore, in the same subjects, we measured visual recognition memory performance for faces, places and objects and used a battery of behavioral tests to evaluate each participant's performance on several perceptual and cognitive tasks.

To address these questions, we performed fMRI while adolescents and adults viewed blocks of images of children's faces, adults' faces, abstract sculptures, cars, indoor and outdoor scenes and scrambled images that appeared in pseudo-random order. Subjects were instructed to fixate on a central crosshair and indicate if two consecutive images were identical (1-back task). Outside the scanner, each subject also participated in a battery of behavioral tests including (i) a recognition memory test for faces, objects and scenes; (ii) a perceptual discrimination test for face identity (Benton et al., 1978); (iii) a facial affect recognition test (*NEPSY-II*); and (iv) an abbreviated IQ test (*WASI*). For each participant, we functionally defined their face-, object- and place selective regions in the VTC to examine the spatial extent of these regions, and determined the amplitude and selectivity of their responses to visual stimuli from an independent data set. We also measured the distributed multivoxel patterns (MVP) of responses across the entire VTC to face and non-face stimuli. We quantified the within-category reproducibility and across-category distinctness of MVPs to determine if there are between age group differences that extend beyond functionally defined ROIs. We related these measures to the age of subject and age of face stimuli. Importantly, we controlled for non-specific face selective confounds that may affect fMRI measurements (Grill-Spector et al., 2008), such as greater motion, lower performance during scan, or less variance explained by the general linear model in adolescents versus adults. Finally, we related our fMRI results to behavioral measures in the same subjects.

MATERIALS AND METHODS

SUBJECTS

Fourteen adolescents ages 12–16 years (7 females), and 11 adults ages 18–40 years (6 females) participated in these experiments. Subjects had normal or corrected vision with no past or current neurological or psychiatric conditions. Adolescents were recruited from the Palo Alto school districts through advertisements in school newspapers. Adult subjects were university affiliates. Informed consent was obtained according to the requirements of the Panel on Human Subjects in Medical Research at Stanford University.

Adolescents were invited to a practice session to exercise motion control in a simulated scanner environment. All subjects were acclimated to the scanning environment for fMRI by participating on a previous day in an anatomical scanning session.

SCANNING

Brain imaging was performed on a 3 Tesla whole-body General Electric Signa MRI scanner (General Electric, Milwaukee, WI, USA) at the Lucas Imaging Center at Stanford University.

STRUCTURAL MRI

We acquired four whole brain anatomical scans (total scan time ~15 min; high-resolution 3D Fast SPGR: 166 sagittal slices, 0.938 mm × 0.938 mm, 1.5 mm slice thickness, 256 × 256 image matrix) using a birdcage headcoil.

FUNCTIONAL MRI

We acquired functional images using a surface coil and a T2*-sensitive gradient echo spiral pulse across 32 slices oriented perpendicular to the calcarine sulcus and extending from the occipital pole to the anterior temporal lobe (TR = 2000 ms, TE = 30 ms, flip angle = 76°, field of view = 200 mm, 3.125 mm × 3.125 mm in-plane resolution). Applying the same slice prescription, we acquired anatomical T1-weighted images used to co-register each subject's functional data to the subject's whole brain anatomy.

STIMULI

Images were projected onto a mirror mounted on the MRI coil (visual angle ~ 15°). Images were presented and responses were recorded via a Macintosh MacBook Pro computer using Matlab (Mathworks, Natick, MA, USA) and Psychtoolbox extensions[1].

EXPERIMENT 1: fMRI

During fMRI, subjects viewed gray-scale images of the following types: faces of male children and adolescents (ages 6–16 years), faces of male adults (ages 18–40 years), abstract sculptures, cars, indoor scenes, outdoor scenes, and scrambled images (created by randomly scrambling pictures into 225, 8 × 8 pixel squares). We exclusively used male faces in order to reduce potential gender effects, as some behavioral studies suggest that females' advantage in face recognition memory performance over male subjects is mostly explained by their better recognition memory for female faces than male faces (Lewin and Herlitz, 2002). The exclusive use of male faces also enabled us to relate present results to our previous findings (Golarai et al., 2007). We used a range of ages for each type of face stimulus (as opposed to matching the age stimuli across subjects, which would introduce between-subject stimulus effects, and potentially increase between-subject variability in fMRI results). Furthermore, we reasoned that if face responses reflect years of prior experience with faces, this prior experience might be best reflected in adolescents' responses to faces of younger children. Thus, boy faces consisted of both children and adolescents, while man faces consisted of adult faces only [albeit ranging across 22 years (18–40)] and matched the age range of the adult participants. Faces of boys and men were presented in separate blocks in order to test for interactions between age of stimuli and age of subjects. All face images were collected from advertising web sites for models, and were matched for distinctiveness and attractiveness by four adult observers.

Cars were of various modern makes, mostly in 3/4 frontal views. We use cars as common objects, with which both children and adult subjects had extensive experience, and have nameable parts that share a typical first order configuration. We used abstract objects as they are relatively novel stimuli with which neither age group has extensive prior experience, and enable us to relate the present study to our previous report (Golarai et al., 2007).

Indoor scenes of buildings were mostly of empty rooms and corridors, devoid of furniture. Outdoor scenes were of natural settings that were (with a few exceptions) devoid of buildings. All scenes were devoid of people, animals or salient objects.

Stimuli were presented in 12 s blocks followed by 12 s of a blank screen with a fixation at a rate of 1 Hz. Subjects participated in two 396-s runs with different images. Images were presented once, except for image pairs that were randomly repeated within a block (~17% of images). Subjects were instructed to fixate and press a button using their right thumb, whenever they detected identical images appearing successively (referred to as a 1-back task), which occurred on ~17% of the images per block. We report subjects' accuracy during performance of the 1-back task (corrected for guessing) using the following formula:

$$\text{Accuracy }[\%] = 100 \times \frac{\text{Hit Rate} - \text{False Alarm Rate}}{1 - \text{False Alarm Rate}}.$$

Behavioral response during scans

There were no significant between age group differences across categories in performance accuracy for the 1-back task ($F_{1,20} = 0.05$, $P = 0.82$). There were significant effects of category between age groups ($F_{6,120} = 4.3$, $P = 0.001$), but there was no significant interaction between age group and category ($F_{6,120} = 1.7$, $P = 0.13$, **Figure 1A**). Adolescents' response times on hit trials were significantly slower than adults' ($F_{1,20} = 3.2$, $P = 0.05$, one-tailed), but there was no age group by category interaction ($F_{6,120} = 0.31$, $P = 0.93$, **Figure 1B**).

Data analysis

All imaging data were analyzed using MATLAB and our in-house software, *mrVista*[2].

Structural MRI

High-resolution anatomical whole brain images from each subject from four scans were averaged into one volume. Using an in-house version of ITK-SNAP[2], white and gray matter were segmented. For each subject, the cortical surface was grown to include 4 mm of gray matter, creating a uniform whole brain gray matter thickness in all subjects. The total volume of this gray matter in the right hemisphere was significantly higher among adolescents than in adults (**Figure 2E**, right: $t_{23} = 2.16$, $P = 0.04$, left: $t_{23} = 1.62$, $P = 0.13$), suggesting a higher surface area in adolescents, which is consistent with several reports of gray matter decrease during this period of development (Sowell et al., 2002), especially in the prefrontal cortex. In contrast, the gray matter volume in an anatomical ROI of the VTC was similar among age groups (*Ps* > 0.6, **Figure 2D**, see below).

Preprocessing

In-plane anatomical images were aligned to the high-resolution 3D whole brain images using a rigid body transformation, and the same transformation was applied to the functional images.

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[1] www.psychtoolbox.org

[2] white.stanford.edu/software

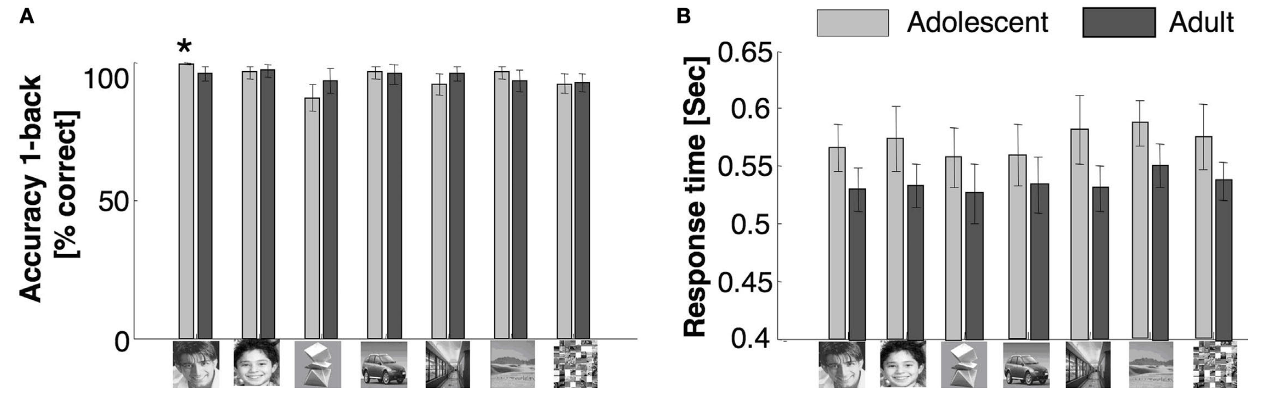

FIGURE 1 | Behavioral data during scan. (A) Accuracy and **(B)** response times in a 1-back task during the scan. Light gray: adolescents ages 12–16 years; Black: adults ages 18–40 years. Error bars show standard error of mean (SEM) for each age group. Asterisk indicates significantly higher accuracy in adolescents than adults ($P < 0.05$, *t*-test).

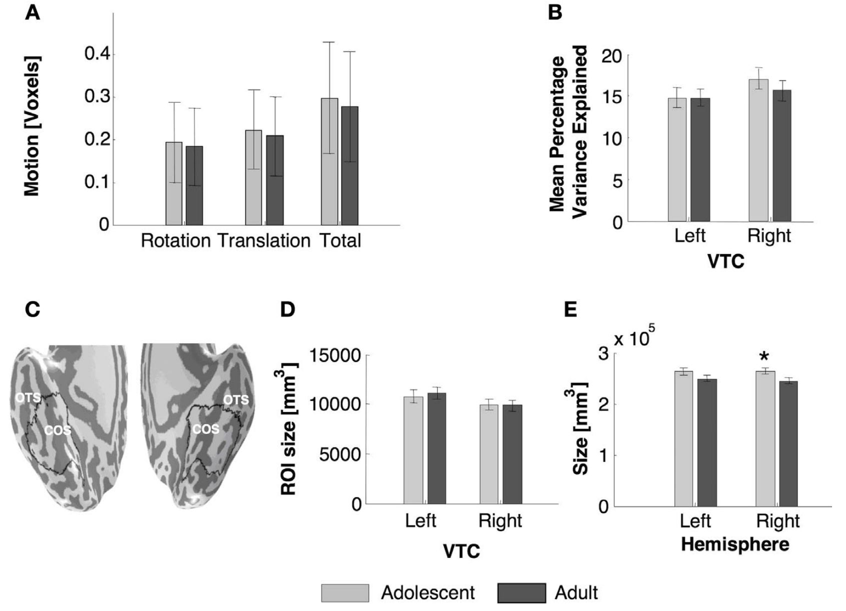

FIGURE 2 | Measurements of BOLD-related confounds and anatomical size of VTC and total hemispheric gray matter across age groups. (A) Motion during scan for each age group. **(B)** Average variance explained by the GLM model in the anatomical ROI of ventral temporal cortex (VTC) as shown in **(C)**. The variance explained was measured for each voxel and then averaged across the ROI and across subjects. **(C)** Representative VTC ROI in an adolescent, OTS: occipito-temporal sulcus; CoS: collateral sulcus. **(D)** Average volume of the VTC in each age group. **(E)** Average volume of the total gray matter in each hemisphere for each age group. All bars represent the average across each age group (11 adults and 14 adolescents), error bars show standard error of the mean (SEM) for each age group. Asterisk indicates significantly higher volume in adolescents than adults ($P < 0.05$, *t*-test).

Functional images were motion corrected and motion parameters were estimated for each participant. The participants' motion was typically less than a voxel and there was no significant difference in total within-scan motion across adults and adolescents ($t_{23} = 0.28$, $P = 0.8$, **Figure 2A**). Data were then detrended using a temporal high-pass filter with a 1/20 Hz cutoff. The time course of each voxel was converted to percent signal change by dividing the response amplitude at each TR by the mean amplitude of blanks. Data were not spatially smoothed.

General linear model

Standard general linear model (GLM) analyses were used to create voxel-by-voxel activation maps (**Figures 3, 6A and 7A**). Predictors were the stimulus conditions convolved with the hemodynamic impulse response function (HRF) used in SPM2[3]. We estimated the BOLD response amplitudes for each stimulus category by computing the beta coefficients from a GLM applied to the

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[3] http://www.fil.ion.ucl.ac.uk/spm/

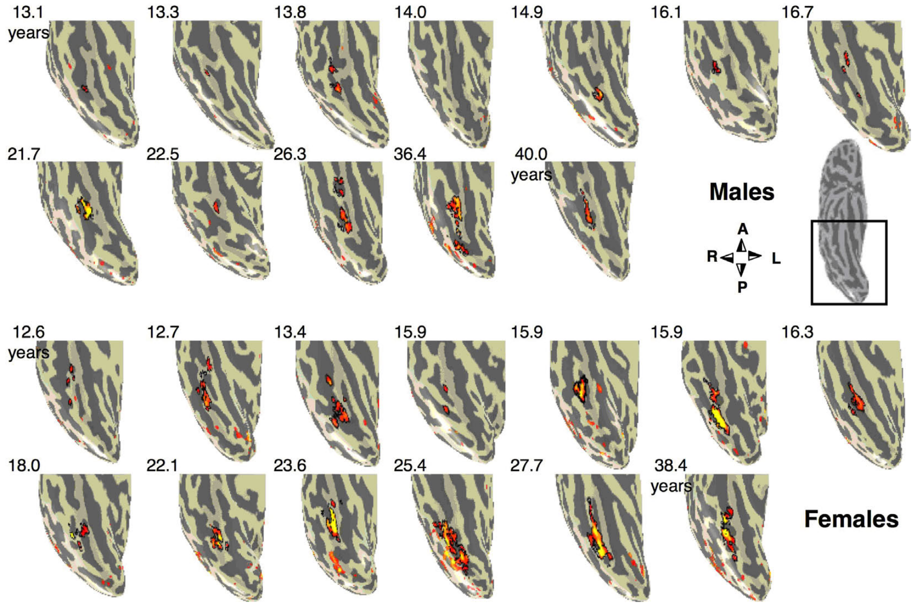

FIGURE 3 | Face selective activations in the right fusiform gyrus for each of the 25 subjects. Face selective activations for the contrast man and boy > cars and abstract objects, $P < 10^{-3}$, *uncorrected,* are projected on the inflated cortical surface of each individual from the right hemisphere for all subjects in the study. Brain images show the posterior aspect of the ventral surface of the right hemisphere as indicated by the inset in a sample brain on the right. Numbers indicate subjects' age in years. The boundaries of the FFA are shown in black. Compass orients to anterior (A), posterior (P), right (R) or left (L). Top two rows show males' and bottom rows show females' brains.

preprocessed time course. We calculated three separate GLMs: (i) GLM of data concatenated from both runs (ii) GLM of run 1 alone and (iii) GLM of run 2 alone.

ROI creation

Three types of ROIs: anatomical, functional-cluster, functional-non-cluster were created for each subject. (1) *Anatomical ROIs of the right and left VTC* were defined as a region between the lateral border of the occipito-temporal sulcus (OTS), the medial border of the parahippocampal gyrus and the anterior edge of the retinotopic regions V1 to V4. Measuring from this caudal border, the ROI extended in the anterior direction to include approximately 2/3 of the remaining extent of the temporal lobe's ventral surface. This ROI was restricted to include only regions with substantial mean BOLD signals (raw scanner values > 1000) in order to exclude regions of signal drop off due to susceptibility artifacts behind the ear canal (**Figure 2C**). Anatomical ROIs were created by GG and evaluated by two other observers (KGS, AL). There were no between age group differences in the volume of the right or left VTC (*Ps* > 0.7, **Figure 2D**). The size of the left VTC was larger among males than females (left VTC: $F_{1,21} = 4.12$, $P < 0.03$ one-tailed), but there were no interactions between factors of age and gender ($P = 0.99$). (2) *Functional Cluster ROIs* were based on a GLM applied to data from run 1 and run 2 and were defined in each subject as one or more contiguous supra-threshold voxels as follows: (a) Face selective ROIs were defined along the fusiform gyrus, often overlapping with the OTS. Three separate face selective ROIs were defined using each of the following contrasts: (i) FFA: man and boy > cars and abstract objects, $P < 10^{-3}$; (ii) Man faces > cars and abstract objects, $P < 10^{-3}$; (iii) Boy faces > cars and abstract objects, $P < 10^{-3}$. Two additional ROIs were created to determine the non-overlapping regions of the latter two ROIs. (b) pFus/OTS: An object selective region overlapping the posterior fusiform gyrus and OTS was defined as cars and abstract objects > scrambled, $P < 10^{-3}$. (c) PPA: A place selective ROI overlapping the collateral sulcus and the parahippocampal gyrus was defined as indoor and outdoor scenes > cars and abstract objects, $P < 10^{-3}$. (3) *Functional Non-cluster ROIs* were based on a GLM applied to data from *run 1* and included all supra-threshold voxels (regardless of clustering) within the VTC for the contrast of interest at five statistical thresholds ($10^{-2} < P < 10^{-6}$).

Measure of BOLD-related noise and goodness of fit

We estimated the percentage variance of the time course explained by the GLM (from concatenated runs 1 and 2) in each voxel as:

$$\text{Variance Explained }[\%] = 100 \times \left[1 - \frac{\text{Variance(residual Error)}}{\text{Variance(time course)}}\right].$$

We then calculated the mean variance explained across the anatomical ROI of VTC (**Figure 2B**) in each subject. There were no between group differences in the variance explained by the GLM across age groups (**Figure 2B**) indicating that our hemodynamic model is adequate for both adult and adolescent data.

Mean response across ROIs

To provide an unbiased estimate of response amplitudes we used data from *run 1* to define the ROI and data from *run 2* to estimate response amplitudes. Using *run 1* data, we defined functional non-cluster ROIs within the VTC as all the voxels in the VTC that passed the threshold of $P < 10^{-3}$ for each of the following contrasts: (1) Man and boy > cars and abstract objects (**Figure 5**). (2) Cars and abstract objects > scrambled (**Figure 8**). (3) Indoor and outdoor > cars and abstract objects (**Figure 9**). Using *run 2* data, we calculated the percent signal change based on the beta estimates from the GLM for each condition relative to the blank baseline from the supra-threshold voxels.

Mean category selectivity across ROIs

To provide an unbiased estimate of category selectivity, we used data from *run 1* to define face-, object- and place selective functional ROIs (see above), and data from *run 2* to estimate the mean *t*-value for the relevant GLM contrast across the supra-threshold voxels (**Figure 5B**).

Multivoxel pattern (MVP) analyses

We determined the multivoxel distributed pattern (MVP) of response to each stimulus type in the anatomical ROI of VTC by calculating at each voxel the relative response amplitude to the stimulus type (i) as $Z\text{-score} = \frac{\beta_i - E(\beta)}{\sigma/\sqrt{\mathrm{df}}}$, where β_i is the beta coefficient from the GLM for the i-th stimulus, E(β) is the mean of all βs, σ is the square-root of the residual variance of the GLM and df is the degrees of freedom. No thresholding or spatial smoothing was applied. Using Z-scores minimizes between-voxel effects. That is, the MVP reflects differences in category selectivity in each voxel, rather than amplitude differences across voxels. For each stimulus type, MVPs were generated across the anatomical ROI of VTC, separately for data from run 1 and run 2 (**Figure 10A**). Each MVP was represented as a vector of length *n*, where *n* is the number of voxels in each subject's VTC. For each subject we calculated the Pearson correlation (*r*) between these vectors from run1 vs. run 2 for each pairing of categories. Within-stimulus type correlations represent the reproducibility of the MVPs between run 1 and run 2 (which used different stimuli): $r_w(\mathrm{i})$ = correlation [MVP_i*(run1)*, MVP_i*(run2)*], where MVP_i is the vector representing the *Z*-scores across VTC voxels in response to stimulus type *i*. Between-stimulus correlations were calculated in a similar manner: $r_b(\mathrm{i,j})$ = correlation [MVP_i*(run1)*, MVP_j*(run2)*], and reflect the degree of similarity between MVP of different categories. Positive values ($r_b > 0$) indicate similar MVPs, and negative values ($r_b < 0$) indicate distinct activations for different stimuli. Within- and between-stimulus type correlations were calculated for all combinations of stimuli in each subject, and averaged across subjects from each age group (**Figures 10B–D**).

Analysis of inter-subject similarity of correlation matrices

Each subject's MVP correlation matrix showing the correlations between MVPs in run 1 and 2 (as in **Figure 10B**, but for each subject) was transformed to a vector of length 36 (number of elements in the MVP correlation matrix). We then calculated the correlation between each pair of subjects' vectors. This inter-subject correlation reflects the degree of similarity between the MVP correlation matrices across subjects.

EXPERIMENT 2

Subjects participated in several behavioral tasks outside the scanner, usually on a different day from the fMRI session. The memory recognition task was most often administered within ~2 weeks of fMRI (often prior to fMRI and during the scanner simulator session). The other behavioral tests were administered after fMRI (on average 96 days later).

Recognition memory task

During encoding, subjects viewed eight images (never seen before) of the same types used in the fMRI experiment: faces of men and boys, cars and abstract sculptures, and scenes. All stimuli were gray-scale images similarly prepared as in Experiment 1 and presented in random order. Subjects were instructed to perform a one-back task while viewing the images. Subjects performed an unrelated visual task for approximately 5 min by viewing a black and white rotating wedge, while fixating on a central point and responding when the fixation point changed color. Next, during a surprise self-paced subsequent recognition memory test, subjects were presented with all the images from the encoding session plus an equal number (eight) of new images per category. Image categories and old and new pictures were randomly presented. Subjects were instructed to indicate whether or not they had seen the image before by pressing one of two buttons, as accurately and quickly as possible. In the few cases where the recognition memory task was administrated after fMRI, subjects were informed that none of the images were from the previous fMRI session. Accuracy for the subsequent recognition memory task was calculated separately per image category and subject:

$$\text{Correct }[\%] = 100 \times \frac{\text{Hit Rate} + \text{Correct Rejection Rate}}{2}.$$

Benton Facial Recognition Task (Psychological Assessment Resources, Inc.)

This test measured subjects' proficiency in perceptual recognition of facial identity and had no memory component. Subjects were presented with one target photo and six comparison photos simultaneously. We asked subjects to make a perceptual judgment based on the identity of the individual in the target photo. All stimuli were black and white photographs of adults of both genders. The task became progressively harder as the viewing angle or lighting was manipulated in the six comparison photographs. Subjects received one point for each correct match (54 total possible points). We report the percentage of total correct trials for each subject. No age or education correction was used. Adults performed significantly better than adolescents on this task ($P < 0.001$, *t*-test, **Table 1**).

NEPSY-II Affect Recognition Task (Harcourt Assessment, Inc.)

This test is designed to measure proficiency in perceptual recognition of facial affect using children's faces. Subjects were shown color photographs of child faces and asked to make a judgment based on the emotional expression of the individual in the target photo, ignoring identity. Photographs included both genders and several races. This task included both perceptual and memory components, in which subjects were asked either

Table 1 | Subject demographic information and behavioral task performance: Data are split by age group. Sample size (N) is indicated for each measure (mean ± standard deviation).

Averages	Age	IQ	Block design % correct	Matrix reasoning % correct	Affect recognition % correct	Benton % correct	Visual recognition memory % correct		
							Face	Scene	Objects
Adolescents	15 ± 2 $N = 14$	126 ± 9 $N = 11$	81 ± 18 $N = 11$	86 ± 6 $N = 11$	86 ± 7 $N = 13$	82 ± 6 $N = 13$	70 ± 10 $N = 13$	76 ± 11 $N = 13$	66 ± 14 $N = 13$
Adults	28 ± 7 $N = 11$	130 ± 8 $N = 7$	89 ± 5 $N = 7$	89 ± 9 $N = 7$	85 ± 6 $N = 9$	92 ± 3 $N = 9$	81 ± 10 $N = 10$	75 ± 11 $N = 10$	72 ± 10 $N = 10$

to match photographs based on emotion, or to remember an emotion and subsequently (~5 s later) match test images to the remembered emotion. Subjects received a point if they correctly matched all affect photos per trial (35 total possible points). The total percentage of correct trials was reported for each subject. There were no differences in performance between adults and adolescents ($P > 0.97$, **Table 1**).

Wechsler Abbreviated Scale of Intelligence (WASI, Harcourt Assessment, Inc.)

This test was used to measure IQ and compare groups. Subjects received three IQ scores (Full Scale IQ, Performance IQ, and Verbal IQ) based on four subtests: Vocabulary, Block Design, Similarities, and Matrix Reasoning. Each of the four subtests tested a different component of intelligence (i.e. verbal knowledge, spatial reasoning, etc.) and was normalized by age. Performance IQ was calculated using the Performance Scale, which was composed of Block Design and Matrix Reasoning scores. Verbal IQ was calculated using the Verbal Scale, which was composed of Vocabulary and Similarities scores. There were no differences in Full-Scale IQ ($P > 0.36$), Performance IQ ($P > 0.11$), or Verbal IQ ($P > 0.96$) between adults and adolescents.

Statistical methods for between age group comparisons

Subjects' data were averaged for each of the age groups. Between group differences were evaluated by a two-tailed ANOVA, repeated measures ANOVA and *t*-tests, unless otherwise noted.

For between group comparisons of the size of the functional or anatomically defined ROIs, we used one- or two-way ANOVAs with the factors of age and (in some cases) gender. For between group comparisons of the size of the functional ROIs, subjects who showed no activations fulfilling the definition of the particular functional ROI were assigned zero for the size of the ROI and included in the analysis. When there was a significant between group difference in the estimates of variance, we report the adjusted T and P values and indicate "non-equal". For between group comparisons of BOLD responses to the various image categories within ROIs, we used a GLM, with responses across categories as the within-participant repeated measure and report the relevant F and P values (**Figures 5A, 8B and 9B**). Where we found a significant interaction between the factors of group and stimulus type, we used subsequent *t*-tests to determine which stimulus types were significantly different between groups.

RESULTS

THE VOLUME AND RESPONSE AMPLITUDES OF THE FUSIFORM FACE AREA (FFA) IN ADOLESCENTS AND ADULTS

To examine the hypothesis that the FFA continues to develop during adolescence, we measured the volume of the FFA across adults and adolescents (see Materials and Methods). We defined the FFA in each subject as clusters of voxels within the fusiform gyrus (sometimes overlapping the OTS) that responded more to faces than to objects (man and boy > cars and abstract objects, $P < 10^{-3}$, uncorrected). We found the FFA in 11/11 adults and 14/15 adolescents (see **Figure 3**). The right FFA's volume was significantly larger in adults than in adolescents by about 1.7 fold ($t_{15} = 2.47$, $P = 0.028$, non-equal variance; **Figure 4A**) and was correlated with subjects' age ($r = 0.55$, $P = 0.004$, **Figure 4B**). There was a similar trend in the left FFA (lFFA), but between age group differences were not significant ($t_{23} = 1.28$, $P = 0.21$, **Figure 4A**). Nevertheless, there was a significant correlation between lFFA's volume and age ($r = 0.41$, $P = 0.04$, **Figure 4B**).

We asked if age-related changes in FFA volume varied with subjects' gender. The rFFA volume was significantly larger in females than males in both age groups (rFFA: $t_{19} = 2.78$, $P = 0.01$, unequal variance). Thus, rFFA volume was determined by each of the factors of gender ($F_{1,24} = 8.74$, $P = 0.008$) and age (rFFA: $F_{1,24} = 7.65$, $P = 0.012$), but there was no significant age by gender interaction ($P = 0.65$). Furthermore, rFFA volume was significantly correlated with age within each of the gender groups (in females: $r = 0.69$, $P = 0.009$; in males: $r = 0.76$, $P = 0.004$, **Figure 4B**). The lFFA was also larger among females than males (lFFA: $t_{18} = 1.78$, $P = 0.04$, one-tailed *t*-test, unequal variance). However, there were no significant effects of age ($P = 0.23$) or interaction between age and gender ($P = 0.9$). Although the correlation between lFFA volume and age was significant when all subjects were included, this correlation did not reach significance in either gender when examined separately ($Ps > 0.08$, **Figure 4B**). Thus, the spatial extent of the rFFA increased with age from adolescence to adulthood in both genders.

Next, we tested if adolescents' smaller rFFA reflected less clustering of face selective voxels, or depended on threshold. We measured the total volume of face selective activations regardless of voxel contiguity at five different thresholds ($10^{-6} < P < 10^{-2}$, uncorrected) in an anatomical ROI of the ventral temporal cortex (VTC, see Materials and Methods). In the right VTC, the total volume of face selective activation was significantly higher in adults than in adolescents ($F_{1,23} = 5.8$, $P = 0.02$; repeated measures ANOVA

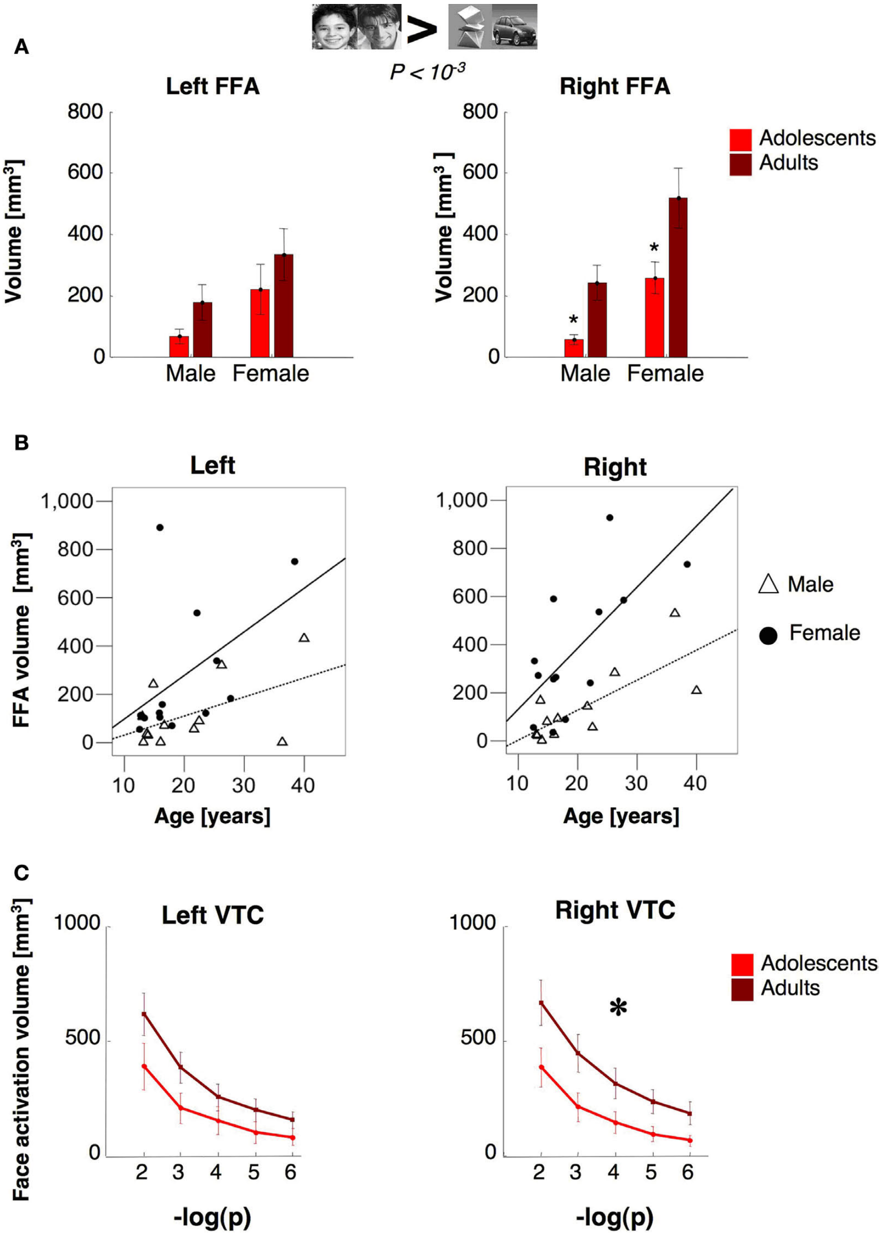

FIGURE 4 | FFA volume as a function of age and gender. (A) The volume of the FFA (man and boy > cars and abstract objects, $P < 10^{-3}$, uncorrected) as a function of age and gender. Bars show the volume of the FFA, as defined in **Figure 3**, averaged across 14 adolescents (ages 12–16 years, *light red*) and 11 adults (18–40 years, *dark red*). Error bars show group SEM. Asterisks indicate significantly smaller volume than adults ($P < 0.05$, *t*-test across age groups). **(B)** The correlation between age and FFA volume among males and females in each hemisphere (rFFA vs. age in females: $r = 0.69$, $P = 0.009$; in males: $r = 0.76$, $P = 0.004$; lFFA vs. age in females: $r = 0.48$, $P = 0.09$; in males: $r = 0.52$, $P = 0.08$). *Open triangles*: males, $n = 12$; *Filled circles*: females $n = 13$. **(C)** The total volume of face selective voxels in VTC, defined as man and boy > cars and abstract objects at five different statistical thresholds (minus logarithm base 10). Volumes are measured irrespective of voxel contiguity. *Light red*: adolescents (ages 12–16, $n = 14$); *dark red*: adults (ages 18–40, $n = 11$). Error bars show group SEM (11 adults and 14 adolescents). Large asterisk indicates significantly larger volume in adults, $P < 0.02$, repeated measures ANOVA).

Figure 4C), and at every threshold tested ($P < 0.033$, *t*-test). In the left VTC, however, between age group differences in the overall volume of face selective activations did not reach statistical significance ($F_{1,23} = 2.60$, $P = 0.12$; **Figure 4C**). Thus, the total volume of face selective activations was significantly higher in the right VTC of adults than in adolescents, regardless of clustering or statistical threshold.

RESPONSE AMPLITUDES OF FACE SELECTIVE ACTIVATIONS IN VTC

We asked if in the face selective regions of VTC, response amplitudes to visual stimuli varied with age group. For each subject, we defined the face selective voxels (man and boy > cars and abstract objects, $P < 10^{-3}$) in the VTC using data from run 1 and measured response amplitudes from run 2 data (**Figure 5A**, see Materials and Methods). Face selective voxels were found in

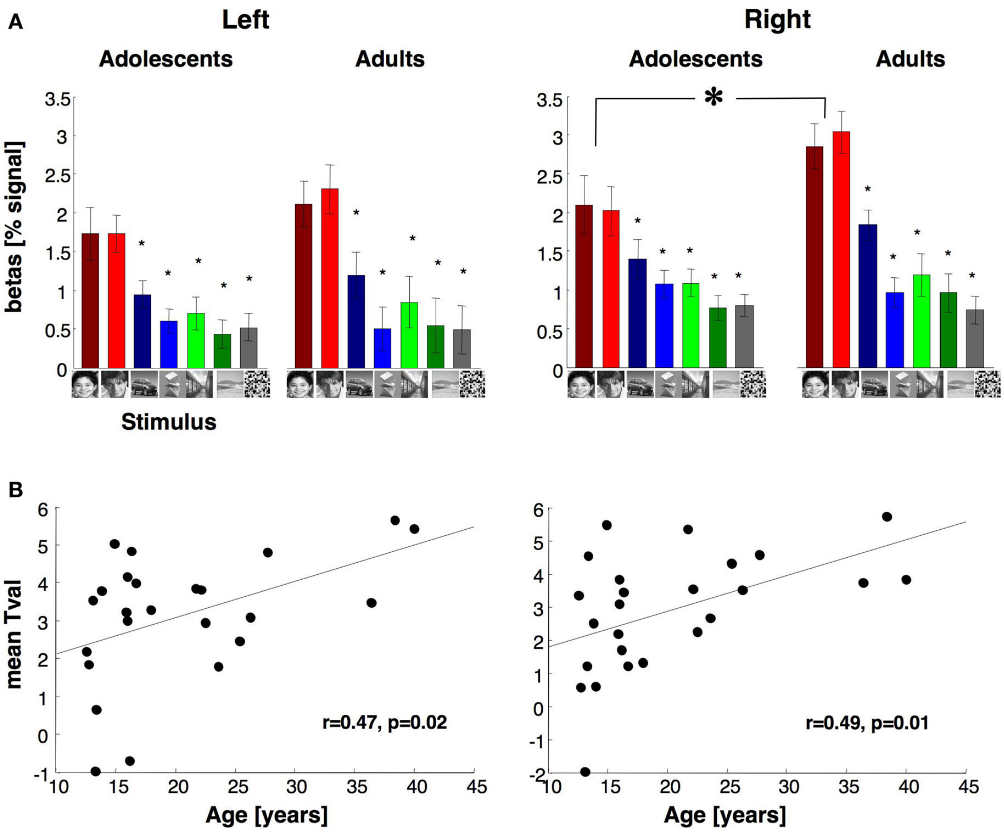

FIGURE 5 | Average response to different stimuli and face selectivity within face selective regions of fusiform gyrus. (A) Average responses to different image categories (relative to the blank baseline) of face selective voxels during run 2. Data are averaged across 14 adolescents and 11 adults in the right hemisphere and 13 adolescents and 11 adults in the left hemisphere. Face selective voxels were independently defined using run 1 data with the contrast: man and boy > cars and abstract objects, $P < 10^{-3}$, uncorrected. Error bars indicate SEM across subjects. Small asterisks indicate responses that are significantly less than faces ($P < 0.05$, *t*-test across subjects) and are corrected for multiple comparisons using Bonferroni correction. Large asterisk indicates significantly lower responses to faces in adolescents than adults ($P < 0.05$, *t*-test across subjects). **(B)** Face selectivity is plotted as a function of age. Each point represents a subject's mean *t*-value for the contrast faces vs. objects from run 2 data across all face selective voxels in VTC as in **(A)**.

run 1 in the right and left hemisphere of all subjects except the left hemisphere of one adolescent. In both age groups, response amplitudes revealed face selectivity, namely higher responses to faces than other stimuli (**Figure 5A**). The overall response amplitudes in face selective voxels of the right VTC were not significantly different between age groups ($F_{1,21} = 1.4$, $P = 0.24$) or genders ($F_{1,21} = 1.8$, $P = 0.2$). However, there was a significant interaction between age and condition ($F_{1,23} = 9.37$, $P = 0.006$), as responses to faces were higher in adults than in adolescents ($t_{23} = 1.7$, $P = 0.05$, one-tailed). There were no interactions between age-of-subject and age-of-face stimuli ($F_{1,21} = 1.29$, $P = 0.27$). Importantly, there were no significant differences between age groups in response amplitudes to non-face stimuli ($Ps > 0.13$), indicating that between age group differences were specific to responses to faces.

In the left VTC, the response amplitudes of face selective voxels were not significantly different across age groups ($F_{1,20} = 0.32$, $P = 0.58$) or genders ($F_{1,20} = 0.00$, $P = 0.99$), and there was no significant interaction between factors of age and gender ($F_{1,20} = 0.02$, $P = 0.88$, **Figure 5A**).

The between age group differences in the response magnitude to faces suggests that face selectivity may be higher in adults than in adolescents. To test this possibility, we measured each subject's mean selectivity to faces using run 2 data from the face selective voxels defined by run 1 data, as above. The mean selectivity was defined as the mean *t*-value across all face selective voxels for the contrast man and boy > cars and abstract objects in run 2. The mean *t*-value was higher in adults (right: 3.7 ± 1.3, left: 3.7 ± 1.2, mean selectivity ± std deviation) than in adolescents (right: 2.3 ± 1.98, left: 2.64 ± 2, mean selectivity ± std deviation). These differences reached statistical significance in the right hemisphere (right: $t_{23} = 2.1$, $P = 0.02$; left: $t_{23} = 1.5$, $P = 0.07$, one-tailed test). Furthermore, there was a significantly positive correlation between subjects' age and the mean face selectivity in both hemispheres (right: $r = 0.49$, $P = 0.01$; left: $r = 0.47$, $P = 0.02$, **Figure 5B**). In sum, development of face selective activations during adolescence manifests as an increase in the total volume of face selective regions, as well as increased response amplitude to faces (but not non-faces) in face selective voxels and consequently, increased face selectivity.

RIGHT FFA IS SMALLER IN ADOLESCENTS REGARDLESS OF AGE OF FACE STIMULI

We asked if the smaller FFA volume in adolescents depends on the choice of face stimuli. In each subject we redefined face selective ROIs, once as the clusters of voxels that responded more to faces of boys than to objects (boy > car and abstract objects, $P < 10^{-3}$, uncorrected) and separately using men faces (man > car and abstract objects, $P < 10^{-3}$, uncorrected, **Figure 6A**). Both contrasts detected face selective voxels in the fusiform gyrus and OTS with significantly larger rFFA volumes in adults than in adolescents ($F_{1,23} = 4.84$, $P = 0.04$, repeated measures ANOVA, **Figure 6B**). However, there was no significant effect of age of face stimulus ($P = 0.62$) and no interaction between age of face stimulus and age of subject ($P = 0.77$). Results were similar when we measured the total volume of all supra-threshold voxels in the right VTC across various thresholds (man > cars and abstract objects: $F_{1,23} = 4.6$, $P = 0.04$; boy > cars and abstract objects: $F_{1,23} = 6.58$, $P = 0.02$, repeated measures ANOVA **Figure 6C**). In contrast, between age group differences in lFFA volume did not reach statistical significance ($F_{1,23} = 2.22$, $P = 0.15$), regardless of the face stimulus used to define the ROI (**Figures 6B,C**). The lFFA volume was larger when defined by faces of boys rather than men, across all subjects ($F_{1,23} = 6.49$,

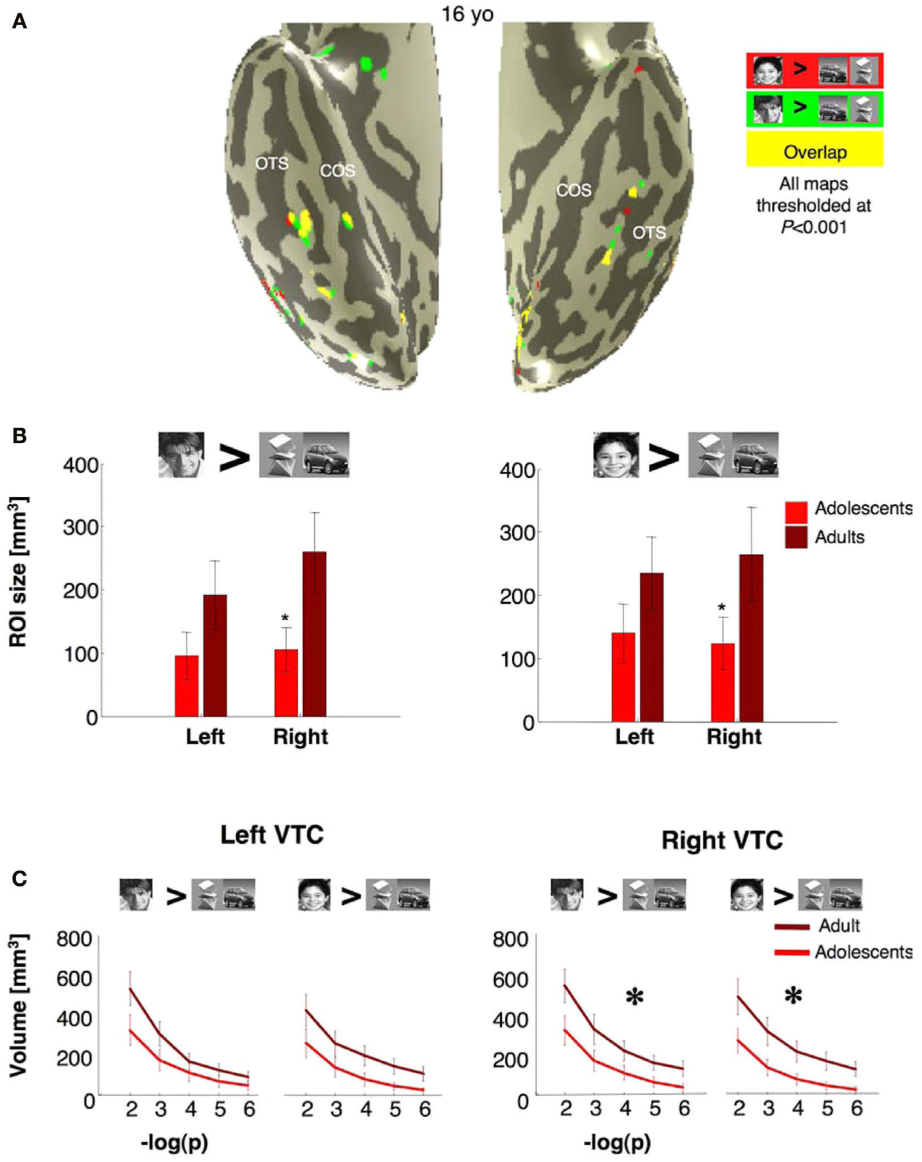

FIGURE 6 | Volume of face selective activations defined using either man or boy faces. (A) Activation maps for boy selective voxels (*boy faces* > cars and abstract objects, $P < 10^{-3}$), red, man selective voxels (*man faces* > cars and abstract objects, $P < 10^{-3}$), green, and overlapping voxels (yellow). Data are shown for a representative adolescent brain. **(B)** Average volume of the cluster of voxels defined by the same contrasts as in **(A)**. Error bars show group SEM. **(C)** The volume of all man selective or boy selective voxels in the VTC regardless of contiguity at five different statistical thresholds (10^{-2} to 10^{-6}, uncorrected). *Light red*: adolescents (ages 12–16 years, $n = 14$); *dark red*: adults (ages 18–40 years, $n = 11$). Error bars show group SEM. Large asterisks indicate significantly larger volume in adults vs. adolescents ($P < 0.02$, F-test repeated measures ANOVA).

$P = 0.02$, **Figure 6B**). However, there was no interaction between age of face stimulus and age of subject ($P = 0.96$). Thus, age of face stimuli did not determine between age group effects on the volume of face selective activations in VTC.

THE SPATIAL OVERLAP BETWEEN FFA REGIONS SELECTIVE FOR FACES OF BOYS OR MEN

We asked if the boy and man face stimuli activated spatially segregated regions in the fusiform gyrus, perhaps to varying degrees depending on the subjects' age. We examined the extent of the spatial overlap between the two ROIs that were separately defined as clusters of activation by faces of men vs. objects (man > cars and abstract object, $P < 10^{-3}$, uncorrected) or faces of boys vs. objects (boy > cars and abstract objects, $P < 10^{-3}$, uncorrected). There was approximately 50% overlap between the two ROIs in both age groups (see **Figure 6A**). Importantly, there was no interaction between age of subject and the size of the non-overlapping volume that responded more to man faces than objects (but not boy faces than objects) and vice versa, in either hemisphere ($Ps > 0.7$). Likewise, response amplitudes from these non-overlapping regions showed no significant interactions between age of subject and age of stimulus ($P > 0.14$). Thus, we found no evidence that the spatial extent, response amplitudes, or spatial location of selectivity depended on the age of face stimuli.

THE VOLUME AND RESPONSE AMPLITUDES OF OBJECT SELECTIVE ACTIVATIONS

We asked if developmental changes in the VTC are specific to face selective regions, or reflect a more general process involving other regions that are selective for non-face categories such as objects and scenes (**Figure 7A**). Thus, we measured the volume (**Figures 7B and 8A**) and response amplitudes of object selective activations in the VTC (**Figure 8B**).

We defined an object selective region along the posterior fusiform gyrus, often extending into the occipito-temporal sulcus referred to as pFus/OTS (see Materials and Methods). The volume of the right pFus/OTS was significantly larger in females than in males ($F_{1,23} = 6.39$, $P = 0.02$), but there were no between age group differences ($P > 0.22$, **Figure 7B**), and no interactions between the factors of age and gender in either hemisphere ($P > 0.31$). Similarly, there were no significant between age group differences in the total volume of object selective activations (cars and abstract objects > scrambled) at five different thresholds ($10^{-6} < P < 10^{-2}$, uncorrected) in either the right or left VTC ($Ps > 0.22$). Additionally, we did not find significant between age group differences in activation volumes in either hemisphere when we separately defined the activations for abstract objects versus scrambled images or cars versus scrambled images at five different thresholds ($Ps > 0.15$).

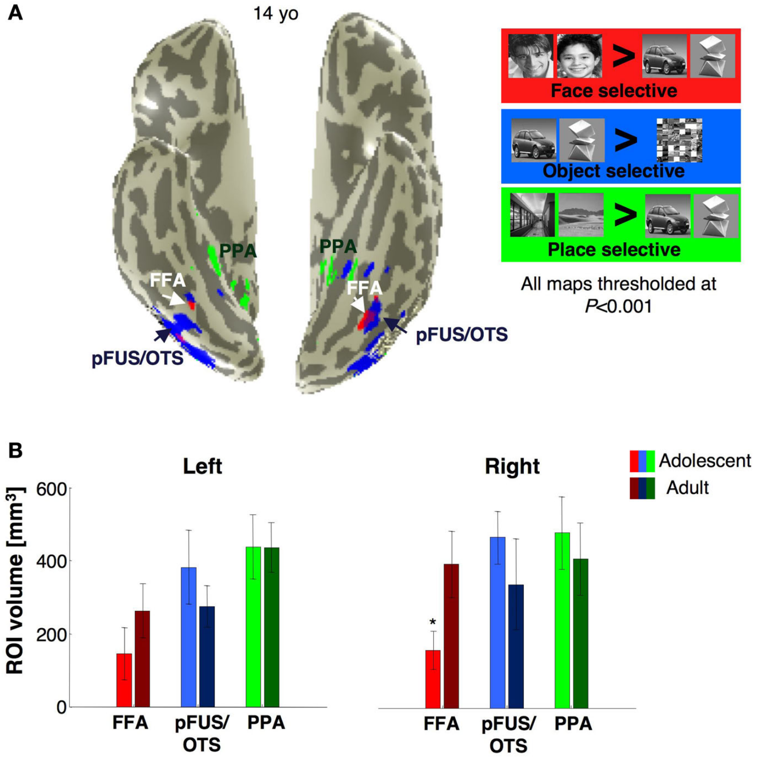

FIGURE 7 | Face-, object- and place selective activations in ventral cortex. **(A)** Face (red), object (blue) and place (green) selective activations in a representative 14-year-old subject. All activation maps are plotted at the $P < 10^{-3}$ threshold, uncorrected. Overlapping face and object activations are shown in magenta. The spatial relation among face, object and place selective activations and also their location relative to anatomical landmarks were similar across adults and adolescents. **(B)** The volume of FFA, PPA and pFus/OTS averaged across 11 adults (light bars) and 14 adolescents (dark bars). Error bars indicate between subjects SEM. Asterisks indicate significantly larger volume in adults than in adolescents ($P < 0.05$, *t*-test).

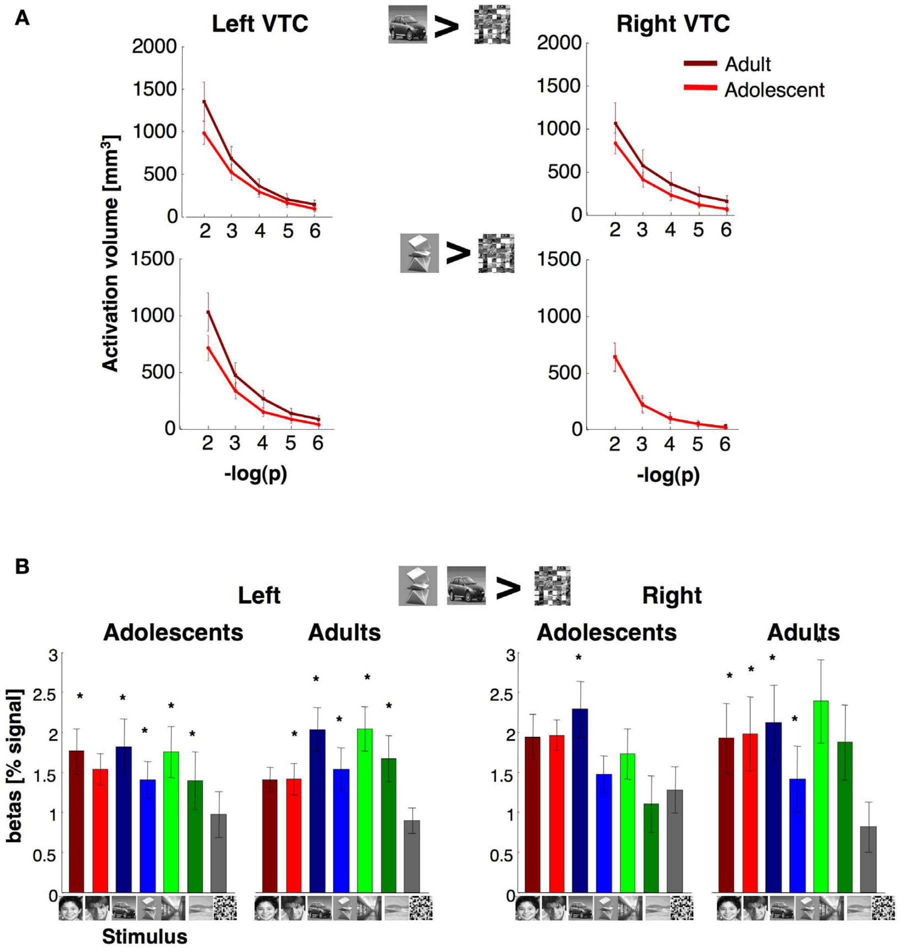

FIGURE 8 | Volume and response amplitudes of object selective activations in VTC. (A) The total volume of activated voxels for cars > scrambled, or abstract objects > scrambled at five different thresholds (10^{-2} to 10^{-6}, shown as minus logarithm base 10, uncorrected). *Light red*: adolescents (ages 12–16 years, $n = 14$); *dark red*: adults (ages 18–40 years, $n = 11$). **(B)** Percent signal change for object selective voxels. We defined in each subject object selective voxels in the VTC using the contrast: cars and abstract objects > scrambled, $P < 10^{-3}$, from run 1 data. Object selective voxels were found in all subjects from run 1 data. Signal amplitudes were extracted from these voxels in run 2 data. We calculated the average response across each subject's voxels and then averaged across subjects. Error bars show group SEM (11 adults and 14 adolescents). Asterisks indicate significantly higher than scrambled ($P < 0.05$, *t*-test across subjects) and are corrected for multiple comparisons using Bonferroni correction.

Response amplitudes were generally higher for objects, faces and scenes compared to scrambled images, and there were no significant effects of age (right VTC: $F_{1,21} = 0.64$, $P = 0.43$; left VTC: $F_{1,21} = 0.03$, $P = 0.86$, **Figure 8B**) or interaction between age and response amplitude to image categories ($P > 0.19$) in either hemisphere.

Response amplitudes were significantly higher among females than males in the right VTC (right VTC: $F_{1,21} = 6.31$, $P = 0.02$; left VTC: $F_{1,21} = 0.46$, $P = 0.51$), but there was no interaction between age and gender in either hemisphere ($Ps > 0.13$), or between either factors of age or gender and image category ($Ps > 0.2$).

The degree of selectivity among the object selective voxels in VTC was measured as the mean *t*-value for the contrast cars and abstract objects > scrambled in run 2. This selectivity was higher in the left hemisphere of adults (2.5 ± 1.3, mean selectivity ± std deviation) than in adolescents (1.7 ± 1.1, mean selectivity ± std deviation, $t_{23} = 1.7$, $P = 0.05$, one-tailed test). In contrast, in the right hemisphere, this mean *t*-value among the supra-threshold object selective voxels was not statistically different across age groups (mean selectivity in adults: 2.3 ± 1.5, in adolescents: 1.6 ± 1.1, $t_{23} = 1.2$, $P = 0.24$). Furthermore, there were no significant correlations between age and magnitude of object-selectivity among the supra-threshold object selective voxels in the right or left VTC ($rs < 1.7$, $Ps > 0.42$), in contrast to the positive correlation between age and face selectivity among the supra-threshold face selective voxels in VTC.

THE VOLUME AND RESPONSE AMPLITUDES OF PLACE SELECTIVE ACTIVATIONS

We examined place selective activations in the VTC (**Figure 7**) to test if the volume and selectivity of place selective activations increase during adolescence in tandem with the development of face selective activations.

We defined the PPA as a scene selective region along the parahippocampal gyrus, often extending into the collateral sulcus (indoor and outdoor scenes > cars and abstract objects, $P < 10^{-3}$, **Figure 7A**). The PPA volume in both hemispheres was similar across age ($Ps > 0.58$) and gender groups ($Ps > 0.70$, **Figure 7B**). Likewise, there were no between age group differences in the volume of place selective activations in the VTC at five different thresholds (right: $F_{1,23} = 0.10$, $P = 0.75$; left: $F_{1,23} = 0.64$, $P = 0.43$; **Figure 9A**), suggesting that the PPA reaches its adult volume by adolescence and earlier than the FFA.

Response amplitudes of place selective voxels in the VTC were higher for scenes than all other categories (**Figure 9B**). There were no significant effects of age (right VTC: $F_{1,21} = 0.36$, $P = 0.56$, left VTC: $F_{1,21} = 0.02$, $P = 0.91$), gender (right VTC: $F_{1,21} = 0.42$, $P = 0.52$; left VTC: $F_{1,21} = 1.74$, $P = 0.20$), or interaction between these factors ($Ps > 0.17$).

The degree of selectivity among the place selective voxels in VTC, as measured by their mean T value, was similar in adults (right: 3.7 ± 1.0, left: 3.7 ± 1.2, mean selectivity ± std deviation) and adolescents (right: 3.1 ± 1.4; left: 3.2 ± 1.3, mean selectivity ± std deviation) and not significantly different in either hemisphere ($Ps > 0.27$). Despite some trends, the correlation between subjects' age and the magnitude of place-selectivity among place selective voxels did not reach statistical significance in either hemisphere in the VTC ($rs < 0.35$, $P > 0.08$).

DISTRIBUTED RESPONSES TO OBJECT CATEGORIES IN VTC ACROSS AGE GROUPS

We asked if the development of face selectivity in the VTC also manifests as changes in the spatial pattern of its distributed responses. It is well documented that category information is also present in the distributed responses across the VTC, as different categories elicit distinct distributed response patterns (Haxby et al., 2001; Cox and Savoy, 2003). In other words, the correlation between MVP of responses across different categories is typically lower than the correlation between MVPs for the same category. Thus, MVP analysis is a useful approach for measuring any age-related changes in the distributed response patterns. Specifically, our findings of fewer face selective voxels in the adolescent VTC, lower response amplitudes elicited by faces (but not objects or scenes) and lower face selectivity suggests that fewer voxels in the adolescent VTC differentially respond to face versus object stimuli. Consequently, face MVPs may be less distinct than non-face MVPs (i.e. more positively correlated) in adolescents than in adults.

MVPs for different categories across the VTC are shown in **Figure 10A** from one representative adolescent subject. In general, the spatial pattern of response was similar across adolescents and adults. For the stimuli presented in our experiment, face MVPs showed responses above the mean in the lateral VTC (red and yellow in **Figure 10A**) and below the mean in the medial VTC (blue

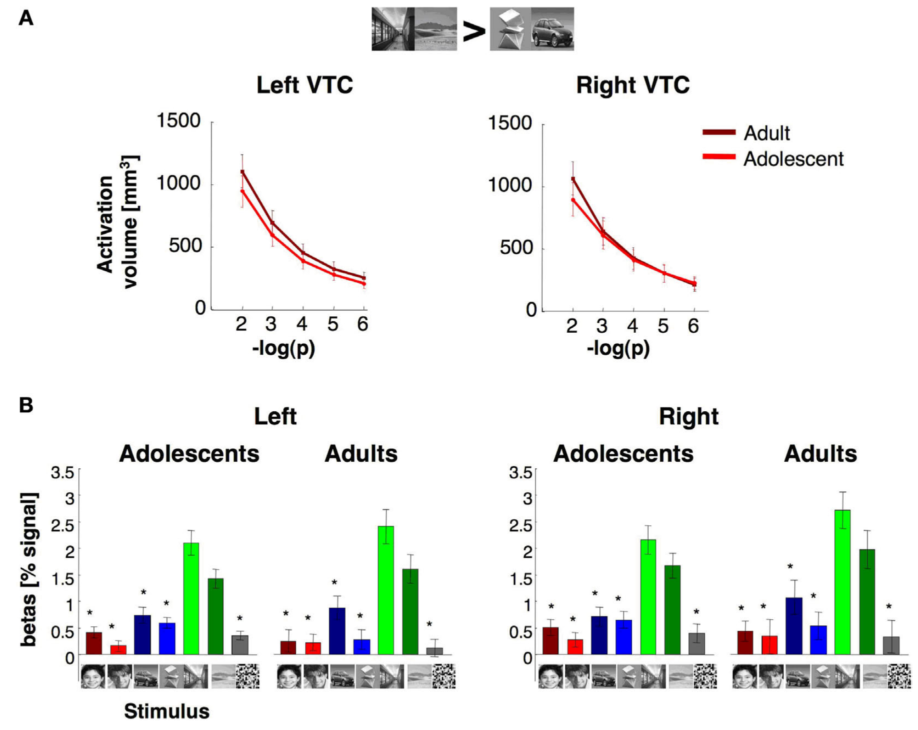

FIGURE 9 | The volume and response of place selective activations in the ventral temporal cortex. (A) The total volume of place selective activations for indoor and outdoor scenes > cars and abstract objects at five different thresholds (10^{-2}–10^{-6}, uncorrected, shown in minus log base 10 of p). *Light red*: adolescents (ages 12–16 years, $n = 14$); *dark red*: adults (ages 18–40 years, $n = 11$). **(B)** Response amplitudes to visual stimuli across place selective VTC voxels. Place selective voxels in the VTC were defined by the contrast scenes > objects, $P < 10^{-3}$, in run 1. Percent signal changes were extracted from run 2 (mean and SEM). Asterisks indicate responses that significantly less than response to indoor and outdoor scenes ($P < 0.05$, t-test across subjects) and are corrected for multiple comparisons using Bonferroni. Error bars reflect SEM across subjects.

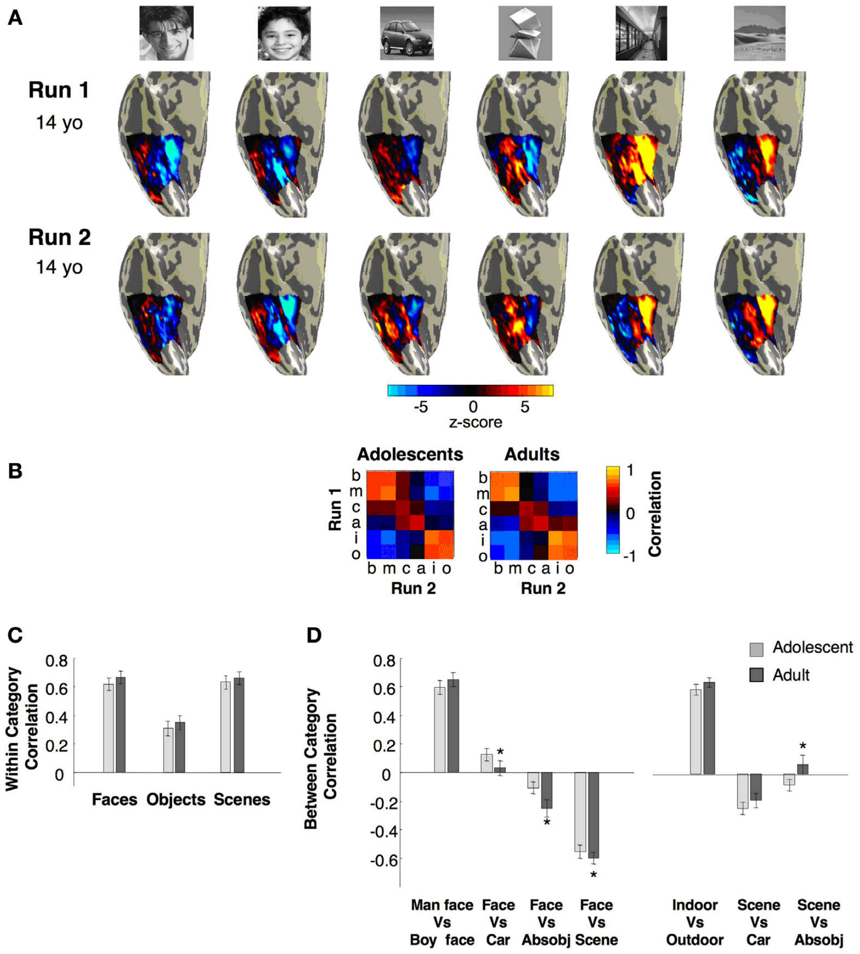

FIGURE 10 | Multivoxel pattern (MVP) analysis of distributed responses to categories across the right VTC. (A) Visualization of the MVP to stimulus categories in run1 and run2 in a representative 14-year-old adolescent. **(B)** Group averaged Pearson correlations within and between categories from run1 versus run2. The on-diagonal components indicate the within-category reproducibility of the MVPs across runs, using different exemplars of the same stimulus category. Off-diagonal components indicate between-category correlations. **(C)** Summary of within-category correlations from **(B)**. **(D)** Across-category correlations from **(B)** between faces and non-faces and between scenes and objects. *Light grey*: adolescents $n = 11$, ages 12–16 years; *Dark grey*: adults, $n = 11$, ages 18–40 years. Asterisks indicate significantly different correlations in adults vs. adolescents ($P < 0.02$, *t*-test across subjects). Error bars denote SEM across subjects.

and cyan in **Figure 10A**). Scene MVPs showed the opposite spatial pattern. The MVPs of responses to cars and abstract objects were more diffuse in both age groups.

We calculated the Pearson correlation between MVPs across all pairings of stimuli across runs 1 and 2 in each subject and then averaged across subjects in each age group.

Results are summarized in a correlation matrix (**Figure 10B**). The diagonal of this correlation matrix shows the within-category reproducibility of MVPs across the two runs for each of the stimulus categories. In both adults' and adolescents' within-category correlations for all stimulus types were significantly positive (all $ts > 4$, $Ps < 0.001$). Within-category correlations were particularly high for faces and places (mean $r_w > 0.6$, **Figure 10C**-left), with lower but significantly positive within-category correlations for objects (mean $r_w > 0.3$, $Ps < 0.001$). Thus, within-category MVPs were highly reproducible between runs. Critically, there were no between age group differences in the reproducibility of the MVPs ($Ps > 0.1$, $ts < 1.7$). Furthermore, the between-category correlations between MVPs for different face types (man vs. boy, **Figure 10C**) were similar to the within-category correlation for the same face types (man vs. man or boy vs. boy, adults: $r_b = 0.65$, adolescents: $r_b = 0.57$, $t_{48} = 1.6$, $P = 0.11$). Thus, there was no evidence for an age of stimulus by age of subject interaction.

Next, we compared the between-category correlations for face vs. non-face MVPs in each subject (**Figure 10C**). In adults, the correlations between face MVPs and non-face MVPs (cars, abstract objects and scenes) were either close to zero (in case of cars), or negative (in case of abstract objects and scenes, **Figure 10C**), suggesting distinct spatial patterns of response to face and non-face stimuli. Similarly, in adolescents, the correlation between face and non-face MVPs were significantly lower than the within-category correlation among face MVPs (**Figure 10C**), indicating that these MVPs were distinct in this group as well. However, the degree of this distinctness was significantly lower in adolescents compared to adults. Specifically, the mean correlation between face vs. car MVPs was near zero in adults, but significantly positive in adolescents (between group difference, *t*-test, $P < 0.02$). Likewise, the between-category correlations for face vs. abstract object MVPs, and also across face vs. scene MVPs were significantly less negative in adolescents ($Ps < 0.02$). Thus, face MVPs become more distinct from non-face MVPs across the VTC with age.

To test the category specificity of these developmental changes, we examined the correlations between the MVPs to scenes and objects across age groups. In both age groups there were highly positive correlations between indoor scene MVPs and outdoor scene MVPs. In both age groups the correlation between scene MVPs and car MVPs were negative, showing distinct patterns of response to scenes versus cars across the VTC (**Figure 10C**). However, the correlation between MVPs for scenes vs. abstract objects was significantly more negative in adolescents than in adults (**Figure 10C**). Thus, the distributed responses to scenes vs. abstract objects were less distinct in the VTC of adults compared to adolescents, contrasting the more distinct pattern of face vs. object responses in adults.

Finally, we asked if there are age group differences in the inter-subject variability of MVP correlations. Thus, we calculated the inter-subject correlation between MVP correlation matrices across all pairs of subjects (see Materials and Methods). In each age group, inter-subject correlations were high (adult-adult: $r = 0.91 \pm 0.04$; adolescent-adolescent: $r = 0.91 \pm 0.03$), indicating highly similar correlation matrices and low between-subject variability within each age group. Importantly, the mean inter-subject correlation between pairings of adult and adolescents' matrices was also high (adolescent-adult, $r = 0.9 \pm 0.04$), indicating that, overall, the correlations among category MVPs were highly similar across the age groups, despite the face- and scene- specific differences that we found.

RELATING THE DEVELOPMENT OF RFFA TO IMPROVEMENTS IN FACE RECOGNITION MEMORY

To determine how our fMRI findings relate to visual recognition performance, we first asked if there were age-related improvements specifically in face recognition memory performance, or more generally for non-face categories as well. Face recognition memory was significantly higher in adults than in adolescents ($t_{23} = 2.75$, $P = 0.01$). In contrast, recognition memory performance for non-face stimuli was not different across age groups ($P > 0.3$). Females were significantly better than males at recognition memory for indoor and outdoor scenes ($P = 0.001$), but there was no interaction between factors of age and gender for any of the stimulus categories ($P > 0.73$). Thus, age-related recognition memory improvements during adolescence were specific to faces.

Next, we asked if rFFA volume is correlated with face recognition memory performance. Notably, face recognition memory performance was significantly correlated with rFFA volume ($r = 0.54$, $P < 0.008$, significant after Bonferroni correction for multiple comparisons, **Figure 11**) with similar trends in the lFFA ($r = 0.52$, $P < 0.01$). In contrast, place and object recognition memory was not correlated with either the right or left FFA volume (**Figure 11**, **Table 2**). Furthermore, there were no significant correlations between face recognition memory and the volume of either the pFus/OTS or the PPA (despite a trend for the lPPA, which was not statistically significant after Bonferroni correction, **Table 2**).

We also asked if performance on other face tasks outside the scanner were correlated with rFFA volume. We used the Benton face recognition task to assess perceptual face discrimination, and an affect perception task to examine recognition of facial expressions of emotion. Performance on the Benton task was significantly better among adults than adolescents, but there were no between group differences in their performance on the affect task (**Table 1**). Furthermore, FFA volume did not predict performance on either of these tasks (**Table 2**). These findings suggest a substantial degree of specificity in the FFA's role in the development of recognition memory for face identity.

DISCUSSION

We found evidence for development of face selectivity in the VTC during adolescence. This development manifested as an age-related growth of the rFFA volume, increased rFFA response amplitudes and selectivity for faces, and increased differentiation between distributed patterns of activations to face versus non-face stimuli in the VTC of adults compared to adolescents. Importantly, rFFA size was correlated with subjects' performance on a face recognition memory task, but not recognition performance for objects or places. In contrast to the rFFA, the volumes and response amplitudes of object- and place selective activations in the VTC were

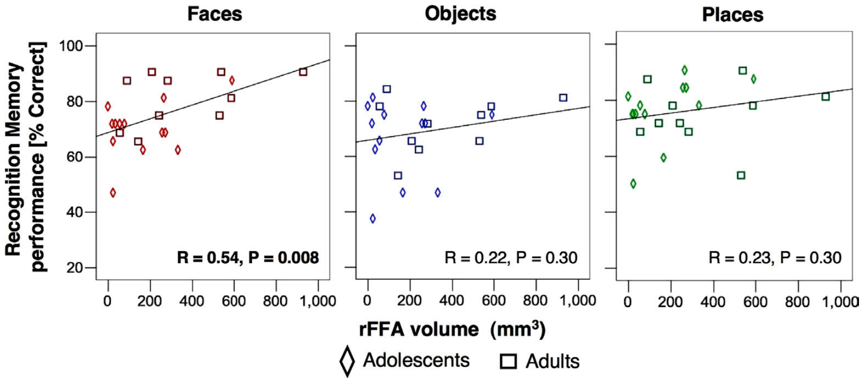

FIGURE 11 | Correlations between rFFA volume and recognition memory performance for faces (*Red*), objects (*Blue*) and places (*Green*). *Diamonds*: adolescents, *n* = 14 ages 12–16 years; *Squares*: adults, *n* = 11, ages 18–40 years.

Table 2 | Correlations between ROI volumes, subject's age and behavioral measures of visual processing. Right and left hemisphere are indicated by (r) and left (l) respectively. Face selective fusiform face area (FFA), object selective pFus/OTS region (pFus), parahippocampal place area (PPA). Bold entries indicate significant correlation after Bonferonni correction for multiple comparisons.

	Age	Affect recognition	Benton	Visual recognition memory		
				Face	Scene	Objects
rFFA	***R* = 0.55**	R = 0.31	*R* = 0.39	***R* = 0.54**	R = 0.23	*R* = 0.22
	***P* = 0.004**	P = 0.16	*P* = 0.08	***P* = 0.008**	P = 0.3	*P* = 0.3
lFFA	*R* = 0.41	*R* = 0.41	*R* = 0.42	*R* = 0.51	*R* = 0.3	*R* = 0.2
	P = 0.045	*P* = 0.06	*P* = 0.05	*P* = 0.01	*P* = 0.17	*P* = 0.37
rpFus	*R* = –0.21	*R* = 0.36	*R* = 0.02	*R* = –0.16	*R* = 0.01	*R* = –0.21
	P = 0.31	*P* = 0.1	*P* = 0.94	*P* = 0.48	*P* = 0.98	*P* = 0.33
lpFus	*R* = –0.05	*R* = 0.01	*R* = –0.39	*R* = –0.36	*R* = –0.24	*R* = –0.28
	P = 0.81	*P* = 0.98	*P* = 0.08	*P* = 0.1	*P* = 0.28	*P* = 0.2
rPPA	*R* = –0.09	*R* = 0.15	*R* = 0.06	*R* = 0.16	*R* = 0.1	*R* = –0.07
	P = 0.69	*P* = 0.51	*P* = 0.8	*P* = 0.47	*P* = 0.66	*P* = 0.77
lPPA	*R* = –0.01	*R* = 0.01	*R* = 0.23	*R* = 0.41	*R* = 0.24	*R* = 0.2
	P = 0.95	*P* = 0.98	*P* = 0.31	*P* = 0.05	*P* = 0.28	*P* = 0.37

similar across age groups. Our findings demonstrate that the development of the rFFA continues during adolescence, after the PPA and pFus/OTS have reached their adult size.

We addressed several methodological factors that may produce non-specific face selective differences and potentially confound comparison of fMRI results across age groups (Grill-Spector et al., 2008). First, we based our functional analyses on individually defined ROIs without spatial normalization in order to avoid confounds due to possible between group differences in brain shape. This approach precluded direct between group comparisons of the location of the FFA, but our subject-by-subject analysis suggests that such differences are likely to be subtle. Second, we improved the spatial resolution of our study relative to previous reports by prescribing smaller voxels during scanning and by avoiding any spatial smoothing of the data. Previous studies of adolescent VTC which employed spatial smoothing (Golarai et al., 2007) and normalization (Scherf et al., 2007) did not find significant differences between adult and adolescent FFA size, despite some trends (Golarai et al., 2007). Thus, the improved spatial precision of our study was likely a critical factor in our ability to detect developmental changes in the FFA of adolescents. Third, subjects across different age groups were matched on several factors that could potentially affect BOLD signals. These include performance on a 1-back task during scan, total motion during the scan, and the percentage of variance across the time-course data that is explained by the GLM. Fourth, we examined whether the functional differences were by-products of between age group differences in the anatomical size of the VTC. Although we found evidence for a larger right hemisphere in adolescents than in adults, the size of the anatomical ROI of the VTC (to which we restricted most of our analyses) was similar across age groups. Thus our functional results in the VTC cannot be explained by between group differences in anatomical size. Our methods involved reconstructing the gray matter by including a uniform gray matter thickness across the whole brain in all subjects. Thus, our measurements are proportional to the surface area of the gray/white matter boundary and preclude direct assessment of any potential between group differences in gray matter volume that might arise from variations in gray matter thickness. However, previous studies suggest that gray matter thickness in the VTC is relatively stable during childhood to adulthood (Sowell et al., 2002), consistent with our anatomical measurements. Fifth, we examined the volume of face-, object- and place selective activations across a range of statistical thresholds and found consistent results across this range, indicating that our findings are not dependent on the choice of the statistical threshold on contrast maps or voxel clustering. Sixth, we examined the reproducibility of distributed responses to visual categories in the VTC across two independent runs, and found them to be highly reproducible in both adults and adolescents, suggesting that our fMRI data were equally reliable across age groups. Thus, neither between age group differences in performance during the scan, BOLD-related confounds, size of the anatomical VTC, nor data reliability can account for our results.

The rFFA volume was substantially larger in adults compared to adolescents across several analyses. Our results are consistent with previous fMRI studies that found evidence for a prolonged development of the rFFA among children ages 7–11 years (Golarai et al., 2007; Scherf et al., 2007; Peelen et al., 2009). However, previous studies did not clarify whether or not this development continued through adolescence. Specifically, in our previous report (Golarai et al., 2007), we found a trend for smaller rFFA size in adolescents versus adults (using a different group of subjects), which was inconclusive, perhaps due to the lower spatial resolution in that study. Another study found a positive correlation between age vs. size or selectivity of rFFA among a group of subjects ranging from age 7–17, however children and adolescents were not separately compared to adults (Peelen et al., 2009). In contrast, a third study found no between group differences in the size or response amplitudes of the rFFA when comparing adults and adolescents (Scherf et al., 2007). The methodological improvements in our current study enabled us to detect substantial developmental changes in rFFA size and face specific responses during adolescence. The smaller rFFA size in adolescents was coupled with lower responses to faces (but not objects) and lower face selectivity in this region. These data are consistent with our previous findings of lower responses to faces (but not objects) in a penumbral region adjacent to the nascent FFA (Golarai et al., 2007).

The development of face selective activations in the VTC was evident in both males and females, although FFA size was larger among females than males in both age groups. This latter effect of gender might be due to our choice of male face stimuli, although previous behavioral studies suggest that females' better face recognition memory performance is only apparent for female faces (Lewin and Herlitz, 2002). Thus, full characterization of gender effects on the size of the FFA will require presentation of both male and female faces in future experiments. Nevertheless, our data show that the rFFA undergoes a substantial development during adolescents in both genders.

Some behavioral studies suggest better face recognition memory performance when older adult observers view faces of their own-age group compared to faces of younger adults (Anastasi and Rhodes, 2005; Perfect and Moon, 2005). These findings have led to the possibility that own-age faces may produce stronger brain responses than faces of other age groups, analogous to the differential FFA responses to faces of other races (Golby et al., 2001). However, we found no evidence for an own-age bias. First, the volume of face selective activations was larger in adults than adolescents whether we defined them using both types of faces, or only men or boy faces. Second, response amplitudes to faces of men and boys were similar in both age groups, whether we defined face selective activations by one or both face stimuli. Third, there was no interaction between the age of subject and size of the non-overlapping activations elicited by man or boy faces alone. Fourth, the distributed patterns of activations across the VTC for faces of men and boys were highly correlated in both age groups, suggesting that our findings are not limited to discrete regions of the VTC, and our current measurements do not distinguish between patterns of responses to faces of men or boys across the VTC.

We did not precisely match the age of face stimuli with the age of participants. Although such a precise matching could increase our sensitivity to potential effects of age of face stimuli, it would also involve perceptual variability across the stimulus sets and potentially introduce additional unwanted variability in the fMRI signals across subjects, rendering data interpretation more difficult. Thus, we chose to minimize this later source of variability by using a single set of stimuli from a range of ages, so that men faces overlapped the age range of the adult subjects and boy faces overlapped the age range of the adolescent subjects and included younger age groups as well. We reasoned that if the FFA size reflects years of prior experience with faces, then adolescents' response to children's faces would reflect this relatively recent experience with children's faces, and bias our experiment towards finding a more adult-like FFA size in this group (i.e. opposite to our results). Given the absence of any trends towards an own-age bias in our data, it is highly unlikely that age of face stimuli could explain the substantially smaller rFFA size in adolescents.

Developmental changes in the VTC were specific to the face selective region of FFA, as place- and object selective regions were similar in their volume and response properties across age groups. We previously reported that the left PPA also undergoes a prolonged development during the first decade of life and is larger in adults than children (ages 7–11 years). In the previous study we found no significant differences between adults and adolescents' PPA volumes, but adolescent PPA volumes were intermediate between child and adult volumes. Here, we found that the size of the PPA and its response profile to visual stimuli were similar across adults and adolescents. Our present findings are consistent with (Scherf et al., 2007) who reported no differences in place selective activations in 11–14 year olds compared to adults using a group analysis. Here we extend those findings as we measure volumes of activations in individual subjects as well as perform independent analyses of response amplitudes and selectivities, which were not performed in previous studies.

Similarly, we found no age-related differences in the size or response properties of an object selective region (pFus/OTS), which is anatomically close to (and sometimes overlaps) the FFA. Thus, it is unlikely that our finding of a smaller rFFA in adolescents is due to anatomical or BOLD-related confounds in the vicinity of the rFFA. Consistent with this interpretation, a recent study found evidence for substantial age-related increases in the rFFA size, but stable size of a nearby body-part selective region in the fusiform gyrus (fusiform body area, FBA) in the same subjects (Peelen et al., 2009). Furthermore, our results are consistent with previous reports that the volume and amplitude of object selective responses across the entire lateral occipital complex are adult-like by the age of 7 years (Golarai et al., 2007; Scherf et al., 2007). Here, we extend previous findings by showing that the lack of development of object selective responses was independent of the level of prior familiarity with the choice of object category in our study, as we found similar results when we separately examined object selective activations to cars (a familiar object category with identifiable parts and a common configuration of those parts) or abstract sculptures (varied exemplars of novel objects). These findings do not preclude the possibility that the PPA and pFus/OTS might show age- or experience-dependent changes, if probed with more sensitive designs such as fMRI-adaptation (Grill-Spector and Malach, 2001). Nevertheless, our findings underscore the differential development of face selective activations as compared to nearby object- or place selective activations during adolescence.

We found developmental changes in the distributed pattern of responses to faces across the VTC. Importantly, the distributed patterns of responses to faces vs. non-face stimuli were more distinct in adults than adolescents. This observation is consistent with our findings of fewer supra-threshold face selective voxels and lower face selectivity of these voxels in adolescents. Whether the results of our pattern analyses reflect wide spread changes in face selectivity across the entire VTC or a region localized to the vicinity of the nascent FFA remains to be determined in future studies. Our previous finding of lower face responsiveness and selectivity in a region surrounding the nascent FFA in children (Golarai et al., 2007) suggests that our current findings may reflect localized changes in the vicinity of the nascent FFA. Future studies are needed to determine how anatomically widespread or restricted the age-related changes in the distributed VTC responses are. Nevertheless, our findings reveal that developmental changes in the VTC responses during adolescence are reflected in the distributed patterns of responses to faces, and are not limited to a modular view of the VTC's functional organization.

The age-related increase in the distinctness of the MVPs was specific to faces vs. objects, as the overall spatial organization of the distributed responses were highly consistent within and across age groups, and the increased distinctness was not a general trend in the VTC for all categories. For example, the between-category correlations for scenes versus most other stimuli were similar across age groups. Meanwhile, the between-category correlation of scenes vs. abstract objects was *less* distinct in adults than adolescents. Given that we found no differences in the size or selectivity of the PPA across age groups, one interpretation of these findings is that with age, more (sub-threshold) VTC voxels outside the PPA respond similarly to scenes and abstract objects. Thus, the age-related *decrease* in the distinctness of distributed responses for scenes vs. abstract objects was opposite to the *increased* distinctness of responses to faces vs. objects. Whether or not these opposite shifts are causally related remains to be determined. However, our findings emphasize the specificity of the developmental increases in the distinctness of the distributed patterns of responses to faces vs. objects.

The age-related increases in rFFA size were associated with behavioral improvements in face recognition memory, consistent with our previous findings (Golarai et al., 2007). Face recognition memory performance was correlated with rFFA volume, but not with the size of object- or place selective regions, and rFFA size was not correlated with recognition performance for objects and places. It is unclear how larger rFFA size subserves better face recognition memory. Nevertheless, we can speculate that, if a larger FFA contains more face selective neurons which are tuned to more face-exemplars, it may produce a better cortical representation of the range of human faces, which in turn may improve face recognition memory. Future studies utilizing concurrent measurements of FFA responses and face recognition performance, using both familiar and non-familiar faces may provide important new insights into the relationship between FFA volume and face recognition performance. Likewise, examination of recognition performance with simultaneous fMRI and electrophysiological recordings in animal models (Tsao et al., 2006) will be essential in elucidating the specific relationship between development of face selective regions and behavioral performance.

An important question is why face selectivity in particular undergoes such a prolonged development during adolescence. One intriguing possibility is that this reflects the ongoing experience-dependent neural changes that accompany encoding and recognition of newly encountered faces, which most socially active adolescents frequently experience. Developmental theories often refer to faces as a special type of visual stimulus, partly due to the early onset of face processing shortly after birth (Johnson et al., 1991). Our findings complement this notion by suggesting that the particularly prolonged development of face selective responses in the VTC may be an additional special feature of face processing, providing a basis for continued plasticity at least throughout the second decade of life. Alternatively, this prolonged plasticity may be a more widespread characteristic of the VTC, which may be evident also after expert learning of new visual categories (Gauthier et al., 1999, 2000). An intriguing possibility is that the fusiform gyrus remains plastic across the entire life span as individuals continuously encounter new faces throughout their lives and recognize them later. These possibilities are not mutually exclusive and could be addressed in future studies.

In conclusion, we found evidence for a prolonged development of face selective activations in the right VTC through adolescence that was associated with increases in the size of face selective regions, increases in responsiveness and selectivity for face stimuli, greater differentiation of distributed responses to face and object in the VTC, and behavioral improvements in face recognition memory. This development occurred in both male and female participants and was not dependent on the age of face stimuli. Our findings of differential development across the VTC provide important constraints for developmental theories of high-level vision, suggesting that development of face recognition occurs over a surprisingly long time, and is likely to require extensive experience. These findings set the stage for understanding the neural basis of developmental disorders in face processing.

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Personal familiarity influences the processing of upright and inverted faces in infants

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Infant face processing becomes more selective during the first year of life as a function of varying experience with distinct face categories defined by species, race, and age. Given that any individual face belongs to many such categories (e.g. A young Caucasian man's face) we asked how the neural selectivity for one aspect of facial appearance was affected by category membership along another dimension of variability. 6-month-old infants were shown upright and inverted pictures of either their own mother or a stranger while event-related potentials (ERPs) were recorded. We found that the amplitude of the P400 (a face-sensitive ERP component) was only sensitive to the orientation of the mother's face, suggesting that "tuning" of the neural response to faces is realized jointly across multiple dimensions of face appearance.

Keywords: face recognition, familiarity, ERPs, perceptual learning

INTRODUCTION

Selectivity is a key feature of mature representations of facial appearance. As demonstrated by a substantial body of behavioral results obtained from adult observers, faces are processed and recognized by distinct processes that do not appear to be readily applied to control stimuli (including inverted, scrambled, or contrast-negated faces). Such features of face processing include high-fidelity encoding of so-called "2nd-order" features of the face (Rhodes et al., 1993) and holistic processing of face patterns as revealed by phenomena like the composite-face effect (Young et al., 1987) and the part-whole effect (Tanaka and Farah, 1993). That these features of adult face processing are generally evident in unaltered faces, but absent or reduced in faces that have been transformed by disorientation or other image-level manipulations indicates that the visual system has learned important distinctions between classes of face-like stimuli. That is, true faces are distinguished from non-face images that share many of the same low-level properties. Presently, we examine how such distinctions are acquired (and thus how selective representations are constructed) by investigating the selectivity of infants' neural response to faces belonging to distinct categories.

The perceptual processes that contribute to face recognition in infancy become increasingly selective during the first year of life. Specifically, infants show poorer recognition of face exemplars within categories they are not exposed to frequently. This process has been termed "perceptual narrowing" (Nelson, 2001; Scott et al., 2007) and has been observed in the context of face categories defined by species (Pascalis et al., 2002; Sugita, 2008), gender (Quinn et al., 2002), and race (Kelly et al., 2005, 2007). In each case, younger infants are able to successfully individuate faces in a broader set of categories than older infants, with older infants being worse at discriminating between faces belonging to infrequently encountered categories. Perceptual narrowing results demonstrate that experience shapes selectivity during infancy, leading to profound perceptual consequences. The first year of life (specifically the 6- to 9-month age range) is thus a critically important time period for studying how distinctions are made between faces and non-face control stimuli, as well as between distinct categories of faces defined at varying levels of granularity. Our aim in the present study was to characterize how distinct types of face selectivity may interact during this period of development. This is distinct from any effort to characterize true perceptual narrowing, but shares the same broad goal of perceptual narrowing research insofar as we wish to more fully understand the developmental timecourse of differential processing for various kinds of faces and face-like stimuli. Specifically, we ask how differential processing of faces at a sub-ordinate level (personally familiar vs. unfamiliar face) may lead to differential processing at a basic level (face vs. inverted face).

What do we mean when we say that faces may be distinguished at different levels of granularity or "scales?" Intuitively, we suggest that degrees of face selectivity can be considered hierarchically. A coarse face processing strategy (that is not very selective) may only differentiate between faces and non-faces (such as inverted, photo-negative or distorted faces (**Figure 1**, top row). A more sophisticated and selective representation may distinguish between face categories on the basis of species, gender, race, or age (**Figure 1**, middle row). Finally, a very sophisticated representation of facial appearance may be able to make distinctions between individual exemplars within a category. This naïve ontology is

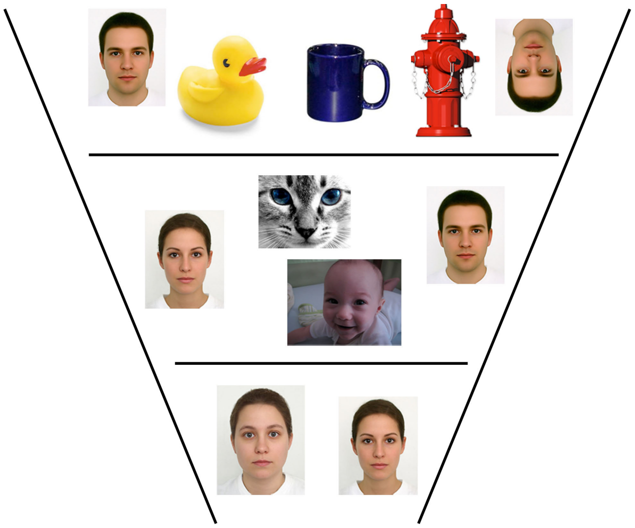

FIGURE 1 | A Hierarchical ontology for face categories. A schematic view of multiple levels of face specificity. At the broadest level on top of this diagram, faces may only be differentiated from non-faces. Continuing downwards, faces are grouped by categories like race and species, ultimately grouped within those categories by individual identity. The goal of the current study is to determine how these varying levels of specificity affect one another in terms of the neural response to faces.

not motivated by any *a priori* understanding of how face perception is behaviorally or neurally organized. For example, we do not suggest that we can clearly state where category boundaries lie between the face and non-face stimuli we have included in **Figure 1**. Nonetheless this framework serves as a useful starting point for asking important questions about how selectivity develops. How (if at all) do the representations used to make the basic distinction between upright faces and other stimuli depend on the representations used to make distinctions between individuals? Do increasingly specific representations of facial appearance develop hierarchically, so that increasingly "fine-grained" distinctions between faces can be made as development progresses and "coarser" distinctions are mastered? Alternatively, does experience with particular individuals drive selectivity across all scales? That is, does extensive experience with a particular face lead to an enhanced ability to make distinctions between that face and its counterparts at all levels of granularity? Do infants learn a neural representation of facial appearance solely by accumulating and applying general principles of selectivity (e.g. "young, white, female faces are processed differently than other faces") or by applying the information if representations can also be learned from specific exemplars (e.g. "Faces that don't look like Mom aren't processed like her").

In the present study, we asked if the magnitude of a neural "inversion effect" for faces was contingent on face familiarity. Face recognition performance suffers more than generic object recognition following a 180-degree planar rotation of the image (Yin, 1969), and ERP waveforms also exhibit a marked inversion effect. Specifically, the N170 component in adults, for example, tends to be delayed and of larger amplitude in response to inverted faces (Rossion et al., 1999). The distinction between upright and inverted faces is an example of differential processing at a "coarse" grain, while face familiarity obviously represents a comparatively much "finer" grain at which differential processing may be realized. Further, both face orientation and face familiarity have been studied behaviorally in infants, making their interaction an attractive starting point for investigating the neural basis of perceptual narrowing across multiple aspects of face variability. Newborn infants show a behavioral response suggestive of selectivity for upright vs. inverted faces (Farroni et al., 2005), an ability that improves dramatically over subsequent months (Turati et al., 2004). Further, within the first few months infants can visually discriminate the mother's face from that of a stranger (Pascalis et al., 1995; de Haan et al., 2001). Given that infants of sufficient age can differentiate between faces along these dimensions, how do familiarity and orientation interact at the level of the neural response? We used event-related potentials (ERPs) to examine the extent to which face category membership defined at one "level" (as in **Figure 1**) modulated the selectivity of the neural response at another level.

We varied face orientation (upright vs. inverted) in a within-subjects design, varying face familiarity across participants. Our index of the neural response to faces was the P400 component, which is a face-sensitive response observed over occipital electrode sites (de Haan and Nelson, 1999; Halit et al., 2003). This component begins to emerge as early as 3 months of age (Halit et al., 2003, 2004; Scott and Nelson, 2004; Macchi Cassia et al., 2006) and is thus a useful way to examine neural face processing during the first year of life. Our question is whether or not the P400 exhibits differential processing for upright and inverted faces as a function of face familiarity, or if there appears to be no influence of face familiarity on the magnitude of the inversion effect. The former case would suggest that face orientation selectivity for this component is initially driven by individual exemplars, while the latter would suggest that selectivity has been acquired and applied generally to wider subsets of faces by 6 months of age. We find evidence of an intriguing interaction between these levels of face categorization, suggesting that the exquisite selectivity exhibited by adult observers may be built up in an exemplar-driven fashion.

MATERIALS AND METHODS

PARTICIPANTS

Infants were recruited from a metropolitan area. All infants were born full-term with no known pre- or perinatal complications. Informed consent was obtained from the parents and infants were assigned to one of two experimental groups. Infants belonging to the "Mother's Face" group were presented with their mother's face during the task, and infants belonging to the "Stranger's Face" group were presented with a stranger's face (see Stimuli for details). The final sample in the "Mother's Face" group consisted of 20 participants (8 males) with a mean age of 183 days (range 177–191 days). An additional 40 infants were tested but not included in the final sample due to eye and/or body movements that resulted in excessive artifact ($n = 29$) or fussiness that resulted in too few trials being recorded ($n = 11$). The final sample in the "Stranger's Face" group consisted of 14 participants (9 males) with a mean age of 184 days (range 177–188 days). An additional 13 infants were tested but not included in the final sample due to excessive eye and/or body movements that resulted in excessive artifact ($n = 11$) or fussiness that resulted in too few trials being recorded ($n = 2$). We emphasize that participants were only excluded for reasons relating to data quality (ERP artifacts) and not due to boredom or disinterest in the task. Further, our attrition rate (though high) is consistent with the 50–75% dropout rate typically reported for infant ERP studies (DeBoer et al., 2007).

STIMULI

The stimuli consisted of color images of female faces displaying neutral expressions. Each woman was photographed wearing a gray scarf in order to conceal any clothing while seated in front of a gray background. Infants in the "Mother's Face" group viewed pictures of their own mother's face presented multiple times in both upright and inverted orientations. Infants in the "Stranger's Face" group viewed pictures of an unfamiliar female face (mothers of "Mother's Face" infants) that was judged to be dissimilar-looking to their own mother, but matched for the presence of eyeglasses and/or race of the model. This face was also presented in upright and inverted orientations.

PROCEDURE

Testing took place in a dimly lit, quiet room after application of the sensor net. Infants were tested while sitting on their parent's lap. Stimuli were presented using E-Prime software v1.2 (Psychology Software Tools Inc., Pittsburgh, PA, USA). The faces were presented on the center of the screen on a white background. The computer monitor was 48 cm wide and 31 cm high. When viewed from a distance of 60 cm, the faces on average subtended a horizontal angle of approximately 16° and a vertical angle of 15°. A video camera mounted above the monitor and centered on the infant's face allowed for observation of the infant at all times during the testing session. On-line judgments were made to present the pictures only when the infant was attending to the monitor. Trials were immediately marked for deletion by the experimenter if the infant looked away during stimulus presentation.

Stimuli were presented for 500 ms followed by an experimenter controlled inter-stimulus interval of at least 1500 ms during which time the screen was white. The two orientations were randomly presented with equal probability, with the constraint that stimuli from the same orientation were not repeated more than three times in succession. Stimulus presentation continued until the infant became bored or too fussy to attend, with a maximum of 100 trials. The average number of total trials viewed by infants was 71 and 80, for the "Mother's Face" and "Stranger's Face" groups respectively.

ERP RECORDING AND ANALYSIS

ERPs were recorded using a 64-channel Geodesic Sensor Net v2.0 (Electrical Geodesics Inc., Eugene, OR). EEG was recorded continuously and referenced to a single vertex electrode (Cz). Signals were amplified using an EGI NetAmps 200 amplifier with a band-pass filter of 0.1–80 Hz and a sampling rate of 200 Hz. Impedances were checked on-line prior to beginning the session and were considered acceptable if lower than 50 KOhm.

Continuous EEG data were processed offline using NetStation v4.1.2 (Eugene, OR). A 30-Hz lowpass filter was applied and trials were constructed that consisted of a 100 ms baseline period and 1500 ms period following stimulus onset. Data were baseline corrected to the average voltage during the 100 ms prior to stimulus onset. Segmented data were edited for EOG and motion artifact. Data from individual sensors were rejected if there was artifact resulting from poor contact or movement. The entire trial was excluded if more than nine sensors had been rejected, or if an eyeblink or other significant artifact had occurred. Of the remaining trials, individual channels containing artifact were replaced using spherical spline interpolation. Individual subject averages were constructed separately for the upright and inverted faces ($M = 22$ trials per condition in both groups), and data were re-referenced to the average reference.

Inspection of the grand-averaged waveforms revealed a well-defined P400 component that was subsequently analyzed within a time window 345–599 ms. Electrode groupings and time windows were selected based on previous reports of this component and through visual inspection of the grand-averaged and individual waveforms. Ten occipital electrodes were identified for our analysis of the P400 (32, 33, 36, 37, 38, 39, 40, 41, 44, 45) which were further partitioned into left, midline and right regions for analysis (**Figure 2A**).

RESULTS

The peak amplitude (maximum value attained within the previously mentioned time window) and latency of the P400 was analyzed for each group separately using a 2 × 3 repeated measures analysis of variance (ANOVA) with Orientation (upright, inverted) and Region (left, mid, right electrode groupings) as within-subjects factors. Greenhouse-Geisser corrected degrees of freedom were used and *post hoc* paired t-tests were conducted using a Bonferroni correction for multiple comparisons.

"MOTHER'S FACE" GROUP

Amplitude

There was a main effect of Orientation, $F(1, 18) = 5.83$, $p = 0.027$, such that the mother inverted ($M = 15$ μV, $SD = 5.6$) elicited a significantly larger P400 peak amplitude than mother upright ($M = 12.6$ μV, $SD = 6$; see **Figure 2B**). We note that in particular that there were no interactions of Region (left, midline, or right sensor groups), which is why we have chosen to present an average waveform over all 10 electrodes in **Figure 2**.

Latency

For latency to the P400 peak, there were no main effects or interactions of Region or Orientation.

"STRANGER'S FACE" GROUP

Amplitude

For the amplitude of the P400, there were no main effects or interactions of Orientation or Region. (see **Figure 2C**).

Latency

For latency to the P400 peak, there was a main effect of Region, $F(2,12) = 5.23$, $p = 0.019$. Paired comparisons revealed that the midline electrode group ($M = 478$ ms, $SD = 39$) peaked marginally faster than the left electrode group only ($M = 506$ ms, $SD = 34$), $t(13) = 2.67$, $p = 0.057$.

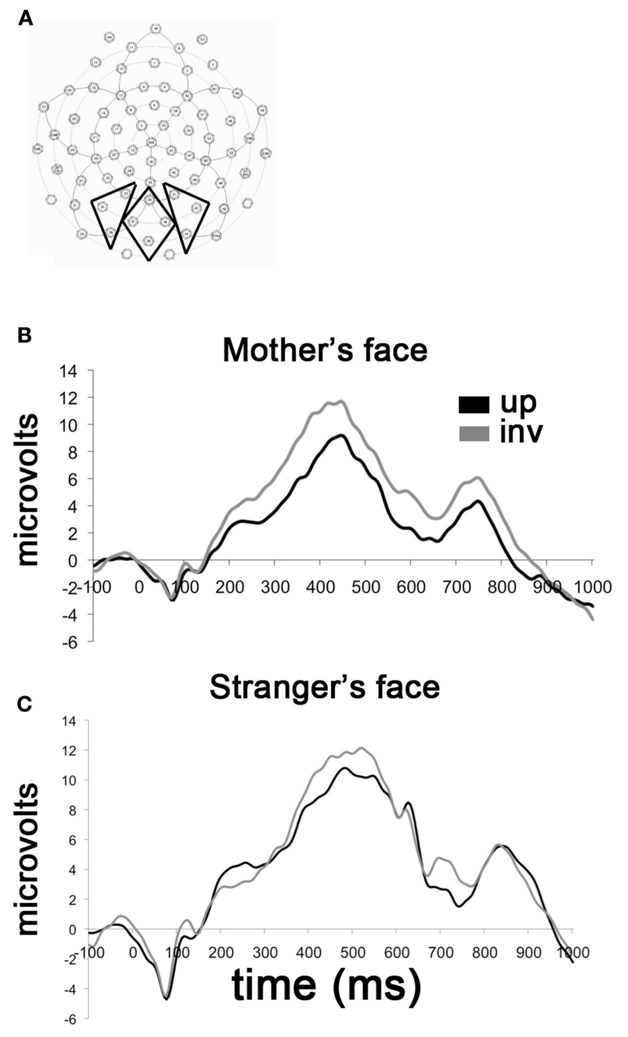

FIGURE 2 | Responses to upright and inverted faces as a function of personal familiarity. (A) The P400 waveform depicted here represents averaged activity over 10 occipital electrodes included in the analysis. These 10 electrodes were grouped into three distinct regions for statistical analysis of regional effects. **(B)** Grand-averaged waveforms obtained for mother's face upright and mother's face inverted. **(C)** Grand-averaged waveforms obtained for a stranger's face upright and inverted. Note the absence of a differential response to upright and inverted faces in the "unfamiliar face" group.

In summary, we find evidence of a differential response to upright and inverted faces only in the "Mother's Face" group. Specifically, the amplitude of the P400 is larger to inverted faces than to upright faces. In **Figure 3**, we display the amplitude data across subjects as a function of stimulus type, sensor region, and subject group. There were no effects of face orientation on latency in either group, which may be due to fairly large variability across subjects (pooled SD across all conditions and groups = 36 ms).

Given the difference in the number of subjects included in the "Mother's Face" and "Stranger's Face" group, we also conducted an additional Monte Carlo analysis to rule out the possibility that a significant effect was observed in the larger group ("Mother's Face") simply due to the larger sample size. We carried out a "jackknife" procedure in which 14 participants were randomly sampled with replacement 10,000 times from the full set of 19 participants in the Mother's Face group. At each iteration, the *F*-statistic for the main effect of face inversion was computed. This allows us to compare the distribution of *F*-ratios obtained from this procedure to the observed *F*-ratio obtained from the Stranger's Face group. We find that this procedure yields an *F*-value for face inversion that is both significantly larger than one and also significantly larger than the F-ratio obtained from the Stranger's Face group as determined by the 5th quantile of the empirical jackknife distribution. We therefore conclude that the observed difference between the groups is likely not due to the difference in sample size.

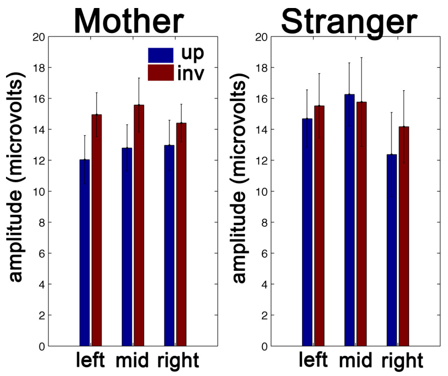

FIGURE 3 | Average P400 amplitudes by subject group and face orientation. At left, we present the between-subjects average of the P400 amplitude to the Mother's face in upright and inverted orientations for each of our three sensor groups. At right, the same results are presented for the Stranger's Face group. Error bars represent+/–1 sem. calculated over the subjects in each group.

DISCUSSION

Infants in the current study were presented with digital images of either their mother's face or a stranger's face presented in an upright and inverted orientation. ERP data revealed that the amplitude of the P400 was greater to mother inverted vs. mother upright. However, no amplitude differences for this component were observed for the upright vs. inverted stranger manipulation. In terms of our initial question regarding how face selectivity is realized across different conceptual scales, even as early as 6 months, evidence for exemplar-driven influence on face processing can be observed. We take this as evidence that face selectivity may indeed be learned in an exemplar-driven fashion. That is, increasing selectivity for facial appearance emerges by repeated exposure to particular individuals, resulting in an enhanced capacity for differential processing at multiple scales of selectivity.

Our result is consistent with adult data from a number of studies (Bruce, 1986). Most directly, Balas et al. (2007) reported a familiarity effect for judging face orientation, suggesting that increased exposure to a face during adulthood facilitates orientation processing. Along other dimensions of face variation, including gender (Rossion, 2002), race (Bruyer et al., 2004), and expression (Kaufmann and Schweinberger, 2004) similar familiarity effects are obtained. Narrowing may thus be driven by and constrained by the individual faces that are seen most often. It is also noteworthy that increased training with non-face categories including

"Greeble" stimuli (Gauthier and Tarr, 1997) and texture patterns (Husk et al., 2007) leads to inversion effects in adults. This may mean that selectivity in *any* domain may be driven primarily by the individual items experienced which subsequently cut deeply across scales of stimulus categories.

How do our data relate to other studies of the face inversion effect in infancy? A key point of comparison is to consider our results alongside those reported by de Haan et al. (2002), who presented 6-month-old infants and adults with upright and inverted images of human and monkey faces. While they report a species-specific inversion effect on the N170 component in adults, they also report the absence of any inversion effects on the "infant N170" (a negativity between 200–350 ms) and a non-specific inversion effect that is evident at the P400. This latter result is not consistent with the data we have reported here, insofar as monkey faces should certainly be unfamiliar to the infants in their task, and the models used in the human face trials were also not familiar to the infants. In neither case would our data suggest that an inversion effect should be evident at the P400. Moreover, there is an additional important discrepancy between our results and this previous study: The inversion effect reported at the P400 in this previous study goes in the opposite direction as our inversion effect. Inverted faces (both human and monkey) elicit a smaller positive peak at the P400, and effect which would appear broadly consistent with other results concerning the "narrowing" of face representations. Can we reconcile our results with this previous report? It is difficult to speak conclusively about the mutual relationship between such distinct results, but we briefly point out two important points that may be relevant to the differences we have described above. First, we note that while we did not observe any topographical differences between conditions, the de Haan paper reports that the inversion effect for Monkey faces was only evident over the left hemisphere, but obtained over both hemispheres for human faces. Second, it is perhaps important that the present study used only one model face per child (presented multiple times in each orientation) while the de Haan, Pascalis and Johnson study reports the use of 20 model faces per condition. The use of more model faces is laudable, and strengthens the generality of their results. In our case, obtaining a wide range of highly familiar faces was not practical, constraining the number of faces we could use in the "Stranger's Face" condition. We suggest that it is possible that the repetition of the same model face repeatedly may have important consequences on the components we have examined here, an issue that has not yet been studied in great depth.

Finally, what are we to make of the differing directions of the face inversion effects reported here and in the de Haan study? If we are to begin by comparing the results of each study to the established pattern of inversion effects in the adult literature we must decide if the important feature of the adult face inversion effect at the N170 is the fact that it is of greater absolute amplitude, or the fact that it is more negative. The former would appear to be consistent with our result, while the latter would be more consistent with an inversion effect in the opposite direction (a more negative positive deflection implies a smaller peak). One way to potentially resolve this ambiguity is to consider data from other components used to study adult face processing. Both the P1 and the P2 exhibit larger amplitudes for inverted faces (Itier and Taylor, 2002) suggesting that perhaps the hallmark of inversion effects is an overall increase in the absolute amplitude of the ERP signal, rather than increased negativity. Though this provides a tentative suggestion that our data are broadly consistent with adult ERP results, future study of the consistency of inversion effects at the P400 during the first year of life will ultimately prove invaluable. Finally, we note that changes in the sign of the inversion effect have been observed in longitudinal studies of infant memory (Bauer et al., 2006), indicating that the same child may "flip" the inversion effect between test sessions. This suggests that it is possible to find inversion effects of differing sign within a relatively short timespan of development.

We close by suggesting potential extensions of this study that would further clarify the consequences of differential processing at multiple scales of face categorization. First, an important extension of this work (that is unfortunately difficult to achieve due to limitations on infant ERP study designs) is the inclusion of multiple stimulus categories representing a more comprehensive sampling of "face space". (Valentine, 1991). Our comparison of familiar to unfamiliar female face processing across distinct subject groups is a fairly crude tool for examining the rich multi-dimensional code for facial appearance. As we have already noted, our desire to use personally familiar faces severely necessarily limited the number of distinct exemplars we could present to our infant subjects with, potentially limiting the generality of our results. Should exploring a larger set of stimuli within individual subjects prove practical (perhaps including father's faces, or other siblings), further elaboration of interactions across "scales" of face specificity would be vital to understanding the development of face recognition. Specifically, further characterizing how categories defined by race, age, or gender all interact with personal familiarity or basic-level face/non-face distinctions would be a crucial step towards describing the acquisition of selective face responses. Second, a similar means of extending the present results would be to consider the impact of personal familiarity (or any other categorical distinction between faces) on the processing associated with other kinds of transformed face stimuli. Here, we have only examined the inversion effect as one example of a "coarse-scale" distinction between face and non-face control images. There are, of course, a wide range of similar transformations that can be carried out on face images that leave many low-level features of the face intact, including color or luminance negation (Galper, 1970; Kemp et al., 1990), or scrambling of face parts. Indeed, many other aspects of face recognition (such as the acquisition of view-invariant recognition) are developing during the first year of life, making them interesting candidates for a similar analysis. Examining how differential processing at the level of exemplars or other face categories impacts differential processing of these other kinds of transformation would represent an additional important contribution.

Finally, we also point out that in our current data we were unable to consistently identify the so-called "infant N170," an additional face-sensitive component that is frequently reported at ~290 ms (de Haan et al., 2002). Should it prove possible to obtain a sufficiently robust response that this component could also be analyzed in the same manner as we have examined the P400 here, it would be useful to determine the extent to which these two components index similar or distinct aspects of face selectivity behave similarly. Similarly, in terms of further understanding the neural substrate supporting our effects, limits in the spatial resolution of ERPs preclude us from drawing inferences as to where in the brain these processes are taking place. Extending the study presented here using Near-Infrared Spectroscopy (NIRS) would permit more robust localization and provide an important complement to this work (Otsuka et al., 2007).

To conclude, our findings suggest that by 6 months of age, the neural architecture underlying face processing reflects selectivity learned from experience with particular faces. Differential processing at coarse scales appears to be modulated by differential processing at comparatively finer scales. Overall, these data complement the extensive behavioral literature on the development of face processing by revealing that the ability to process information about familiarity and orientation are not fully independent.

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Early visually evoked electrophysiological responses over the human brain (P1, N170) show stable patterns of face-sensitivity from 4 years to adulthood

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Whether the development of face recognition abilities truly reflects changes in how faces, specifically, are perceived, or rather can be attributed to more general perceptual or cognitive development, is debated. Event-related potential (ERP) recordings on the scalp offer promise for this issue because they allow brain responses to complex visual stimuli to be relatively well isolated from other sensory, cognitive and motor processes. ERP studies in 5- to 16-year-old children report large age-related changes in amplitude, latency (decreases) and topographical distribution of the early visual components, the P1 and the occipito-temporal N170. To test the face specificity of these effects, we recorded high-density ERPs to pictures of faces, cars, and their phase-scrambled versions from 72 children between the ages of 4 and 17, and a group of adults. We found that none of the previously reported age-dependent changes in amplitude, latency or topography of the P1 or N170 were specific to faces. Most importantly, when we controlled for age-related variations of the P1, the N170 appeared remarkably similar in amplitude and topography across development, with much smaller age-related decreases in latencies than previously reported. At all ages the N170 showed equivalent face-sensitivity: it had the same topography and right hemisphere dominance, it was absent for meaningless (scrambled) stimuli, and larger and earlier for faces than cars. The data also illustrate the large amount of inter-individual and inter-trial variance in young children's data, which causes the N170 to merge with a later component, the N250, in grand-averaged data. Based on our observations, we suggest that the previously reported "bi-fid" N170 of young children is in fact the N250. Overall, our data indicate that the electrophysiological markers of face-sensitive perceptual processes are present from 4 years of age and do not appear to change throughout development.

Keywords: face recognition, N170, ERP, development

INTRODUCTION

The ability to recognize faces is perhaps the most developed of all visual perceptual skills of the human adult brain (Bruce and Young, 1998). Despite an astonishing level of proficiency in face recognition abilities already in the first days (e.g., Maurer and Young, 1983; Johnson et al., 1991; Pascalis et al., 1995; Valenza et al., 1996; Simion et al., 1998), months (e.g., Fagan, 1972; Maurer and Salapatek, 1976; for reviews, Johnson, 1997; Simion et al., 1998) or years (e.g., Tanaka et al., 1998; Sangrigoli and De Schonen, 2004; McKone and Boyer, 2006; de Heering et al., 2007) of life, behavioral studies have indicated that children's ability to recognize faces shows marked development well into adolescence (e.g., Carey and Diamond, 1977, 1994; Blaney and Winograd, 1978; Ellis and Flin, 1990; for reviews, see Carey, 1992; Chung and Thomson, 1995). Yet, surprisingly little is known regarding the functional developments underlying this improvement and their neural correlates (e.g., Gathers et al., 2004; Golarai et al., 2007; McKone et al., 2009).

Children's improvement on perceptual face discrimination tasks, such as same-different judgments, suggests more than just memory development (e.g., Carey and Diamond, 1980; Mondloch et al., 2002, 2004). However, many functions, such as vernier acuity (Skoczenski and Norcia, 2002), sustained attention (Betts et al., 2006), the ability to narrow the focus of visual attention (Pastò and Burack, 1997), and the ability to use deliberate task strategies (Bjorklund and Douglas, 1997), to name a few, have been shown to improve across childhood and even into adolescence in some cases (see also Flavell, 1985). Consequently, whether age-related improvement on face processing tasks is specific to our ability to *perceive* faces *per se* (Carey and Diamond, 1977; Carey, 1992), or rather a product of age-related improvements in general sensory or cognitive functions, or general visual pattern recognition (Want et al., 2003; Crookes and McKone, 2009) is still highly debated.

Event-related potentials (ERPs) recorded from the scalp are particularly promising for clarifying this issue, and for understanding the developmental course of human face processing in particular (de Haan and Nelson; 1999; de Haan et al., 2003; Taylor et al., 2004) for at least two reasons. First, a behavioral response reflects the output of a large number of sensory, perceptual, cognitive and motor processes, so that variations in response time (RT) and accuracy are difficult to attribute to variations in a specific process (e.g., high-level perceptual processes) (Luck, 2005). In contrast, the excellent temporal resolution of ERPs provides a continuous measure of processing between a stimulus and a response, making it possible to more precisely determine which stage(s) of processing undergo changes with development. Second, ERPs can be recorded non-invasively in experiments that do not require complex task instructions, allowing experimenters to test of a wide age range of participants with the same experimental paradigm.

Since the pioneering work of Jeffreys (1989) on the vertex positive potential (VPP) elicited by faces, studies recording ERPs to pictures of faces in adults have focused on a sequence of well-characterized posterior components, most notably the P1 and the N170. The visual P1 (or P100) is an early occipital component, peaking at around 100 ms following stimulus onset in adults, which is thought to originate from striate and extrastriate visual areas (e.g. Clark et al., 1995; Di Russo et al., 2002). It is known to be sensitive to many low-level properties of visual stimuli and is perhaps the most documented visual evoked potential (see Regan, 1989). It is usually the first visually observed component, although it is sometimes preceded by an early response to large stimuli (C1, Jeffreys and Axford, 1972). The P1 is followed by a N1 component, termed the N170 when elicited by face stimuli. The N170 is a more prominent occipito-temporal negative component, named for its peak latency of approximately 170 ms (Bentin et al., 1996). Typically showing a pronounced right-hemisphere lateralization, the N170 clearly and consistently shows the largest amplitude to faces at lateral occipito-temporal electrode sites (**Figure 1**; e.g., Bötzel et al., 1995; Bentin et al., 1996; for a review of N170 face-sensitivity, see Rossion and Jacques, 2008). Some studies have also reported face-sensitive effects at the level of the earlier P1 component (e.g., Eimer, 1998, 2000; Itier and Taylor, 2004a), but these early effects are less consistent and appear to reflect low-level systematic differences between faces and other complex visual stimuli (Rossion and Jacques, 2008). The N170 thus remains the main focus of interest within the adult face-processing literature.

To date, only a handful of ERP studies have investigated face processing in normal developing populations of children between the ages of 5 and 16 years. A meta analysis of four prominent studies (Taylor et al., 2001; Itier and Taylor, 2004a,b; Batty and Taylor, 2006) which recorded ERPs to faces in children described large decreases in P1 amplitude with age together with smaller, task dependent, decreases in the latency of the P1 across childhood (Taylor et al., 2004). Important age-related changes in the amplitude, latency and scalp topography of the N170 were also reported. Specifically, latency of the N170 decreased with age, as much as 100 ms between 4–5 years and adulthood, with the steepest decrease occurring before 10–11 years. Amplitude of the N170, rather, was reported to have a "U" shaped developmental trajectory, being of least negative amplitude for children of 10–11 years. Children both older and younger than 10–11 years were reported to have larger (more negative) N170 amplitudes. Moreover, the topography of the N170 in children was marked by a dominant posterior positivity, rather than negativity, and adult-like topographical activity emerged starting in the mid-teenage years (Taylor et al., 2004). It was also reported that the N170 was often bifid in young children, having both an early (N170a) and later (N170b) peak. In older children and adults, only a single N170 peak was observed.

The reported age-related changes in children's raw ERPs to faces have been tentatively associated with developmental behavioral changes in the way children perceive faces. In particular, the developmental changes on the P1 have been associated with an increased ability to perceive faces holistically (Taylor et al., 2004), i.e. as a global and integrated representation (Tanaka and Farah, 1993; Rossion, 2008, 2009). Likewise, it was reported that in young children, the N170a and N170b were related to an increased reliance on holistic and configural processing strategies, respectively (Taylor et al., 2004).

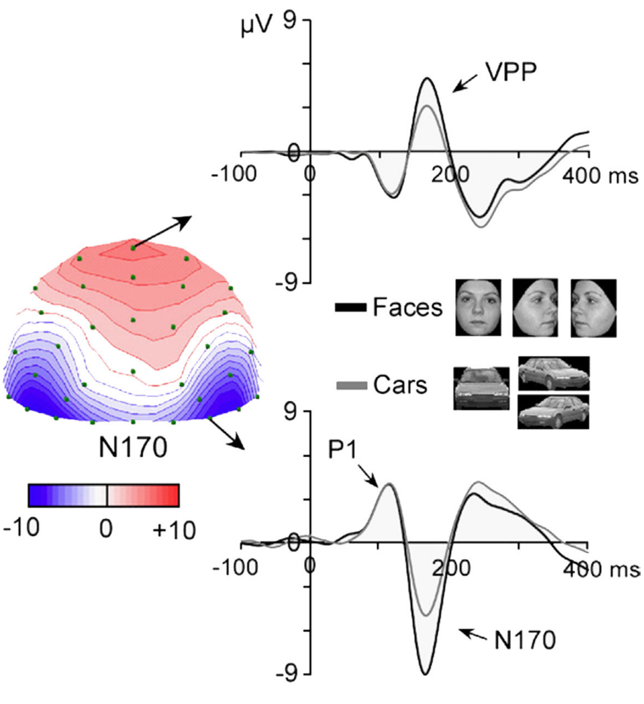

FIGURE 1 | Adapted from Rossion and Jacques (2008). The N170 is a negative component recorded from posterior lateral electrode sites following the presentation of faces and object stimuli from various categories (here pictures of cars). It peaks at about 160–170 ms following stimulus onset and is recorded between 130 and 200 ms. It is most prominent at the lowest occipito-temporal electrode sites, usually maximal on channels P8(T6) or PO8, or lower channels in this area. The component is larger in response to faces than objects in both hemispheres, with usually a larger response in the right hemisphere. The N170 is associated with a temporally coincident positivity on the vertex (CZ), the vertex positive potential (VPP), which shows identical response properties and largely reflect the projection of the occipito-temporal dipolar sources to the vertex (see Joyce and Rossion, 2005). The data presented are grand averages of 20 adult subjects presented with full-front and 3/4 profiles pictures averaged together.

However, while previous ERP studies of face stimulation in childhood (see also Henderson et al., 2003) indicate that visually evoked brain responses to faces change dramatically over the course of development, the magnitude of the changes reported by Taylor et al. (2004), in terms of latency and topography of the N170 in particular, is surprisingly large. Indeed, developmental studies with school age children looking at visually evoked ERPs to simple non-face stimuli have shown that both the amplitude and latency of P1 and N1 decrease across childhood (Buchsbaum et al., 1974; Brecelj et al., 2002), but the delay of latency compared to adult components is relatively small (10–20 ms), in particular for visual stimuli processed in the ventral stream (Coch et al., 2005). In light of this evidence, the interpretations of Taylor et al. (2004) should be subject to caution for several reasons. First, visual ERP parameters (latency, amplitude, topography) may undergo change throughout development and until adulthood due to age-related changes in any number of general factors that are unrelated to brain function (e.g., skin conductivity, skull thickness, head size, …) or to perceptual processes *per se* (e.g., EEG signal-to-noise ratio, attention, …). Second, even if these changes would reflect the development of perceptual processes, there has yet to be any direct evidence that they reflect face-specific perceptual processes rather than general improvements in visual pattern perception (Want et al., 2003). In short, large age-related changes are observed for the amplitude, latency or topography of the P1 and N170 recorded to face stimuli, but the exact same age-related changes might also be observed for ERPs to non-face stimuli.

This issue is important because the face-specific perceptual development theory (Carey and Diamond, 1977, 1980; Carey, 1992; Mondloch et al., 2002) is based essentially on behavioral data, and argues that development of face recognition abilities results from specific, qualitative differences between children and adults' face processing styles (but see Crookes and McKone, 2009; McKone et al., 2009). This theory assumes that we develop perceptual processes to utilize specifically for the identification and/or recognition of faces. These perceptual processes would not develop to the same extent, and therefore would not be as efficient, for the recognition of non-face visual objects.

With respect to supporting the face-specific perceptual development theory, previous developmental ERP studies of face processing appear to stop short in terms of control stimulation and analyses. Indeed, contrary to many studies performed with adults (e.g., Bötzel et al., 1995; Bentin et al., 1996; Rossion et al., 2000) none of the existing studies of face perception in childhood have systematically compared ERPs recorded in response to faces with ERPs recorded in response to complex visual categories, or to simpler stimuli matched for low-level properties (e.g., in adults, Rousselet et al., 2007). To our knowledge, only two previous developmental ERP studies presented non-face stimuli together with pictures of faces (Taylor et al., 1999, 2001). The first study used pictures of faces, cars and scrambled versions of these stimuli. However, the N170 in response to pictures of non-face stimuli was considered minimal and therefore not analyzed (Taylor et al., 1999). Similarly, apart from describing a decrease of N170 latency to pictures of flowers (Taylor et al., 2001), the second study did not focus on the specificity of these electrophysiological responses to faces.

Additionally, in terms of analyses, previous studies have compared ERP components directly across age groups without taking into account the potential confounds of such comparisons. For instance, amplitude of the P1 (or P100) may change substantially across development, even for very simple flashed or pattern reversal stimulation. For instance, Crognale (2002) reported a P100 in response to chromatic and achromatic stimuli that increased in amplitude from infancy until about the age of 9 years, where it sometimes exceeded 50 μV. After 9 years, the P100 slowly decreased until adulthood, where it typically reached amplitudes of less than 10 μV. Thus, although changes in P1 amplitude in response to face stimuli have been interpreted as an increased refinement of the ability to process faces holistically (Taylor et al., 2004), these changes may actually reflect only mere general developmental patterns that have nothing to do specifically with visual pattern perception, let alone face perception.

Furthermore, the general change in P1 amplitude over the course of development is highly problematic for the comparison of the N170 across age groups. Due to the temporal order of the visual evoked potentials, the amplitude of the P1 will inevitably influence the amplitude and topography of the subsequent components, including the N170. In fact, the report of Taylor et al. (2004) that the N170 in children was marked by a dominant posterior positivity, could likely be explained by the large positivity of the P1 in the childhood years. Specifically, the first negative deflection following the P1, the N170, may not deflect below zero if the amplitude of the P1 is too large. A small P1, on the other hand, would ensure that any negative deflection would reach well below zero microvolt. Therefore, it seems important to take into account age-related variations of the preceding P1 activity to make direct comparisons of the N170 amplitude and topography across age groups.

In summary, while the seminal studies of Taylor, Itier and colleagues have provided a wealth of information concerning the electrophysiological markers of face processing and the development of these markers, the absence of systematic comparison between faces and nonface patterns, and the non-consideration of general P1 age-related differences when assessing modulations of the N170, when make it premature to assume that the large modifications in terms of amplitude, latency and brain topography of P1 and N170 responses to faces are face-specific, or even face-sensitive (i.e., larger to faces than other visual stimuli). Thus, it becomes apparent that any suggestions that developmental changes in ERP signal are related to the development of face specific perceptual processing (i.e. configural or holistic processing; Taylor et al., 2004) were beyond the support of previously reported data and could be reconsidered.

The goal of the current study was to offer a stringent test of the face-specificity, or face-sensitivity, of the age-related changes of the two most commonly studies visual components in the face-processing literature, the P1 and the N170. To do that, we recorded ERPs from a higher density of channels than previous developmental studies of face processing (64 scalp electrodes as compared to ≈30 in Taylor et al., 2001; Itier and Taylor, 2004a,b; Batty and Taylor, 2006) from adults and children between the ages of 4 and 17 years, in response to faces and well-controlled visual stimuli, namely phase-scrambled faces. We also recorded ERPs to a familiar nonface object category (pictures of cars), to control for the effect of shape, and its scrambled counterpart. By recording ERPs to faces, cars and their respective scrambled counterparts, we aimed at isolating any face-specific responses, and characterizing their development over childhood and adolescence. Specifically, this paradigm allowed us to subtract the visual responses elicited by the phase scrambled stimuli from the visual responses recorded to faces and cars. This subtraction process serves to remove much of the response to faces and cars that could be attributed to low-level characteristics of the stimuli, leaving responses that were specific to the two stimulus categories. Furthermore, we focused on using methods of data reduction that would isolate, to the greatest extent possible, the N170 response from that of the preceding P1 in order to make reliable comparisons of the stimulus effects at the N170 across age groups.

MATERIALS AND METHODS

PARTICIPANTS

Seventy-two child participants (36 females; age range 4–17 years old; 6 left-handed) and 11 adults (7 males, mean age 23, age range: 20–29, 1 left-handed) were included in the final sample (see **Table 1**). ERP data from seven additional children were recorded but could not be used due to excessive artifacts contamination and low signal-to-noise ratio (SNR). All participants had normal or corrected vision. Written informed consent was obtained from each participant and/or one of their parents, and verbal consent was obtained from each participant prior to the experiment.

STIMULI

Two sets of 42 colored photographs of full front faces and cars were used (**Figure 2A**). Faces were without glasses, facial hair or make-up, and posed a neutral expression. All pictures were trimmed to remove the background and, in the case of faces, the clothing and hairline. Two additional sets of stimuli were created by phase-scrambling the faces and the cars using a Fourier phase randomization procedure (Näsänen, 1999) (**Figure 2A**). The phase randomization procedure replaces the phase spectrum of the images with random values, keeping the amplitude spectrum of the image unaltered. It yields images that preserve global low-level properties of the original image (i.e., luminance, contrast, color distribution, spatial frequency spectrum) while entirely degrading shape information. Thus, the "scrambled" images used here are fundamentally different than the "scrambled" stimuli used by Taylor et al. (1999), which were made of original images divided into randomly organized squares. This latter manipulation disrupts shape as well, but introduces low-level differences (e.g. sharp edges) that may greatly influence early visual responses. Face and car stimuli were embedded in a light grey frame (lighter than the background) before the phase-randomization procedure so that these stimuli and their scrambled counterpart would stimulate an equal portion of the visual field. All stimuli of the present study subtended ~3.15° × 4.30° of visual angle.

PROCEDURE

After electrode-cap placement, subjects were seated at a viewing distance of 100 cm from a computer monitor. Stimuli were displayed on a grey background using E-prime 1.1. In each trial, a fixation point (a small starfish) appeared at the centre of the screen for 400 ms, followed approximately 400 ms (randomized between 300 and 500 ms) later by the presentation of the test stimulus for 500 ms. The offset of this stimulus was followed by an inter-trial interval of about 2250 ms (2100–2400 ms) (**Figure 2B**). For the adult group, the timing values were slightly shorter (initial fixation 300 ms, delay 300 ms, stimulus 300 ms, intertrial interval between 1600 and 1800 ms), which was done to maintain participants' attention during the task. Since this study's interest was in the earliest responses to faces, before 300 ms, the shorter duration of visual stimulation in the adult group was not considered a potential problem for comparison of adult and child data. Participants were asked to judge whether each presented stimulus was an object (face or car) or a "texture" (scrambled versions), and gave their response by pressing either a left or a right keypad with their left or right hand. The hand associated with each response (object, texture) was counterbalanced across participants. Adults responded with two fingers of their dominant hand. Participants were instructed to fixate the centre of the screen for the duration of each block and to respond as quickly and accurately as possible. There were 84 trials per condition (42 stimuli in each set repeated two times each), broken in 14 blocks of 24 stimuli each. The order of conditions was randomized within each block. At the beginning of the experiment, all children completed a minimum of six training trials to ensure that they understood the task.

EEG recording

The EEG was recorded from 64 Ag/AgCl electrodes mounted in an electrode cap (Waveguard, ANT). Electrode positions included the standard 10–20 system locations and additional intermediate positions. Vertical and horizontal eye movements were monitored using four additional electrodes placed on the outer canthus of each eye and in the inferior and superior areas of the right orbit. During EEG recording, all electrodes were referenced to the left mastoid, and electrode impedances were kept below 10 kΩ. EEG was digitalized at a 1000 Hz sampling rate and a digital anti-aliasing filter of 0.27*sampling rate was applied at recording (at 1000 Hz sampling rate, the usable bandwidth is 0 to ~270 Hz). After a 0.1 Hz high-pass and 30 Hz low-pass filtering of the EEG, trials contaminated with eye movements or

Table 1 | Age ranges and mean ages of the children included in each age group as well as the mean number of trials that contributed to the individual averaged data for each condition.

Child age group	n	Age range in years (days)	Mean age in days	Mean (standard deviation) number of trials contributing to averaged data in each condition			
				Face	Car	Scrambled face	Scrambled car
Group 1	9	4–6 (1783–2349)	2022	54.8 (11.6)	55.0 (9.7)	55.9 (8.2)	56.1 (10.0)
Group 2	9	6–8 (2427–2910)	2754	62.0 (10.3)	61.4 (11.3)	59.1 (12.5)	59.7 (12.0)
Group 3	9	8–9 (2931–3411)	3162	59.2 (15.1)	54.8 (14.6)	54.3 (14.5)	55.7 (16.4)
Group 4	9	9–11 (3455–4071)	3837	69.7 (9.1)	66.9 (10.4)	70.4 (9.9)	68.8 (9.0)
Group 5	9	11–12 (4141–4637)	4424	66.2 (12.2)	63.7 (13.8)	64.4 (13.0)	64.1 (10.0)
Group 6	9	12–14 (4646–5254)	4938	62.7 (11.9)	61.9 (10.2)	62.2 (14.1)	61.4 (13.9)
Group 7	9	14–16 (5367–5774)	5340	65.9 (11.7)	63.6 (12.6)	64.7 (14.6)	66.1 (13.3)
Group 8	9	16–17 (5831–6238)	6079	78.2 (5.5)	77.4 (4.5)	77.3 (4.6)	77.2 (4.4)

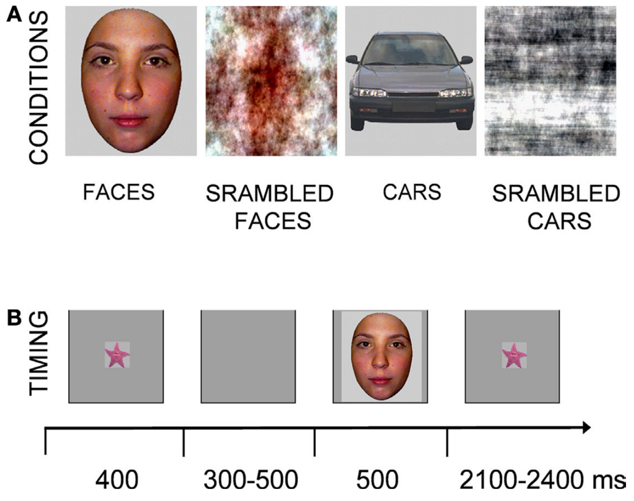

FIGURE 2 | (A) Examples of stimuli used in this study, faces, phase-scrambled faces, cars and scrambled cars. **(B)** Timeline of the events during the recording of event-related potentials.

other artifacts (≥ ± 80 μV) were marked and rejected. When there were too many blink artifacts (21 participants), a correction was applied using a principal component analyses method (Ille et al., 2002). Incorrect trials and trials containing EEG artifacts were removed. The average number of trials remaining in each condition is reported separately for each age group in **Table 1**. Due to a greater number of behavioral errors and artifacts in the EEG, slightly fewer trials were averaged for younger children (4–9 years old) than the older children (9–17 years old) and adults. Fewer trials may be a factor contributing to the lower SNR of the EEG for younger children compared to older children and adults. However, and most importantly, this was true for all four conditions, for which the average number of trials was almost identical in every group tested (**Table 1**). If anything, this small general difference between groups could slightly enhance or reduce general age-related changes, for which we completely replicated previous observations, but not age-related differences between conditions, the main focus of the present study.

Participant averages were baseline corrected using the 200 ms pre-stimulus epoch and then re-referenced to a common average reference.

Electrophysiology

Following visual stimulation, we observed both the *P1*, which was largest on bilateral lateral occipital electrodes (O1/O2, or PO7/PO8) and peaked at around 100 ms for all age groups (see **Figure 3**), and the following *N170*, which, in adults, had an occipito-temporal topography to faces and cars and peaked at about 150 ms, being maximal on lateral electrode sites (P7/P8), with a right hemisphere advantage (see **Figure 4**).

Amplitude values of these two components were measured at different pairs of occipito-temporal electrodes in the left and right hemisphere where they were the most prominent (for the *P1*: O1/2, PO5/6, PO7/8; for the *N170*: P7/8, PO7/8) (**Figures 3 and 4**). Amplitude and latency of the P1 was quantified for each condition and each subject as the peak of the component. Given the large variance in component latency across subjects, the P1 peak was defined as the maximum voltage within a ~50 ms window which was defined separately for each subject by visual inspection of the waveforms (e.g., between 80 and 130 ms in adults). The same temporal window was used for all conditions in each subject. While the P1 peak was quite easy to identify in all participants and conditions, the N170 amplitude and latency values were much more difficult to measure. In fact, the N170 was extremely small for the conditions in which scrambled pictures were presented, and peak measures were not reliable in individual participants. Therefore, we measured this component only for the face and car conditions. Moreover, due to the presence of a "bifid" N170 in the three youngest age groups, as previously reported (Taylor et al., 2004), the identification of the N170 was ambiguous in the grand-averaged data. Importantly though, for a large majority of individual subjects, the first negative deflection following the P1 was clearly visible, and even when it was smaller in amplitude than the second deflection, we considered the N170 to be the first negative deflection following the P1 peak.

Statistical analyses comparing age groups were performed by dividing the child participants into nine age-groups of an equal number of nine participants (see **Table 1**). All effects with two or more degrees of freedom were adjusted for violations of sphericity using the Greenhouse-Geisser correction when appropriate. Even though data were grouped for statistical analyses and for the sake of clarity, they were also displayed at the individual level in several illustrations.

RESULTS

BEHAVIORAL RESULTS

Behavioral accuracy rates and RTs to correct responses in the face/car vs. scrambled stimuli classification were analyzed in an ANOVA with within-subject factors of *Shape* (non-scrambled, scramled) and *Category* (face, car) and the between subject factor of *Age Group* (nine levels). Analyses of Accuracy revealed a main effect of *Category* ($F_{1,74} = 18.29$, $p < 0.001$), due to higher (~1.0%) accuracy for faces/scrambled faces than car/scrambled cars, as well as a significant interaction between *Shape* and *Category* ($F_{1,74} = 10.07$, $p < 0.002$) driven by the fact that accuracy was lower for cars than for the other three stimulus types, which did not differ in accuracy. There was also a main effect of *Age Group* ($F_{1,74} = 5.75$, $p < 0.001$), because accuracy for the youngest group (4–6 years) was significantly lower than for the older age groups, starting with the 3rd age group, (8–9 years), (86.4% vs. >96.1%). Accuracy did not differ between any other age groups. There were no interactions involving the factor *Age Group*.

Analyses of RTs revealed main effects of *Category* ($F_{1,74} = 15.95$, $p < 0.001$), due to faster RTs (~10 ms) for faces/scrambled faces than car/scrambled cars, and *Shape* ($F_{1,74} = 5.76$, $p < 0.019$), due to faster RTs (~12 ms) to the scrambled stimuli compared to the non-scrambled stimuli. These main effects were qualified by a significant interaction between the two factors ($F_{1,74} = 6.04$, $p < 0.016$) which was driven by an RT advantage for scrambled stimuli over non-scrambled stimuli which was much greater for the car category (~18 ms) than the face category (~5 ms) due to the slow RTs to the intact car stimuli. There was also a main effect of *Age Group* due to the general decrease in RTs with age, which stabilized, however, in the 11- to 12-year-old group. In short, the classification task – which

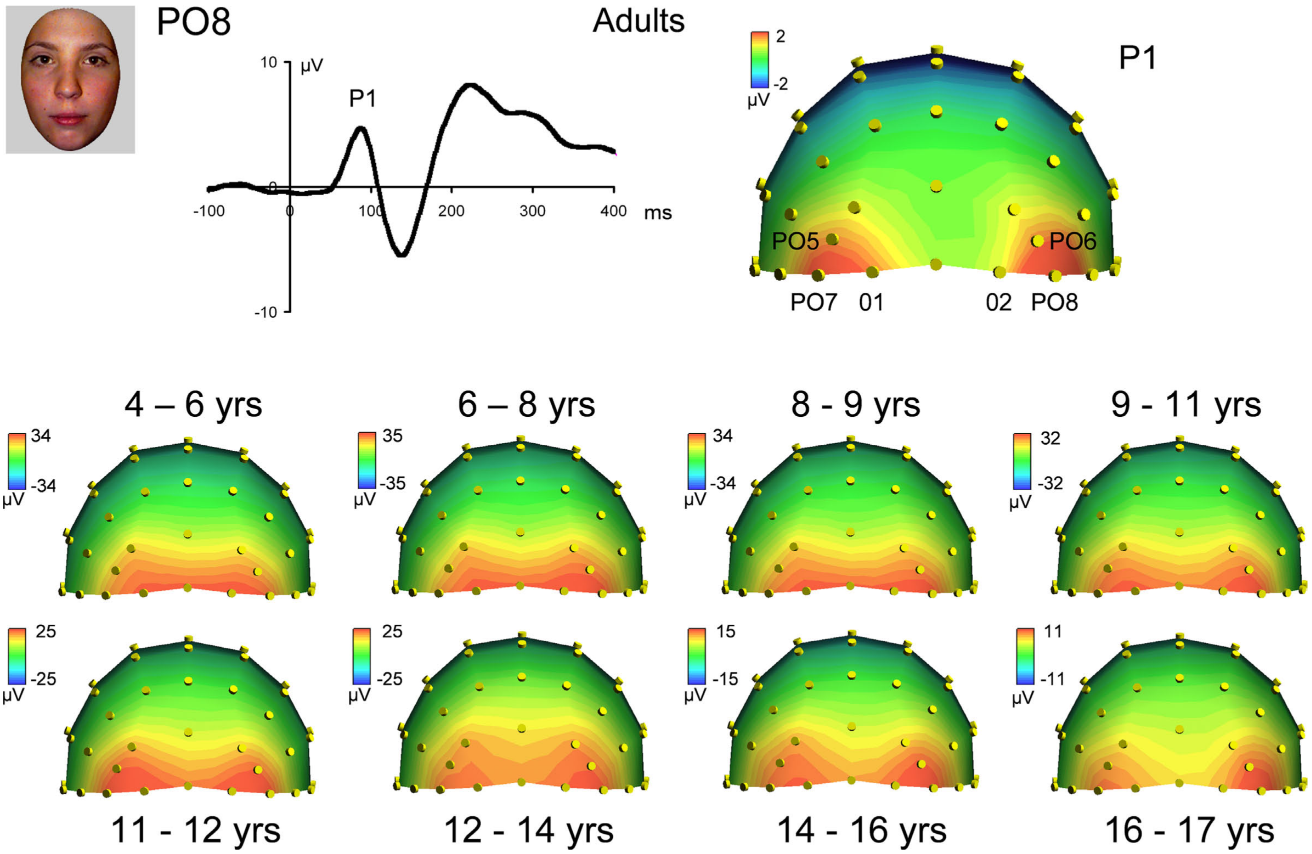

FIGURE 3 | Scalp topographies at the peak of the visual P1 component in responses to faces in adults (top) and all age groups. Note the increasing separation of the lateral occipital sources generating the P1. This variation may be due to changes in head size across development. The topographic plots are snapshots of the P1 response at 90 ms for adults, and at the following latencies for the youngest to oldest age groups, respectively: 109, 108, 111, 104, 105, 108, 95 and 102 ms.

was intended mainly to maintain heightened levels of attention to the stimuli throughout the experiment – was performed easily by participants of all age groups.

EVENT-RELATED POTENTIALS

Visual component P1

Topographical distribution of components on the scalp. The first consistent brain response to visual stimulation that can be observed on the human scalp, the P1, was observed in all participants for all conditions. Visual inspection of the data showed that the topography of the P1 in response to faces appeared to change across development. Specifically, in young children, P1 activity was widespread across occipital electrodes, showing the strongest activation at electrodes O1 and O2 (**Figure 3**). However, from visual inspection, it is apparent that by about 14–15 years of age, the P1 shows more focused, lateral activation as the maximum focus of P1 activity shifts away from O1 and O2, and moves to lateral electrodes PO7 and PO8 (**Figure 3**). Importantly, this increased lateralization with age was found for each of the other conditions (cars, scrambled faces and scrambled cars, **Figure 4** and **Figures S1 and S2** in Supplementary Material).

To provide more substance to these observations, we computed a Pearson correlation between the P1 to faces and each of the other three conditions, separately for each age group (3 correlations × 9 age groups; correlation based on 64 electrode amplitude values at the peak of the component). All of the correlation coefficients were highly significant, ranging from 0.95 to 0.99. This result indicates that the scalp topography, i.e. the distribution of electrophysiological responses on the scalp, at the time of the P1, was virtually identical for faces and nonface stimuli in every age group. Moreover, the topography of the P1 for faces in adults (and therefore for all c onditions) was highly correlated with the topography of the P1 of each age group (8 correlation values ranging from 0.87 to 0.92).

Latency. Visual inspection of the data revealed moderate decreases in P1 latency with age, with the most significant decrease apparent between 9 and 12 years. On average the P1 peaks at about 114 ms in the youngest age group, and 90 ms in adults (**Figure 5**). This was true for scrambled faces also (**Figure 5B**) as well as for pictures of cars and scrambled cars (**Figures S3 and S4** in Supplementary Material). The decrease in latency was fairly linear in all four conditions.

Statistical analyses largely confirmed these observations on P1 latency. An ANOVA with four within-subject factors, (*Electrode* (O1/2, PO5/6, PO7/8), *Hemisphere* (right, left), *Shape* (non-scrambled, scrambled), *Category* (faces, cars) and one between

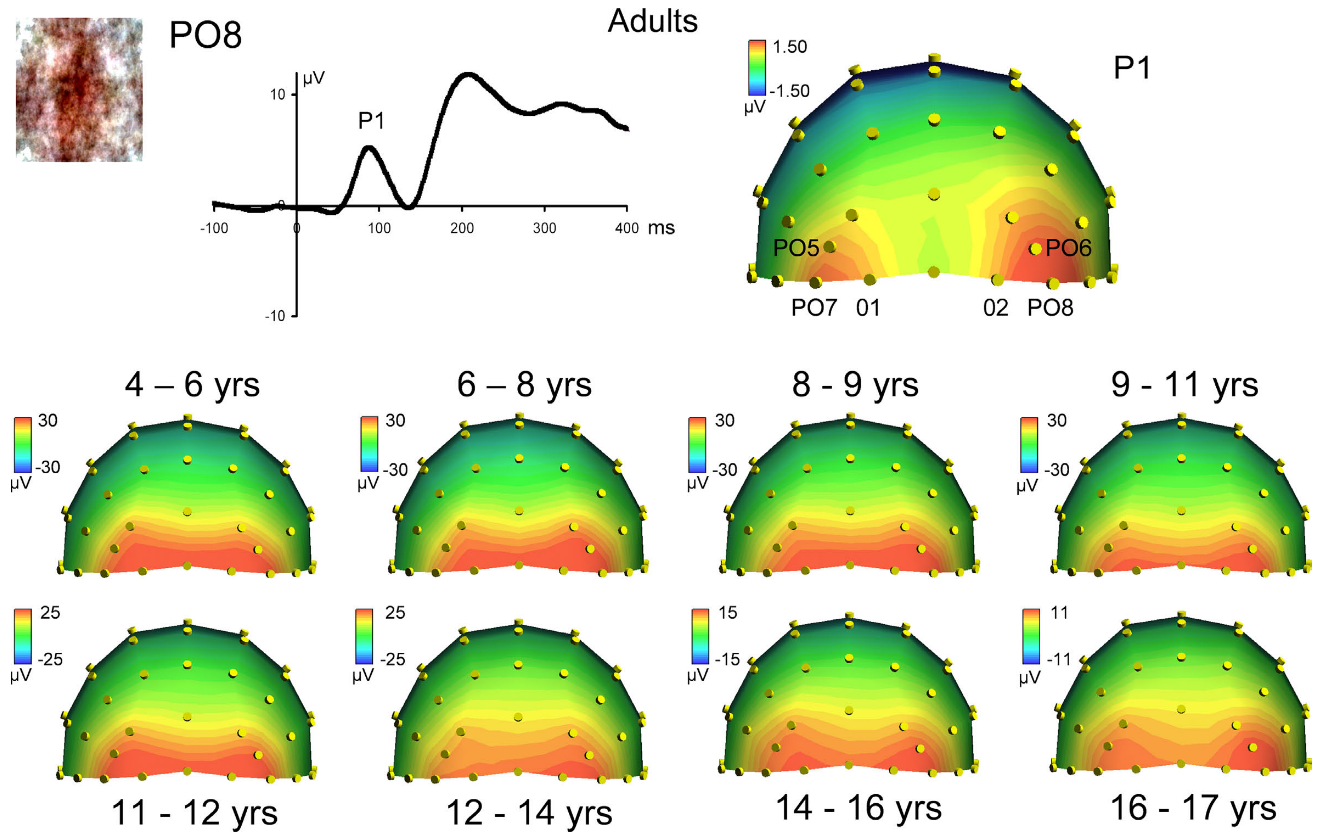

FIGURE 4 | Scalp topographies at the peak of the visual P1 component in responses to scrambled faces in adults (top) and all age groups. Note the increasing separation of the lateral occipital sources generating the P1, and the general similarity with the changes observed for faces (**Figure 3**). The topographic plots are snapshots of the P1 response at 100 ms for adults, and at the following latencies for the youngest to oldest age groups, respectively: 116, 113, 115, 111, 111, 109, 99 and 110 ms.

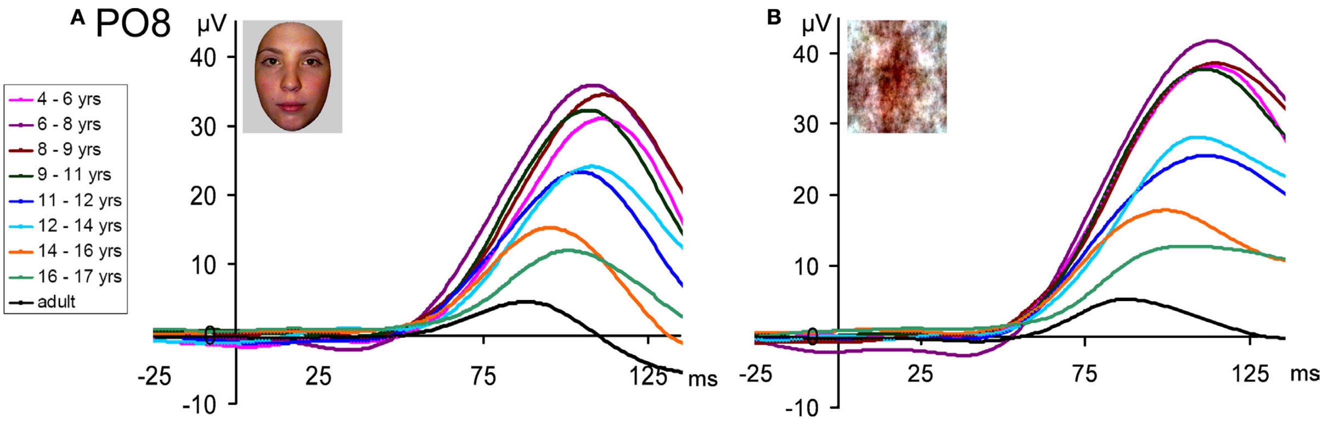

FIGURE 5 | The P1 recorded from PO8 (right lateral occipital site) for the 8 age groups and adults, illustrating the large changes in latency (linear decrease) and amplitude (overall decrease). (A) In response to faces. **(B)** In response to scrambled faces. Note the larger amplitude to scrambled faces, and the overall similarity of developmental changes for faces and scrambled faces. A 30-Hz low-pass filter has been applied to each waveform in this figure.

subject factor, *Age Group* (9 levels) showed main effects of *Category* ($F_{1,74} = 13.16$, $p < 0.001$), due to slightly faster (~1.7 ms) response to faces/scrambled faces than car/scrambled cars, and of *Hemisphere* (~2 ms faster in the right than the left hemisphere; $F_{1,74} = 4.95$, $p < 0.029$). Additionally, the overall decrease in latency was confirmed by a main effect of *Age Group* ($F_{1,148} = 9.50$, $p < 0.001$).

There were two-way significant interactions between *Category* and *Shape* ($F_{1,74} = 7.13$, $p < 0.009$) resulting from faster response to faces than all other three visual stimuli (~2.3 ms); *Shape* and *Electrode* ($F_{1,148} = 4.78$, $p < 0.009$, faster at O1/O2 for non-scrambled stimuli but not for scrambled stimuli); and *Hemisphere* and *Electrode* ($F_{1,148} = 5.80$, $p < 0.01$) due to faster (~3 ms) responses in the right vs. left hemisphere but only on more lateral channels (PO8 vs. PO7, but not vs. PO6 vs. PO5).

The only significant interaction involving *Age Group* was a triple interaction between *Shape*, *Electrode* and *Age Group* ($F_{1,148} = 2.23$, $p < 0.016$) (all other *p*-values >0.08). This was found because responses were faster to meaningful stimuli in the first six age groups on O1/O2, but not in the last three age groups. Most importantly, *Category*, and *Shape* did not interact with *Age Group* ($p = 0.78$), and these three factors did not interact with *Hemisphere* ($p = 0.77$), or *Electrode* ($p = 0.79$). The five way interaction between these factors was also non-significant ($p = 0.51$). This indicates that the P1 latency did not show a specific developmental course for faces: it decreased substantially between 4 years old and adulthood (~20 ms), but equally so for faces, cars and their scrambled counterparts (**Figure 5**; **Figure S4** in Supplementary Material).

Amplitude. Visual inspection of the P1 amplitude in response to faces revealed dramatic changes with age, as indicated by the different scales on **Figure 3** and the different age group waveforms on **Figure 5A**. There was a general decrease in the amplitude of the P1 with age. The largest decreases are visible between the 9–11 year olds and 11–12 year olds, and again between the 12–14 year olds and the 16–17 year olds. The P1 amplitude decreased even further, until adulthood. Importantly, this pattern of amplitude decrease was also found for scrambled faces (**Figure 6**), and the nonface conditions (**Figure S5** in Supplementary Material). The P1 also

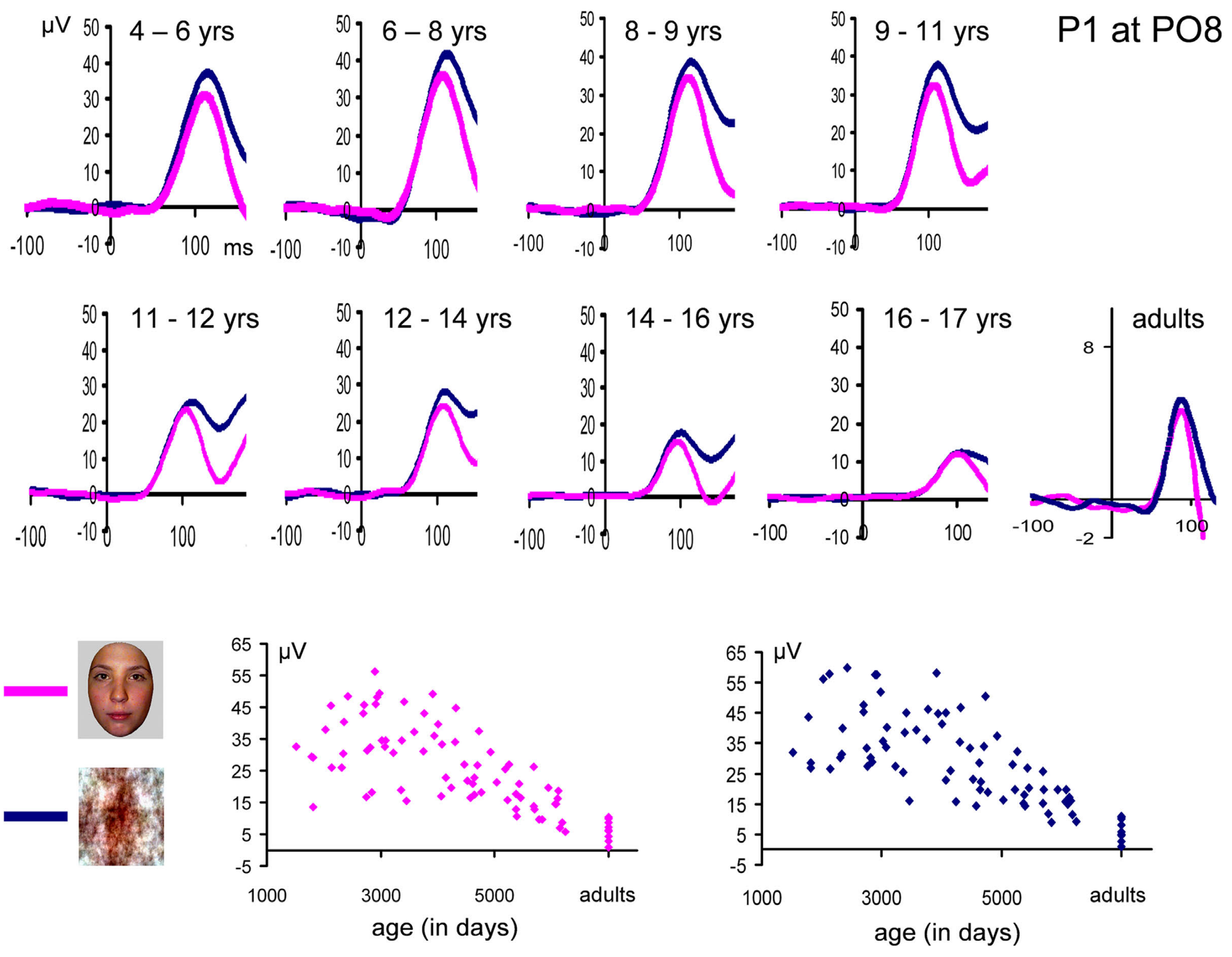

FIGURE 6 | The P1 recorded from PO8 (right lateral occipital site) for the 8 age groups and adults, illustrating the larger response to scrambled faces as compared to faces, which remained constant all along development. The waveforms above are based on grand-averaged data from nine subjects in each group, while the individual data of each participant in the study are shown below. Children's data is plotted according to participant age in days at the time of test. Note the progressive decrease in amplitude with age, both for faces and scrambled faces, and the inter-individual variance in amplitude of the component. A 30-Hz low-pass filter has been applied to each waveform in this figure.

appeared slightly larger in response to scrambled faces than faces in all age groups (**Figure 6**), but roughly equal for faces and cars (**Figure S6** in Supplementary Material).

Statistical analyses largely confirmed these observations. An ANOVA with four within-subject factors [(*Electrode* (3), *Hemisphere* (2), *Shape* (2), *Category* (2)] and one between subject factor (*Age Group*, nine levels) showed main effects of *Category* ($F_{1,74} = 22.64$, $p < 0.001$), due to larger (~1.0 μV) responses to faces/scrambled faces than car/scrambled cars, *Shape* ($F_{1,74} = 42.19$, $p < 0.001$) due to larger (~2.1 μV) responses for the scrambled compared to the non-scrambled stimuli, *Hemisphere* (~3 μV larger in the right than the left hemisphere; $F_{1,74} = 20.51$, $p < 0.001$), and *electrode* (amplitude 3–4 μV larger at O1/O2 and PO7/PO8 compared to PO5/PO6). Additionally, the overall decrease in amplitude with age was confirmed by a main effect of *Age Group* ($F_{1,148} = 22.72$, $p < 0.001$).

There were significant two-way interactions between *Shape* and *Hemisphere* ($F_{1,74} = 14.36$, $p < 0.001$) due to the amplitude advantage for scrambled stimuli being greater in the right (~2.7 μV) vs. left hemisphere (~1.5 μV); *Hemisphere* and *Electrode* ($F_{1,148} = 18.69$, $p < 0.001$) for larger (~2.5–4.5 μV) responses in the right vs. left hemisphere but only on more lateral channels (PO8/PO6 vs. PO7/PO5); *Category* and *Electrode* ($F_{1,148} = 34.18$, $p < 0.001$) for larger responses to faces/scrambled faces than cars/scrambled cars only on more lateral channels (PO8/PO6 vs. PO7/PO5); and *Shape* and *Electrode* ($F_{1,148} = 34.18$, $p < 0.001$) for larger responses to scrambled faces than non-scrambled stimuli on all channels but more on the most lateral channels (PO8/P07).

There were two significant two-way interactions involving the factor *Age Group.* The first, *Shape* by *Age Group* ($F_{1,74} = 3.95$, $p < 0.001$), was due to scrambled stimuli having a greater amplitude advantage compared to non-scrambled stimuli only in the youngest age groups (**Figure 6**; **Figure S5** in Supplementary Material). However, as far as face stimuli were considered (**Figure 6**), the larger P1 amplitude to scrambled images was of equal magnitude in all age groups when taking into account the percentages of increases (e.g., 5 μV increase in children who have a 50 μV P1 can be considered as equal as a 0.5 μV increase in adults having a 5 μV P1; **Figure S7** in Supplementary Material). The second interaction, *Electrode* by *Age Group*, ($F_{1,148} = 3.29$, $p < 0.001$), was due to a smaller response at PO5/PO6, compared to the more medial electrodes, only in children under 12 years of age.

Finally, there was a significant triple interaction involving the factors *Category*, *Shape*, and *Hemisphere* ($F_{1,74} = 4.37$, $p < 0.04$), due to the fact that while in the left hemisphere the amplitude advantage for both faces and scrambled faces over cars and scrambled cars was of equal magnitude (~1.0 μV), in the right hemisphere the advantage for scrambled faces over scrambled cars was greater (~1.3 μV) than the advantage of intact faces over intact cars (~0.4 μV). There were also two significant triple interactions involving the factor electrode: *Category*, *Electrode*, and *Hemisphere* ($F_{1,74} = 7.2$, $p < 0.002$), because the larger responses to faces/scrambled faces than cars/scrambled cars was not found in the right hemisphere for the occipital channels (O1/02); and *Shape*, *Electrode*, and *Hemisphere* ($F_{1,74} = 7.2$, $p < 0.002$), because the smaller responses to non-scrambled than scrambled stimuli was not as strong at occipital channels (O1/02).

Most importantly, there was no interaction between *Category*, *Shape* and *Age Group* ($p = 0.853$), or of these three factors with *Hemisphere* ($p = 0.256$), or *Electrode* ($p = 0.43$), or all five factors (five-way interaction, $p = 0.22$). This indicates that the P1 amplitude did not show a specific developmental course for faces.

In summary, there were large changes across development on the first visually evoked response to flashed photographs of faces over lateral and central posterior electrodes, the visual P1 evoked potential: it decreased linearly in latency with age, decreased (non-linearly) with age in amplitude, and its topography evolved from central-occipital to lateral-occipital or occipito-temporal channels. However, none of these latency, amplitude and topographical changes associated with development were specific to faces: these changes also took place for pictures of cars, and even for the meaningless visual control stimuli. Hence, development of the P1 response is not face-specific. Interestingly, there were some stable responses across age for the P1, such as its right lateralization, which is found even in the youngest age group. There were also some differences between categories and shape in P1 amplitude, such as the larger responses to scrambled stimuli over non-scrambled stimuli. However, once again, this effect was constant across age.

N170

At visual inspection, the N170 to faces, identified as the large negative deflection following the P1 at occipito-temporal sites, was observed to change dramatically across development (**Figure 7**). These changes concern its scalp distribution (**Figure 7**), amplitude (the largest in adults and in the youngest age group), and latency (decreasing with age) (**Figure 8**). It also appears to change in width or frequency range, being particularly wide for the youngest age groups (**Figure 8**). These observations are not novel and, as we will see below, largely replicate previous observations (Taylor et al., 2004). However, the question of interest of the present study is whether these N170 modulations with age are face-specific, or rather reflect general developmental effects.

As indicated in the introduction, there are a number of issues to consider when addressing this question, in particular we must take into account the variations that are common to all conditions of visual stimulation, as well as the variations in P1 parameters that can affect the N170 component differently across age groups. We approached our analyses of the N170 with an awareness of several potential problems. First, since there are large variations of P1 parameters across age, the raw measurements of the N170 parameters will be affected indirectly. For instance, the N170 does not cross the zero baseline in many children, due to the large P1, affecting the measure of absolute amplitude and topographies on the scalp (**Figures 7 and 8**). However, the absolute amplitude of the negative deflection could be substantial in these children, and a complementary peak-to-peak measurement and analysis is warranted to characterize the developmental changes of the N170. Second, while the N170 was clearly visible in all children for the conditions where a meaningful shape (face or car) was presented, there was only a small negative deflection for scrambled stimuli at that latency (e.g., **Figures S8 and S9** in Supplementary Material). Thus, the N170 was not measured for these conditions. Finally, due to the presence of a "bifid" N170 in the three youngest age groups,

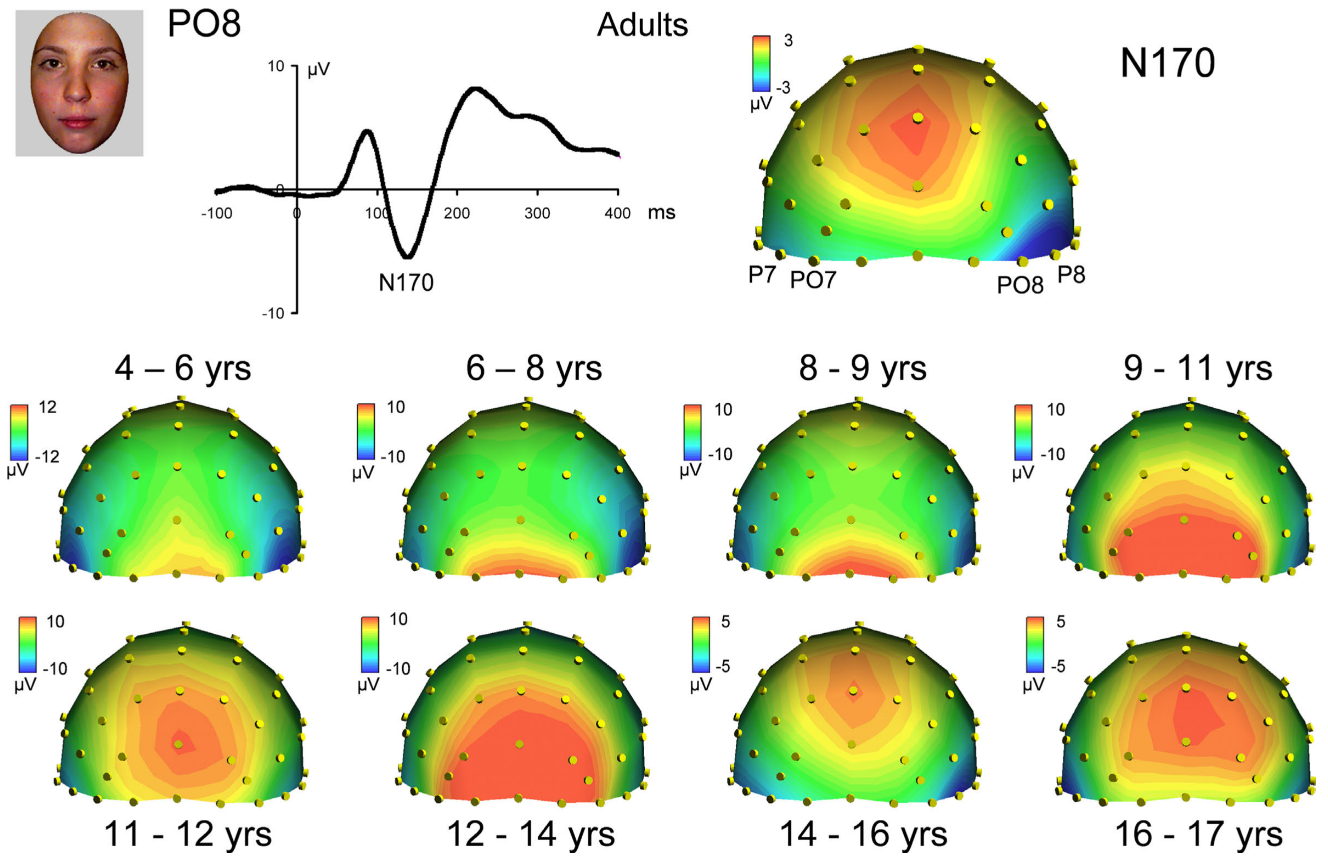

FIGURE 7 | Scalp topographies at the peak of the visual face-sensitive N170 component in responses to faces in adults (top) and all age groups. Note the large variations in scalp distribution of the raw N170 component, which becomes adult-like at about 14–16 years old only. Right lateralization is clear only in adult data on this topography. The topographic plots are snapshots of the N170 response at 139 ms for adults, and at the following latencies for the youngest to oldest age groups, respectively: 166, 180, 185, 156, 150, 153, 143 and 148 ms.

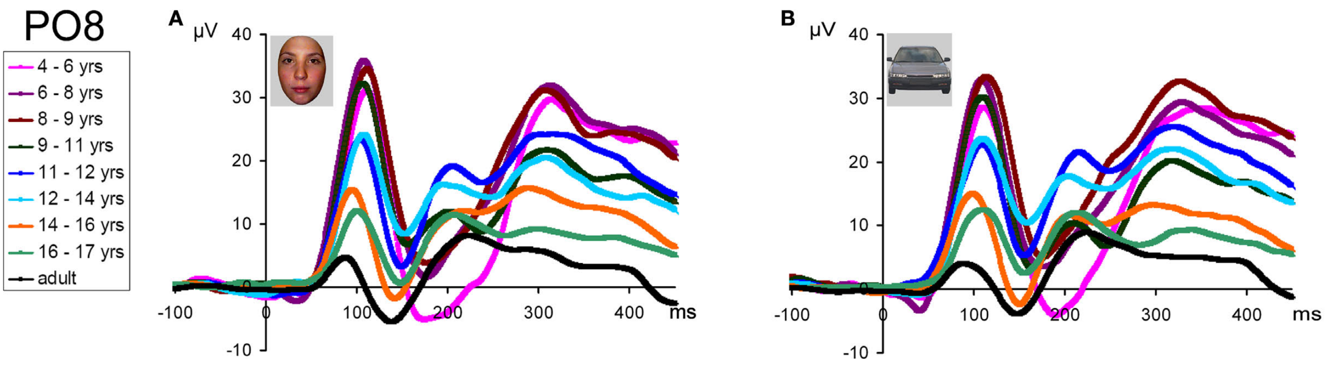

FIGURE 8 | Grand-average waveform in response to (A) faces and (B) cars in all age groups and adults (in black) illustrating the dramatic differences of amplitude, latency and width of the N170 component. Note that, especially evident for the face stimuli, in the three youngest age groups the component is particularly wide due to the merging in grand-averaged data of the N170 with a late negative deflection. This phenomenon can be clearly observed on individual data (**Figure 11**). A 30-Hz low-pass filter has been applied to each waveform in this figure.

identification of the N170 was ambiguous in grand-averaged data. In the large majority of individual subjects however, the first deflection was visible, and even when it was smaller in amplitude than the second one, for consistency across age groups, we considered the N170 to be the first negative deflection following the P1.

Topographical distribution on the scalp. The topography of the raw N170 in response to faces appears to change dramatically across development (**Figure 7**). Specifically, in adults it appears as a bilateral occipito-temporal negativity with a strong right hemisphere advantage, associated with a positivity at centro-parietal sites (VPP;

Jeffreys, 1989), replicating the usual N170 pattern (**Figure 1**). In children however, the N170 was not always visible on the topographical maps and appeared hidden/dominated by a large occipital positive potential, which was particularly prominent for the 9- to 14-year-old children; **Figure 7**). The right lateralization of the N170 was not clear at all, and it is only in the 14–15 year olds that the N170 appeared adult-like. Strangely, it appeared less adult-like in the oldest children tested (16–17 years).

However, the critical question is whether or not these changes in the N170 parameters are specific to faces. We must keep in mind that variations in terms of the topography of the N170 may be caused by general variations in ERP activation with age. These variations can be removed, and the N170 isolated, by subtracting out the general low amplitude responses evoked by scrambled faces from the responses evoked by faces. Therefore, we first removed the amplitude responses to low-level stimuli to understand if there are general developmental changes occurring with the topography of the N170. After removing the general low-level responses from the responses to both faces and cars, we were able to compare these two stimulus categories in order to determine if any developmental changes we may observe would be specific to faces.

When the scrambled face response is subtracted from the face response, the N170 topography appears remarkably similar across development, i.e. as a clear negative deflection centered on occipito-temporal sites (**Figure 9**). Like for the P1, it is much more lateralized in adults than in the non-mature brain, being concentrated on occipito-temporal channels rather than central occipital channels. The N170 shows a right hemisphere advantage, which may be larger in some age groups than others, but without a systematic developmental trend (**Figure 9**).

Thus by subtracting from faces the responses evoked by scrambled faces, the N170 topography appeared much more similar across age groups, as well as more adult-like at all ages. Correlation analyses between topographies substantiated these observations. Pearson correlation values between the raw topography to face stimuli for each age group and the group of adults ranged between 0.085 (NS) and 0.84 (**Figure S10A** in Supplementary Material). However, correlation values of the topographies of the N170 resulting from the subtraction (faces – scrambled faces) were all much higher and ranged between 0.87 and 0.96 (**Figure S10A** in Supplementary Material; all significant at $p < 0.0001$). These correlation values based on differential activities increased with

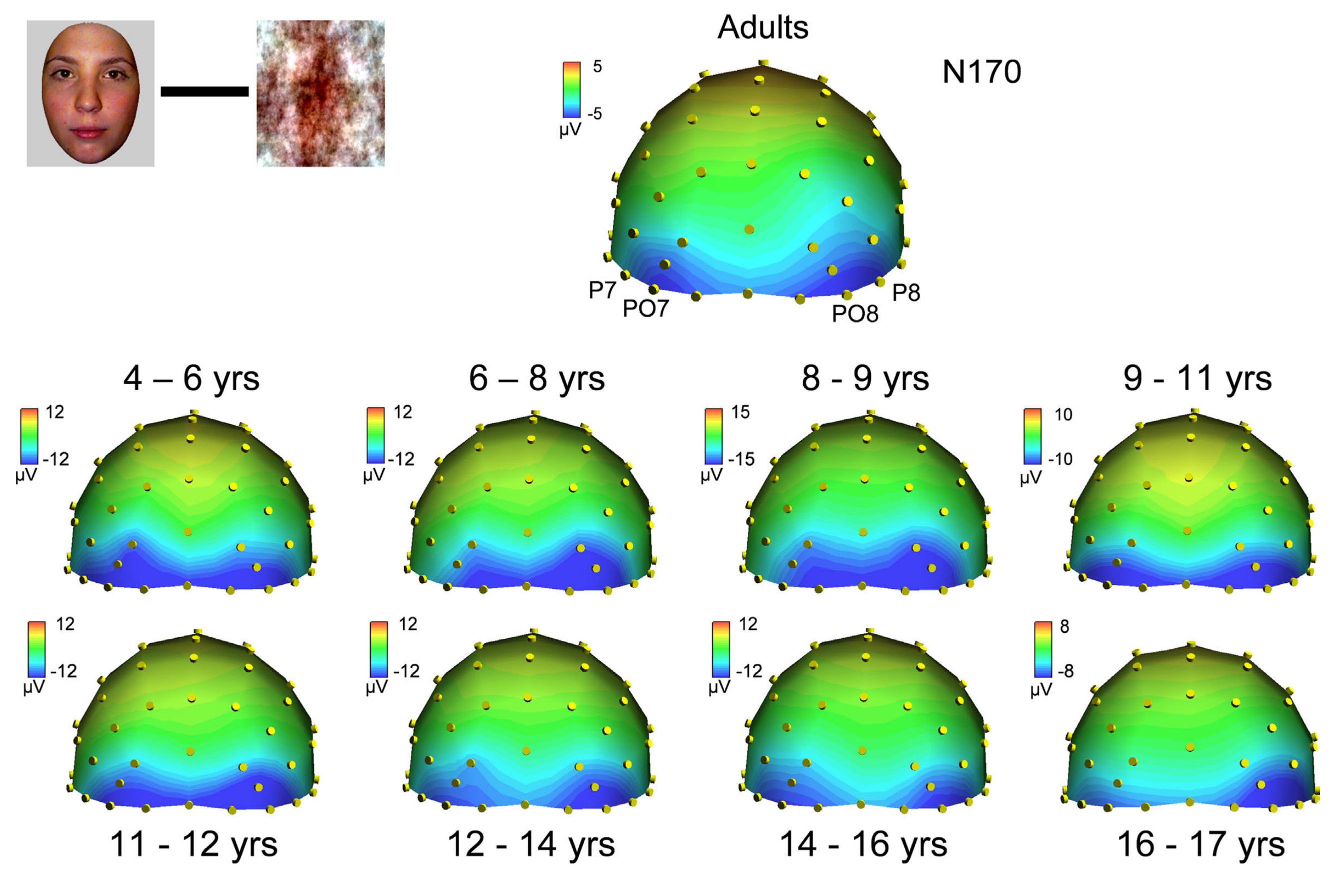

FIGURE 9 | Scalp topographies at the peak of the visual face-sensitive N170 component in responses to faces in adults (top) and all age groups, when the response to scrambled faces at that latency is subtracted out. Compared to the large variations in scalp distribution of the raw N170 component (**Figure 7**), the N170 shows a remarkable stability in terms of topography across all age groups, with a very small refinement with age. Note that the right hemisphere dominance, characteristic of the adult face N170, is visible in most age groups, at least from 6 to 8 years old. The topographic plots are snapshots of the (face – scrambled face) subtracted N170 response at 180 ms for adults, and at the following latencies for the youngest to oldest age groups, respectively: 166, 180, 190, 148, 150, 163, 143 and 159 ms.

age, being maximal for the oldest age group. This indicates a small increase in the adult-like scalp distribution of electrophysiological activity across development at the time window of the N170, which cannot be accounted for by developmental changes to low-level visual stimulation.

Importantly, the same correlation analysis performed on the N170 for pictures of cars compared to the correlation performed on the subtraction (cars – scrambled cars) showed a similar pattern[1] (**Figure S10B** in Supplementary Material). Correlations of the child and adult topographies to pictures of cars were quite low for most age groups (ranging between 0.02 and 0.87, but lower than 0.60 for all but one age group) but improved substantially in all age groups when the variations due to low-level stimuli were removed via the subtraction. If anything, the "adult-likeness" of the N170 topography increased significantly more for pictures of cars than faces across development (**Figure S10B** in Supplementary Material), an observation that counters the idea that the scalp distribution of electrophysiological activity during the N170 time-window undergoes face-specific developmental changes during the course of development. The topographies of cars – scrambled cars across age groups support this statement (**Figure S11** in Supplementary Material).

N170 latency. If one considers the grand-averaged data by age group, the N170 latency to faces appears to decrease with age, peaking at about 200 ms in the youngest age group and reaching about 150 ms in adulthood (**Figure 8**). However, when measured in individual participants, the decrease of N170 latency was much smaller. In fact, mean latencies ranged from about 170 to 145 ms (**Figure 10**), revealing only a 25 ms difference between the youngest age group and adults. In both the grand averaged waveform and the data extracted from individual subjects, the decrease did not appear to be linear, but was substantial between the three youngest age groups (4–9 years old) and the older participants (**Figure 10A**).

This pattern reflects in part the fact that, in the three youngest age groups, the measure of N170 latency was complicated by the merging of the first negative deflection (what we refer to as the real N170) following the P1 with a second negative deflection (**Figure 8**). While Taylor et al. (2004) referred to the second negative deflection as the N170b, we propose that it may actually be the N250. The N250 is negative mid-latency ERP which, in adults, is larger for repeated compared to unrepeated faces over temporal scalp regions, in particular for familiar faces ("N250r", e.g. Begleiter et al., 1995; Schweinberger et al., 1995; Tanaka et al., 2006). We believe that the large amounts of inter-trial variance in young children's data caused these two components to appear to have less temporal separation in the averaged waveform compared to being quite distinct in older child and adult averages. The merging of the N170 and N250 was particularly clear on the grand-averaged data from the three youngest age groups (**Figure 8**). However, in individual participants, even in the youngest age groups, the two negative components could be more easily distinguished, and thus the first negative deflection, identified as the N170, was measured appropriately in our sample (**Figure 11**).

Importantly, all of these observations were also true of the responses to cars, and were thus not specific to faces (**Figures 8B and 10B**).

The statistical analysis on the N170 latency was performed with the within subjects factors of *Electrode* (PO7/PO8, P7/P8), *Hemisphere* (right, left), *Category* (faces, cars) and the between subjects factor *Age Group* (nine levels).

The ANOVA showed main effects of *Category* ($F_{1,74} = 87.43$, $p < 0.001$), due to faster (~8 ms) response to faces than cars. Additionally, the overall decrease in latency with age was confirmed by a main effect of *Age Group* ($F_{8,74} = 15$, $p < 0.001$) which was driven by decreases in latency between age groups 3 and 5 (8–12 year olds), with latencies remaining unchanged from the 11–12 years to adulthood.

The only significant interaction which involved *Age Group* was a triple interaction between *Category*, *Electrode* and *Age Group* ($F_{1,148} = 2.48$, $p < 0.019$) (all other *p*-values involving *Age Group*, $p > 0.15$). This triple interaction appeared to be caused by differences between age groups 2 and 3 vs. all other age groups: for the children

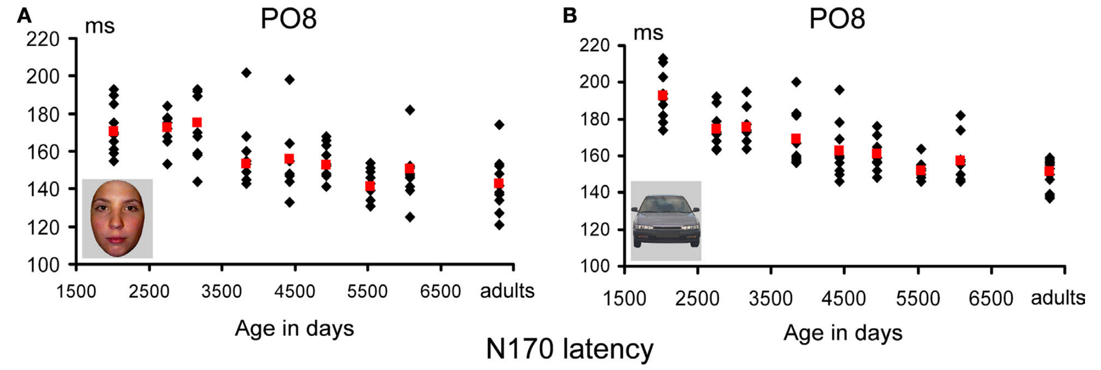

FIGURE 10 | Peak latency of the N170 in response to pictures of faces (A) and cars (B). Individual data points are plotted according to the mean age (in days) of each age group. Mean latency for each age group is shown in red. The overall latency delay for cars was found across all age groups, and the developmental decrease of latency appears to follow a similar pattern for faces and cars. Note the longer latency of the N170 in the three youngest age groups compared to the others, but also the generally smaller variability (25 ms at most). The N170 latency appears to reach an adult-like level at 14–16 years old, both for faces and cars.

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[1] Given the small N170 latency delay in response to cars compared to faces (see latency analysis) which also concerned the small N170 responses to scrambled stimuli, we did not perform a direct subtraction of faces and cars, but compared these conditions indirectly through subtraction of their scrambled counterparts.

of age groups 2 and 3, the most lateral channels (P7/P8), showed earlier peak latency for cars than faces, while the posterior channels (PO7/PO8) showed the opposite pattern. However, ANOVAs comparing the group of adults to each age group of children showed only non-significant trend for a *Category* × *Electrode* × *Age Group* for age groups 2 ($p = 0.06$) and 3 ($p = 0.073$) (all other p-values $p > 0.18$).

N170 latency delay with respect to P1: peak-to-peak analysis. We performed the same statistical analysis with the factors *electrode* (two levels: PO7/PO8; P7/P8), *Hemisphere* (two levels, right and left), *Category* (two levels, faces and cars) and *Age Group* (nine levels) on the measure of the difference in milliseconds between the peak of the N170 and the peak of the P1. This analysis revealed a main effect of *Category* ($F_{1,74} = 29.21$, $p < 0.001$) due to a *longer* (~5 ms) peak-to-peak difference for cars than for faces. There was a main effect of the factor *Age Group* ($F_{8,73} = 3.77$, $p < 0.001$), driven by a moderate decrease in peak-to-peak N170 latency (**Figure 13A**). In total, the peak-to-peak latency of the N170 for faces decreased from 58 ms (youngest age group) to 44 ms (adults), but this decrease was entirely due to the three first age groups. There were no further significant decreases in latency from age group 4 until adulthood (**Figure 12A**). The same developmental trajectory was present for cars, with the only exception of the data of the first age group, for which the peak-to-peak latency was particularly long in response to cars (**Figure 12B**). Nevertheless, interaction between *Age Group* and *Category* was not significant.

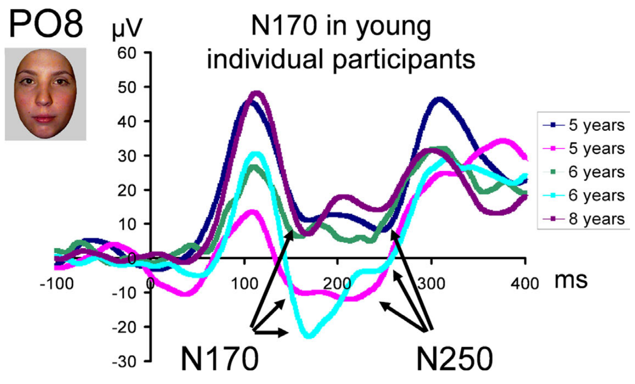

FIGURE 11 | Illustration of young individual participant waveforms showing the two negative deflections which are merged on grand averaged data (Figure 8). The second peak may correspond to the N250 in older populations (see text and Supplementary Material). A 30-Hz low-pass filter has been applied to each waveform in this figure.

There was also an interaction between *Electrode* and *Group* ($F_{8,74} = 3.77$, $p < 0.001$) due to the fact that the peak to peak differences was smaller at P7/P8 than PO7/PO8 for the youngest children (~6 ms), was roughly equal for the two sets of electrodes for age groups 2–7 (6–16 years), and was smaller at PO7/PO8 for the oldest children and adults (groups 8 and 9; ~9 and 5 ms, respectively). The interactions of interest, namely those involving *Category* and *Age Group*, were not significant (*Category* × *Age Group*, $p > 0.32$; *Category* × *Age Group* × *Hemisphere*: $p > 0.47$; *Category* × *Electrode* × *Hemisphere*: $p > 0.29$; *Category* × *Age Group* × *Hemisphere* × *Electrode*: $p > 0.19$).

In summary, we found that the absolute N170 latency in response to faces decreased with age, but to a much lesser degree than previously reported (Taylor et al., 2004), i.e. within a range of 25 ms here. These variations with age were even smaller (about 15 ms) when accounting for P1 latency differences, and were found only when comparing the three first age groups (4–9 year old children) to the older participants. However, this was possibly due to the larger variance in the youngest age groups (**Figure 12**), which may have been caused by the merging of a later occipito-temporal negativity with the N170 for some participants. In any case, this relatively small decrease of N170 latency was found for both faces and cars, and if anything there was a smaller age-related change (i.e., decrease of latency) for faces than cars when considering age group 1.

N170 amplitude. If one considers the grand-averaged data by age group, the N170 amplitude to faces appears to vary with age, but in a non-systematic way (**Figure 8**). This observation is also true when measured in individual participants (**Figure 13**). In both the grand averaged waveform and the data extracted from individual subjects, the amplitude modulation took a form similar to the

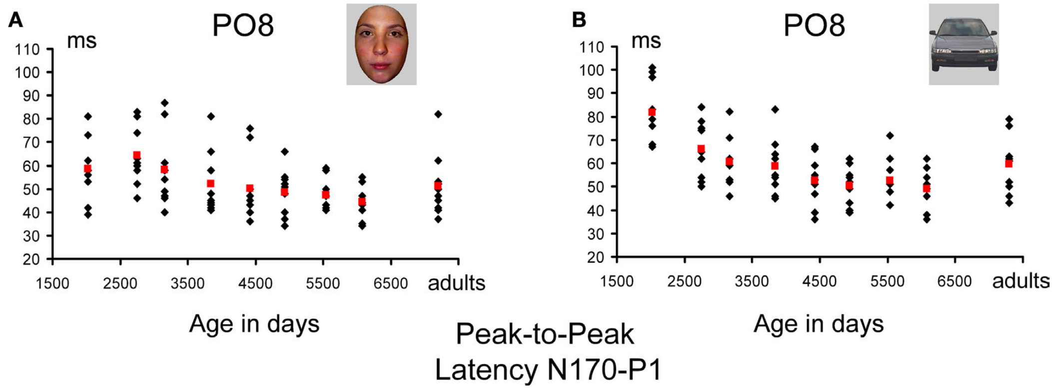

FIGURE 12 | Peak-to-peak latency measurements of the N170 vs. P1 for faces (A) and cars (B). Individual data points are plotted according to the mean age (in days) of each age group. Mean measurements for each age group are shown in red. If anything, the changes of latency with age were larger for pictures of cars (due to the youngest children). For faces, the peak-to-peak difference in latency between the P1 and N170 was of 10–15 ms at most.

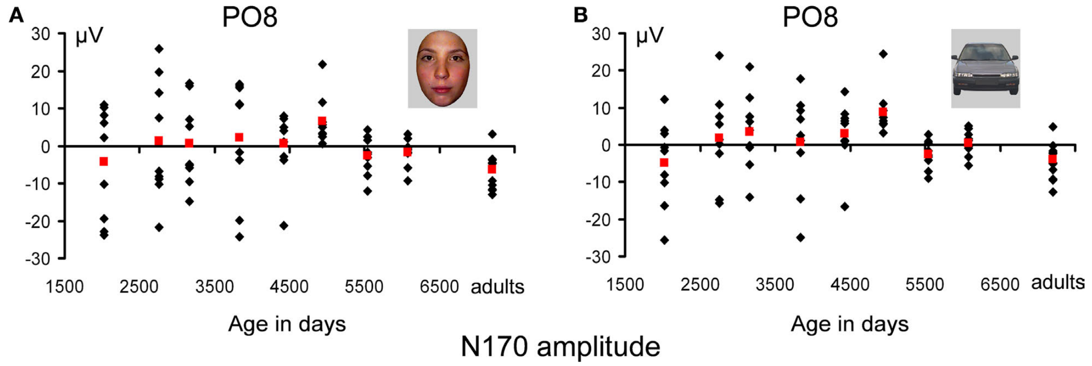

FIGURE 13 | Amplitude measurements of the N170 for faces (A) and cars (B). Individual data points are plotted according to the mean age (in days) of each age group. Mean measurements for each age group are shown in red. The U-shape function of N170 amplitude is not so clear on the figure due to the large scale used to display individual participant's data. Note the remarkable similarity between the amplitude variations of faces and cars with age.

inverted "U"-shaped function (**Figure 13**), previously described by Taylor et al. (2004). This reflects in part the fact that, like latency, in the three youngest age groups, the measure of N170 amplitude was complicated by the merging of the first negative deflection following the P1 (what we refer to as the real N170) with a second negative deflection taking place later (referred to as N170b by Taylor et al., 2004). Again, the confusion between the two negative deflections was particularly clear on grand-averaged data (**Figure 8**), while, in individual participants of the youngest age groups, the two negative components could be more easily distinguished, and thus the first negative deflection, identified as the N170, measured appropriately (**Figure 10**).

Importantly, again, all of these observations concerning amplitude modulations were also made for the waveforms recorded to pictures of cars, and were thus not specific to faces (**Figure 13B**).

The statistical analysis on the N170 amplitude was performed with the factors *Electrode* (PO7/PO8; P7/P8), *Hemisphere* (right and left), *Category* (faces and cars) and *Age Group* (nine levels). The ANOVA showed a trend for an effect of *Category* ($F_{1,74} = 3.26$, $p < 0.075$), due to larger (~0.7 μV) response to faces than cars. Additionally, a main effect of *Age Group* ($F_{8,74} = 3.5$, $p < 0.002$) which was due to the negativity first decreasing with age and then increasing again (**Figure 7**). There was also a main of *Electrode* ($F_{8,74} = 173.37$, $p < 0.0001$) for a larger N170 at P7/P8.

The only significant interaction which involved age group was a two-way interaction between *Electrode* and *Age Group* ($F_{1,148} = 5.71$, $p < 0.001$) (all other *p*-values involving the factor *Age Group* $p > 0.15$), which was due to the N170 decreasing in amplitude on P7/P8. There were also a number of significant interactions involving the factor electrode, but no interaction with age group (*Electrode* × *Category* × *Hemisphere*: $F_{1,148} = 6.71$, $p < 0.012$; *Hemisphere* × *Electrode*: $F_{1,148} = 6.15$, $p < 0.015$).

N170 amplitude with respect to P1: peak-to-peak analysis. To take into account P1 variations, we performed the same statistical analysis with the factors *Electrodes* (two levels: PO7/PO8; P7/P8), *Hemisphere* (two levels, right and left), *Category* (two levels, faces and cars) and *Age Group* (nine levels) on the measure of the difference in amplitude between the peak of the N170 and the P1. This analysis revealed a main effect of *Category* ($F_{1,74} = 16.93$, $p < 0.001$) due to a *larger* peak to peak difference for faces than for cars (face – car difference ≈2 μV). There were also main effects of the factors *Hemisphere* ($F_{1,74} = 11.54$, $p < 0.001$) due to a greater amplitude difference in the right than the left hemisphere (2.7 μV), and *Electrode* ($F_{1,74} = 124.58$, $p < 0.001$), due to a larger difference in amplitude at more posterior channels (PO7/PO8 vs. P7/P8). Finally, there was a main effect of *Age Group* ($F_{8,73} = 17.08$, $p < 0.001$), driven by a significant, marked decrease in peak-to-peak amplitude difference until the 5th age group (11–12 years), with further more modest decrease until adulthood (**Figure 14**).

There was also an interaction between *Electrode* and *Age Group* ($F_{8,74} = 5.367$, $p < 0.001$) due to the fact that the peak-to-peak differences were largest at PO7/PO8 for the youngest age group (4–6 years) and then decreased monotonically (but no significant difference between 16–17 year olds and adults). The interactions of interest, namely those involving *Category* and *Age Group*, were not significant (*Category* × *Age Group*: $p > 0.82$; *Category* × *Age Group* × *Hemisphere*: $p > 0.72$; *Category* × *Electrode* × *Age Group*: $p > 0.31$; *Category* × *Age Group* × *Hemisphere* × *Electrode* $p > 0.81$).

In summary, we observed a larger N170 for pictures of faces than cars in all age groups, and this was clearer on the peak-to-peak analysis, thanks to the fact that differences of the opposite polarity at the level of the P1 were subtracted out. The right hemisphere showed larger amplitude than the left hemisphere, an effect which was again largely significant in the peak-to-peak analysis. These two effects were found in all age groups. The U-shaped function of the N170 amplitude with development was found for both faces and cars, but could, in fact, be attributed to variations at the level of the P1. Rather, peak-to-peak analyses were performed, we observed a small linear decrease of amplitude with age until adulthood, again of the same magnitude for pictures of faces and cars (**Figure 14**).

DISCUSSION

The goal of the present study was to assess whether the large age-related variations in latency, amplitude and scalp topography characterizing visually evoked components in response to simple face stimulation, the P1 and N170, can be taken as evidence for face-specific developmental changes in perceptual processes. This issue is of importance because the nature of the vast improvements

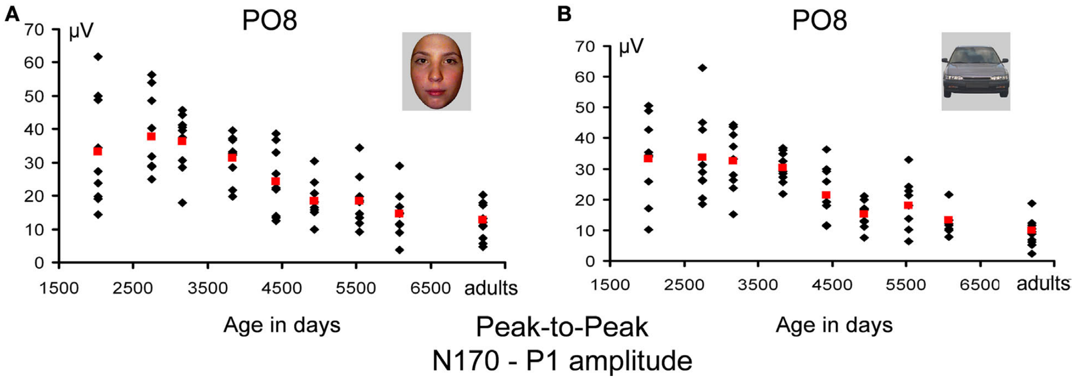

FIGURE 14 | Amplitude measurements of the N170 – P1 for faces (A) and cars (B) (absolute values). Individual data points are plotted according to the mean age (in days) of each age group. Mean measurements for each age group are shown in red. The U-shape function of N170 amplitude is no longer there, and the age-related variations reflecting the processes taking place following the P1 show only a slight decrease with age, similar for faces and cars.

of face recognition abilities throughout childhood and until adulthood is still highly debated (Crookes and McKone, 2009). On one hand, it has long been proposed that age-related improvements on face processing tasks are specific to our ability to perceive faces *per se*, particularly the relationships between facial features (holistic/configural processing), which would develop slowly as we accumulate an increasing amount of visual experience with faces (Carey and Diamond, 1977; Carey, 1992; Mondloch et al., 2002). On the other hand, age-related improvement on face processing tasks until adulthood could result from improvements in general sensory or cognitive factors, or in visual pattern recognition in general (Want et al., 2003; Crookes and McKone, 2009).

As we stated in the introduction, although challenging and rarely done, recording ERPs in developmental populations is potentially invaluable in resolving this debate. Using ERPs, perceptual processes reflected by visual components on the scalp can be relatively well isolated (in time, and space to a lesser extent) from other processes taking place at different moments in the time course of face processing. For instance, the N170 has been associated with early access to high-level representations in the human brain, with a particular sensitivity to faces, while the preceding P1 may show a lesser degree of face-sensitivity that is based on low-level visual features (Rossion and Jacques, 2008, 2010). In contrast, later components (e.g., N250, P3) are generally related to associative and decisional processes. Moreover, differential ERP responses evoked by faces and objects in the adult population have been well characterized, and these effects could be assessed in developmental populations.

However, previous developmental ERP studies reported general (large) changes of early brain responses to face stimuli, without considering whether these changes were specific to faces, or at least substantially different for faces than other visual categories. That is, the extent to which modifications of the parameters of the P1 and N170 can be taken as evidence that perceptual processes for faces change specifically with age (Carey, 1992) remained unknown.

Here, in a study in which we used several non-face visual stimuli in addition to faces, relied on a high-density recording system, topographical and peak-to-peak analyses, and made use of displays of individual ERP data, we made three types of observations:

1. General age-related changes in amplitude of the P1 and N170 in response to faces exist, replicating previous studies. However, decreases in the component latencies with age were much smaller than previously reported, and variations in topographies were minimal when taking each stage of processing into account independently.
2. Differences between conditions (faces vs. objects; shapes vs. non-shapes) in the P1 and N170 parameters were found as early as 4 years. Face-sensitivity on the P1 can be explained by low-level differences between faces and objects (i.e., it was equally large for scrambled stimuli and meaningful stimuli). There was a near absence of N170 to phase-scrambled stimuli, and a larger and earlier N170 to faces than cars. There was a right hemisphere dominance for N170 for meaningful stimuli, with occipito-temporal topography for both faces and cars. The fact that these effects were found as early as 4 years is an original contribution of the present study.
3. None of the differences between conditions (faces vs. objects; shapes vs. non-shapes) in the parameters of the P1 and N170 changed across development. These differences thus can not be related to well-known behavioral performance variations in face recognition.

Altogether, our findings do not offer any electrophysiological evidence that perceptual processes change specifically, or even to a different extent, for faces than non-face visual patterns across development. This does not rule out the qualitative view of the development of face perception, as we discuss below, but indicates that previous evidence seemingly in favor of this view (e.g., Taylor et al., 2004) should be qualified. In addition, throughout the course of our analyses, we identified a number of methodological issues that may be important to consider for future investigations in this area of research. We will now turn to these points in more detail.

GENERAL AGE-RELATED CHANGES IN P1 AND N170 PARAMETERS

With respect to general age-related changes of both the P1 and N170, we largely replicated previously reported observations (Taylor et al., 1999, 2001, 2004; Itier and Taylor, 2004a,b). The P1 decreases dramatically in amplitude with age, and shows modest decreases in latency. With respect to the "raw" N170 amplitude, we observed a similar inverted "U" shaped development previously reported by Taylor and colleagues, with the N170 being of least negative amplitude around 10–12 years of age, but of larger amplitude in younger and older children and adults. In terms of latency, the changes we found on the N170 were much less drastic than those previously reported (Taylor et al., 2004). We also reported general changes of P1 and N170 topography with age with more spatial precision, due in part to our use of a much higher density of electrodes (64 as compared to 32 at most in previous developmental studies, with some studies having even fewer channels, e.g. Taylor et al., 1999; Henderson et al., 2003). While the P1 topography was not much different across age groups, becoming fully adult-like at 16–17 years (**Figure 3**), the N170 topography was dramatically different across development, but stabilized around the same age as the P1 (**Figure 7**).

P1 age-related general changes

The P1 was clearly identifiable in all subjects' data for all four conditions. The latency and amplitude decreases observed with age (**Figures 3–6**) did not differ for the four types of stimuli, indicating that this decrease reflects a general developmental trend. These developmental variations in P1 latency and amplitude are not novel, having been previously reported for simple visual stimuli (e.g., Buchsbaum et al., 1974; Brecelj et al., 2002; Crognale, 2002). The variations in P1 latency suggest increasing general speed and efficiency in early visual processing across childhood and may reflect increasing myelinisation in visual cortical areas (Yakovlev and Lecours, 1967; Nelson, 1997). Although the reasons for changes in P1 amplitude are not entirely clear, it has been documented that absolute EEG power decreases between the ages of 10 and 20 years, with especially marked declines of power in the slow-wave band below 7.5 Hz (Matousek and Petersen, 1973). This decrease in neural activity has been related to changes in brain structure over adolescence, with the age-related reduction of slow-wave activity mirroring an age-related reduction in gray matter volume (Whitford et al., 2007), and synaptic density in particular (Huttenlocher, 1990). However, these amplitude variations may also be due to changes in conductivity of underlying tissues (bone, subcutaneous fat thickness and blood circulation, skin), cortical convolution and position of the sulci and gyri relative to external electrodes, general level of brain activity, and increases in head size. For instance, children's skull tissue normally contains a larger amount of ions and water and may thus have a higher conductivity than the adults calcified cranial bones (Lai et al., 2005).

We also observed, for all conditions, an increasing distinction of the lateral P1 distributions on the scalp, with an increasing lateralization with age. We suggest that this phenomenon is due to an increase in head size with age rather than the generating sources of the P1 becoming more separated with development. In fact, transforming the grand-averaged data in scalp current source density (CSD, Perrin et al., 1989; Tenke and Kayser, 2005), spatially enhancing the representation of the direction, location, and intensity of current generators that underlie an ERP topography, suggested the presence of two quite focal and distinct sources in each hemisphere generating the P1 for all age groups (**Figure S12** in Supplementary Material).

N170 age-related general changes

General age-related variations of the N170 parameters were in agreement with what has been previously reported for amplitude and topography (Taylor et al., 2004), but were much less spectacular than previously described for latency.

While as previously reported (Taylor et al., 2004), the N170 amplitude variations with age showed a U-shaped function, subtracting out the large baseline variations of P1 amplitude revealed a more progressive change in peak-to-peak amplitude of the N170 with age. More precisely, the N170 was large and stable in amplitude until 9 years and then decreased steadily until adulthood (**Figure 15**). This was found for both faces and cars and may be related to a maturation of shape-related processes or general age-related structural and functional brain changes, as discussed for the P1.

When age-related P1 variations are subtracted out, the N170 in response to both faces and cars appears to have a highly similar topography (i.e., occipito-temporal negativity with a right lateralization) across all age groups, even when comparing the youngest age group to adults.

When P1 variations in latencies are subtracted out (peak-to-peak analysis), the decrease of latency of the N170 from 4 years to adulthood is not spectacular, being of 10–15 ms at most, and remaining unchanged from the 4th age group (9–11 years) to adults. This indicates that the transition between the first early visual response detectable on the scalp (P1) over the mid-central and lateral occipital cortices, and the occipito-temporal shape-related processes (N170), undergo only a tiny decrease in time over development, and is stable by about 9 years of age. This is perfectly understandable: while P1 latency variations with age reflect the accumulation of the delay of response of visual pathways from the retina to the striate and extrastriate areas (e.g., Brecelj et al., 2002; Brecelj, 2003), the transition between P1 and N170 should not concern a great deal of visual processing stages or synaptic relays in the visual cortex. In addition, the smaller head size in young children may certainly compensate for age-related delays in speed of transmission of information between visual areas.

TWIN PEAKS

This short latency delay between P1 and N170 in the present study was also found because, contrary to previous studies (Taylor et al., 2001, 2004), we measured the N170 consistently in all age groups as the first negative deflection following the P1. In the three youngest age groups, grand-average data showed a very late and wide N170 (**Figure 8**) compared to the other age-groups. This observation is not new, it was made by Taylor et al. (2001, 2004). In their review paper, they measured the "real N170" at the second peak in younger children, hence describing an extremely late N170 latency in these age groups. However, in our study, we were able to determine the peak of the first negative component in individual data. It seems clear from our data that this "bifid" N170 phenomenon arises from the merging of two negative peaks, the N170 and the N250 (**Figures 11 and 15**).

The definition of the N170 at a much earlier latency than previously reported, resolves discrepancies between the Taylor et al. (2004) and other developmental studies in which the N1 to visual stimuli activating the ventral pathway is only slightly delayed for 6–8 year olds compared to adults (e.g., Brecelj et al., 2002; Coch

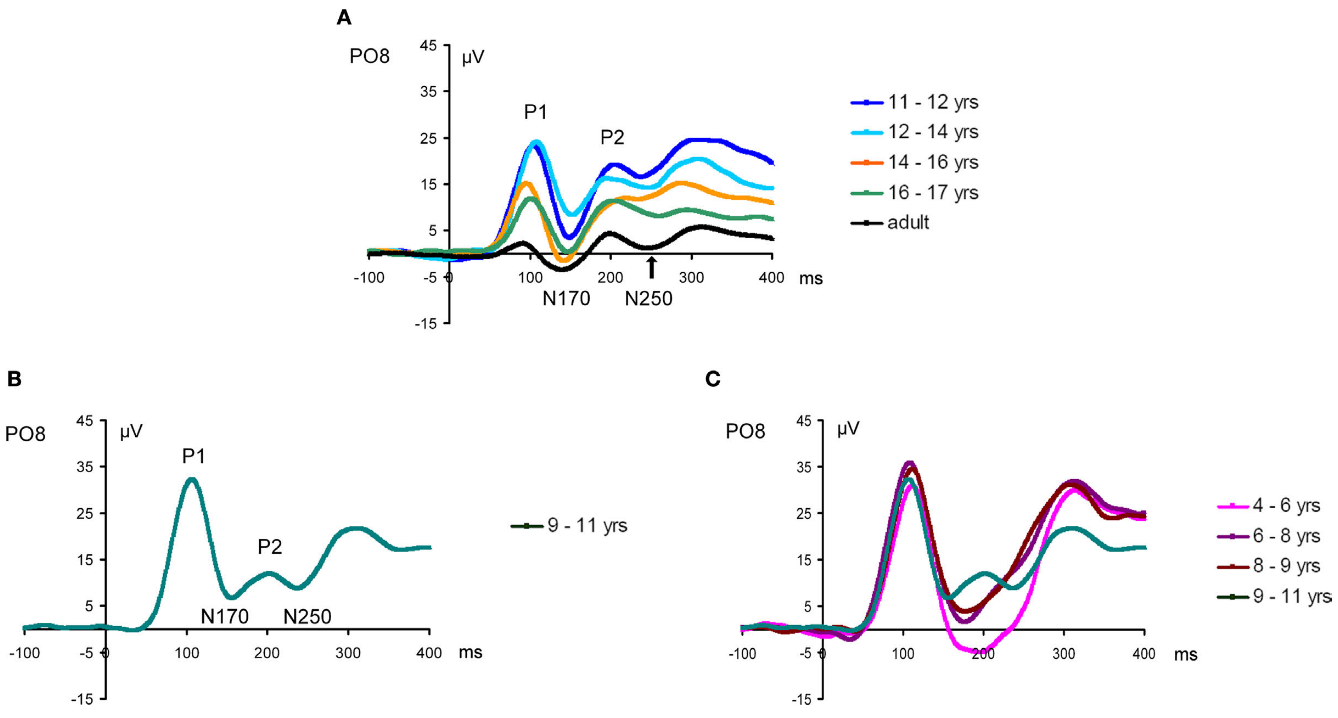

FIGURE 15 | (A) Grand-average waveform recorded at PO8 in response to faces in the four oldest age groups and adults. Note the separation of the N170 and N250 by the clear presence of the P2. **(B)** The grand-average waveform for the 9–11 year olds. Note that the P2 is less pronounced for this age group. **(C)** Grand-average waveforms recorded at PO8 in response to faces in the three youngest age groups (4–6 years, 6–8 years, 8–9 years) compared to the 9–11 year olds. Note that there is no P2 in the three youngest groups and that the "N170", as evident from the grand means, encompasses both the N170 and N250 of the older age group. The peak of the N170 for these three youngest age groups falls close to where the Peak of the P2 is for 9–11 year olds. This leads us to believe that in the grand averaged data, the N170 and N250 are merged in the three youngest age groups due to large inter-subject variability and the absence of a pronounced P2.

et al., 2005). Moreover, it has been reported that sensitivity to faces emerges as early as 290 ms in 12 month old infants (N290, de Haan et al., 2003), a component which, in conjunction with the later occurring P400, has been characterized as a possible precursor to the N170. According to the report of Taylor et al. (2004) of a 270 ms "N170" latency in 4- to 5-year-old children, face-sensitivity would have shifted only 20 ms between age 1 and 4, and then about 100 ms until adulthood, a paradox which was difficult to understand (de Haan et al., 2003). In fact, our data resolve this paradox and suggest the opposite: the latency of face-sensitivity effects decreases dramatically between 1 year of age and 4–5 years of age, but then only moderately until 9–10 years, where it is almost adult-like.

Furthermore, while Taylor et al. (2004) referred to the two peaks of the "bifid" N170 as the N170a and N170b, associating them with distinct face processes (holistic and configural respectively), our present observations lead to us to disagree with this view. Rather, based on topography, latency and right lateralization, we would like to argue that the first negative deflection, which arises relatively early following the P1 even in young children, corresponds to the N170 component in all age groups. The second peak is present in all age groups, including adults. However, in between these negative peaks, one can see the emergence of an increasingly sharper P2 component with age, which clearly distinguishes the two negative components at older ages (**Figure 15**). The second negative peak, the N170b of Taylor et al. (2004) appears to correspond rather to the adult N250, a negative mid-latency ERP which, in adults, is larger for repeated compared to unrepeated faces around 250 ms over temporal scalp regions, in particular for familiar faces ("N250r", e.g. Begleiter et al., 1995; Schweinberger et al., 1995; Tanaka et al., 2006). If this hypothesis is correct, future developmental ERP studies should be able to test – and perhaps demonstrate – the presence of such familiarity-dependent identity repetition effects for faces or objects of expertise in childhood.

STABLE (ADULT-LIKE) DIFFERENCES BETWEEN CONDITIONS IN THE PARAMETERS OF EARLY VISUAL RESPONSES

An important and novel aspect of the current study lies in the fact that we measured the electrophysiological responses not only to faces, but also to phase-scrambled faces, pictures of cars and phase-scrambled cars as well. For the first time, we were able to describe adult-like face-sensitivity of P1 and N170 across development.

P1 stable variations

First, with respect to amplitude, even though this effect is not always found (e.g., Rossion et al., 2003; Boutsen et al., 2006; Rousselet et al., 2007), previous studies in adults have reported larger P1 (or M1 in MEG) in response to faces than to objects (e.g. Eimer, 1998; Eimer and Holmes, 2002; Goffaux et al., 2003; Itier and Taylor, 2004c). Here we observed a larger P1 in response to pictures of faces than cars, replicating the latter observations. However, for the first time to our knowledge, we were able to demonstrate that this effect is, as previously suggested (Rossion and Jacques, 2008), entirely accountable by low-level visual differences between faces and cars, since it was also found for phase-scrambled versions of these stimuli. Most importantly, the larger low-level-related amplitude of the P1 for faces is observed from 4 years to adulthood, which indicates that the sensitivity of the visual system to low-level visual properties of faces, an aspect which certainly plays a critical role in the speed of face detection (e.g., VanRullen, 2006) is present at 4 years of age. Furthermore, the relative magnitude of this face-related P1 increase appeared to remain constant across development.

Second, independently of the category, we also observed a larger P1 response to scrambled stimuli than non-scrambled stimuli, which, again, did not show any significant change with age. Such increases to scrambled visual stimuli in low-level visual regions of the brain have been reported previously in fMRI studies of object processing (e.g., Malach et al., 1995) as well as in ERP/MEG studies (Tanskanen et al., 2005; Rousselet et al., 2008). This may reflect no more than a spread of information across the whole (rectangular) area of the visual stimulus when using phase-scrambled or pixilated images (**Figure 2**).

Stable N170 differences between faces and cars

Across all age groups, we found that the N170 was large in response to shapes (faces and cars) compared to meaningless versions of stimuli conveying the same low-level visual properties. Neither of the two scrambled stimuli elicited a very robust N170, an observation that has been previously made for adults (Tanskanen et al., 2005; Rousselet et al., 2008) and children (Taylor et al., 1999), and supports the interpretation of the N170 as a component reflecting high-level visual processes, particularly sensitive to faces (Rossion and Jacques, 2008, 2010). This indicates that, across all age groups, the N170 reflects in large part high-level visual processes, contrary to the P1.

As stated above, when considering the raw N170 amplitude, we replicated the finding of an inverted "U" shape developmental trajectory (**Figure 14**), with widely varying topographies across age groups (**Figure 7**). However, this finding was true for both faces and cars. Despite the small size of the N170 responses to scrambled stimuli, we used these responses to compute new waveforms for each subject, subtracting the responses of scrambled faces and cars from their meaningful non-scrambled stimulus counterpart. This subtraction process served to remove much of the response to faces and cars that could be attributed to low-level characteristics of the stimuli, leaving responses that were specific to the two stimulus categories. It also removed much of the P1 variations, which were furthermore taken into account by peak-to-peak analyses. These complementary analyses were, to our knowledge, not performed in previous ERP studies of the development of face processing.

When considering the N170, subtracting a portion of the response that can be attributed to low-level characteristics of the stimuli seems fundamental given that the P1, which is highly sensitive to low-level characteristics as discussed above, varies so greatly with age. Thus, to have a true measure of age-related changes on the N170, it was important to subtract the age-related changes happening on the P1. In order to directly take into account the variations of the P1, we analyzed both the amplitude and latency of the peak-to-peak (N170 to P1) values for both faces and cars. This measure is a reflection of the magnitude of amplitude or latency change between the peak of the P1 and the peak of the N170.

While these measures revealed general decreases in peak-to-peak latency and amplitude with increasing age, the decreases were no more pronounced for faces than for cars. Importantly, analyses of the subtraction waveforms revealed highly similar N170 topographical distributions across all age groups. In fact, correlation values with the adult N170 (P1 subtracted topography) were above 0.86 for all age groups, while the same correlation values for the raw N170 topographies were much smaller, and varied greatly by age group (**Figure S10** in Supplementary Material).

Besides general changes at the level of the N170, we reported, for the first time to our knowledge, larger amplitude (in particular when taking into account low-level and P1-related variations) and shorter latency to faces than cars, both remaining stable across age. N170 amplitude for faces was not much larger than for cars in this study (see Rossion and Jacques, 2008 for a discussion of this issue) but significantly so at all ages, which extends the well-known adult face-sensitivity of this component to children as young as 4 years. The shorter latency to faces is sometimes also reported in the adult ERP literature (e.g., Carmel and Bentin, 2002), but not consistently (e.g., Rossion et al., 2000). Remarkably, again, these effects were found here across all ages, and were of equal magnitude.

No evidence for interactions between development and face-sensitivity of early components

The use of multiple stimuli and peak-to-peak analyses allowed us to compare the responses across conditions to understand if any of the observed developmental changes were unique to faces or were also shared with these other stimuli. Our response to this question is clear: all age-related variations in the parameters of the P1 and N170 were not specific to faces. Furthermore, these age-related variations were not at all larger for faces than other visual stimuli.

Overall, these data do not offer any support for the view that perceptual aspects of the face processing system change specifically with development (Carey, 1992). In fact, they do not even support the view that the *speed* of these processes may change specifically for face stimuli (Crookes and McKone, 2009). Nevertheless, our data do not rule out the possibility of observing evidence from ERPs in favor of a qualitative view of the development of face processing in future studies testing sensitivity of these responses to individual faces (see below).

With respect to the P1, we found either massive changes with development that concerned all conditions equally, or differences between conditions that remained stable across development. These observations make it highly unlikely that P1 variations across age reflect specific changes in face processes, such as the ability to process faces as a whole pattern (holistic processing, Tanaka and Farah, 1993), as previously suggested (Taylor et al., 2004). As a matter of fact, recent ERP studies in adults, and behavioral evidence in children support this claim. For example, other P1 amplitude and latency variations in adults, for instance in response to inverted faces (e.g. Linkenkaer-Hansen et al., 1998) appear to be driven mainly by low-level parameters (e.g. subject's gaze fixation, Jacques and Rossion, 2009b), and, contrary to the N170, do not correlate with behavioral effects (Jacques and Rossion, 2007). Additionally, behavioral studies suggest that holistic processes are present at 4–6 years of age (e.g., Tanaka et al., 1998; de Heering et al., 2007) or even earlier in life (Cohen and Cashon, 2001), and may not evolve quantitatively throughout development (Crookes and McKone, 2009).

BASIC-LEVEL FACE CATEGORIZATION IS STABLE BETWEEN 4 YEARS OLD AND ADULTHOOD

The lack of face-specific, or even face-sensitive, changes across development at the level of the N170, by far the most widely investigated response to face stimuli (Rossion and Jacques, 2008, 2010), has several implications for our understanding of the neurofunctional development of face processing, and for future studies in this area of research.

While face recognition abilities improve tremendously with age, one has to be reminded that this concerns the ability of the system to distinguish novel from previously seen individual faces, or discriminate photographs of unfamiliar individual faces. However, as far as we know, there are no massive age-related improvements reported in the ability to categorize a visual stimulus as a face, i.e. to distinguish it from other visual categories. Improvements in figure-background segmentation and closure (Mooney, 1957) may be observed with development, but again, they would reflect general visual recognition improvements rather than being face-specific. Hence, the observation of a stable ERP difference between faces and nonface objects across development, as reported here, is not that surprising. In particular, there is ample evidence indicating that the N170 represents the first stage of access to high-level face representations (Rossion and Jacques, 2008). The present data indicate that this access may be slightly delayed in the youngest age groups, just as for other visually meaningful patterns. Yet, this basic-level face categorization appears functionally and qualitatively equivalent to what is observed in adults.

The observation of a stable N170 face effect across development stands, however, in contrast to the increase of the size of an area responding preferentially to faces in the right middle fusiform gyrus (fusiform face area, FFA; Sergent et al., 1992; Kanwisher et al., 1997) as reported in recent fMRI studies (Golarai et al., 2007; see also Aylward et al., 2005; Scherf et al., 2007), for children between 7 and 16 years old. However, a recent fMRI study reported in this journal showed that face-sensitivity in the right FFA was mature at 7 years of age (Pelphrey et al., 2009), an observation which is in line with our ERP findings. If, rather, a late maturation of the FFA is supported by future fMRI studies, it would suggests either that the right FFA does not contribute much to the N170 recorded on the scalp, or that the age-related effects observed in fMRI reflect late processes taking place in this area, rather than early access to face representations. Even though the N170 certainly originates from many cortical sources interlocked in time in the visual cortex, a large number of source localization studies indicate an important contribution of the middle fusiform gyrus (e.g., Rossion et al., 2003; Deffke et al., 2007; Henson et al., 2007), making the first possibility very unlikely. Our data thus points to a relatively late, differential age-related contribution of the right FFA in face processing that is not captured by the early N170 response. In other words, the significant correlation between the size of the right FFA and recognition memory for faces may be due to memory or attention for faces rather than changes to early perceptual face processes.

Finally, even though our electrophysiological data do not offer any support for the qualitative view of the development of perceptual face processes, they should not be taken as a categorical rejection of this view, and should not dissuade others using ERPs to tackle this issue in the future. While some authors preferentially address this issue by testing the variations of the N170 parameters with simple stimulus variations such as inversion and negation, or isolation of facial features (e.g., Taylor et al., 2001; Itier and Taylor, 2004b), we would rather recommend measuring the sensitivity of the N170 component (isolated from other general age-related variations) in paradigms which can be more directly related to individual face perception abilities. In line with this suggestion, a number of relatively recent ERP studies have shown that the N170 does not only mark early access to face representations in the human brain, but is sensitive for individual face representation (Jacques and Rossion, 2006). In particular, using face identity adaptation, it has been shown that the N170 is sensitive to the repetition of individual faces for upright but not inverted faces (Jacques et al., 2007; Ewbank et al. 2008), with a certain degree of generalization across viewpoint (Caharel et al., 2009a), and based on 3D shape rather than 2D surface-related (texture, color) variations (Caharel et al., 2009b). More pointedly, this early sensitivity to individual faces appears to be due to an early holistic perception of the face stimulus (using the composite face effect, Jacques and Rossion, 2009a), a fundamental aspect of face perception that is at the heart of the debate about the qualitative view of the development of face perception in the human brain (Crookes and McKone, 2009).

In other words, the present observations rule out the interpretation of developmental changes in the basic P1/N170 responses, and their face-sensitivity, as supporting age-related improvements in face processing. They do not at all, however, rule out the possibility that modulations of these responses by, for example, stimulus inversion (Taylor et al., 2001; Itier and Taylor, 2004b), or face identity repetition, show age-related changes that could be related to improvements of face recognition abilities during development. In fact, we would argue that the present work provides a better platform to test these hypotheses, and thus investigate the development of face recognition. In carrying out such studies however, one must be aware of a number of methodological issues and complications that arise during the analysis and interpretation of children's data, in particular the huge amount of variance in amplitude, and latency of raw ERP responses that is evident particularly in young children's data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/humanneuroscience/paper/10.3389/neuro.09/067.2009/

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Location, location, location: alterations in the functional topography of face- but not object- or place-related cortex in adolescents with autism

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In autism, impairments in face processing are a relatively recent discovery, but have quickly become a widely accepted aspect of the behavioral profile. Only a handful of studies have investigated potential atypicalities in autism in the *development* of the neural substrates mediating face processing. High-functioning individuals with autism (HFA) and matched typically developing (TD) controls watched dynamic movie vignettes of faces, common objects, buildings, and scenes of navigation while undergoing an fMRI scan. With these data, we mapped the functional topography of category-selective activation for faces bilaterally in the fusiform gyrus, occipital face area, and posterior superior temporal sulcus. Additionally, we mapped category-selective activation for objects in the lateral occipital area and for places in the parahippocampal place area in the two groups. Our findings do not indicate a generalized disruption in the development of the entire ventral visual pathway in autism. Instead, our results suggest that the functional topography of face-related cortex is selectively disrupted in autism and that this alteration is present in early adolescence. Furthermore, for those HFA adolescents who do exhibit face-selective activation, this activation tends to be located in traditionally object-related regions, which supports the hypothesis that perceptual processing of faces in autism may be more akin to the perceptual processing of common objects in TD individuals.

Keywords: fMRI, ventral visual pathway, fusiform gyrus, occipital face area, superior temporal sulcus, parahippocampal place area, lateral occipital area, development

INTRODUCTION

The recognition of faces may be among the most taxing perceptual challenges confronted by observers in their day-to-day life. Not only must the observer derive precise information about gaze direction, gender and affect of a face, but must also individuate the face perceptually from all other faces so that identity can be accurately assigned. Moreover, all of these computations must be done accurately and rapidly, notwithstanding the ambiguity of the input arising from the commonality of input features (i.e., all faces have two eyes, a nose and a mouth in the same spatial arrangement). The complexity of face processing is reflected by the finding that typically developing (TD) children do not exhibit mature recognition skills for facial identity (Carey and Diamond, 1977; Mondloch et al., 2004) or facial expressions (Herba and Phillips, 2004) until early adolescence. This delayed developmental trajectory may be related to similarly late occurring changes in the emergence of the neural substrate that mediates face processing (Golarai et al., 2007; Scherf et al., 2007). An unfortunate consequence of this prolonged developmental trajectory for the behavioral and neural substrates supporting face processing may be the increased vulnerability to delay or disruption, particularly in individuals with neurodevelopmental disorders.

In one such disorder, autism, impairments in face processing are a relatively recent discovery, but have quickly become a widely accepted aspect of the behavioral profile (for summary see Behrmann et al., 2006b; but see also Jemel et al., 2006). These impairments in autism appear to impact many aspects of face processing, including difficulty remembering faces (Boucher and Lewis, 1992; Scherf et al., 2008a), processing facial expressions (Ashwin et al., 2007; Humphreys et al., 2007), and knowing which components of faces convey especially important communicative information (Joseph and Tanaka, 2003). Although the overwhelming majority of the *behavioral* studies reporting atypical face processing in autism have been conducted with young children and adolescents, the majority of studies investigating abnormalities in the *neural substrate* of face processing have been conducted with adults. Many studies of adults with autism (Critchley et al., 2000; Schultz et al., 2000; Pierce et al., 2001; Hall et al., 2003; Hubl et al., 2003; Ogai et al., 2003; Piggot et al., 2004; Wang et al., 2004; Dalton et al., 2005; Deeley et al., 2007; Humphreys et al., 2008), although not all (Hadjikhani et al., 2004, 2007; Pierce et al., 2004; Bird et al., 2006; Kleinhans et al., 2008), have found hypoactivation in core face-related regions within the ventral visual pathway, including the 'occipital face area' (OFA), fusiform face area (FFA), and the posterior superior temporal sulcus (STS).

Only a handful of studies have investigated potential atypicalities in the *development* of the neural substrates of face processing in children with autism. Consistent with the adult studies, some of these developmental studies report face-related hypoactivation, particularly in the fusiform gyrus (FG), during a variety of face-processing tasks (Wang et al., 2004; Dalton et al., 2005; Grelotti et al., 2005). Most recently, using a 1-back task while scanning with fMRI, Pierce and Redcay (2008) reported a significant reduction in the number of face-selective voxels in the right and left FG and in the magnitude of face selectivity in the active voxels in the left FG in children with autism (mean age of 9.9 years), relative to TD controls, specifically when the children were observing an unfamiliar adult face. Similarly, Corbett and colleagues reported no suprathreshold face-related activation in the amygdala or FG during an emotion-matching task in young adolescents with autism, along with hypoactivation in the FG during a face identity-matching task in the same participants (Corbett et al., 2009). In contrast, using a 1-back task and scanning with magnetocencephalography, Kylliäinen et al. (2006) found that children with autism exhibited striking similarities, with only subtle differences from TD controls in overall signal latencies, amplitudes, topographies, and stimulus sensitivity when observing unfamiliar faces.

The goal of the current study was to investigate three lingering unanswered questions from this existing work, with a focus on adolescence as the critical developmental period of evaluation. Given the inconsistencies in the literature, our first goal was to determine whether there is atypical activation in face-related cortex in adolescents with autism. To achieve this goal, we tested a fairly homogenous population of high-functioning adolescents with autism (HFA) during naturalistic viewing conditions. Participants watched dynamic, real time movie vignettes of faces, common objects, buildings, and scenes of navigation while undergoing an fMRI scan. This task elicits more natural exploration of the visual environment than the static photographs used in previous developmental neuroimaging studies, which translates to greater category-selective activation in the ventral temporal lobe than more traditional tasks with static images (Avidan et al., 2005).

Importantly, we targeted early adolescence (ages 11–14) as a critical time for evaluating the emergence of face-related activation in individuals with autism. We focused on this age range in light of recent findings indicating that in TD individuals, adolescence is an important period of neural plasticity when substantial reorganization occurs in the cortical and limbic networks (i.e., Nelson et al., 2005), especially in terms of the fine-tuning and functional organization of face-related regions in the ventral temporal lobe (Scherf et al., 2007). Also, adolescence is the time when nearly one-third to one-half of individuals with autism experience a deterioration of functioning (Bradley et al., 2004). For example, perceptual development appears to be specifically vulnerable during early adolescence in autism (see Scherf et al., 2008a,b). Investigating the developmental profile of face-related cortex in autism during this important period will shed light on (1) perceptual and brain development particularly when symptoms often worsen, and (2) the design of targeted interventions to induce plasticity in the behavioral and neural substrates supporting face processing.

Our second goal was to evaluate whether the functional topography of the ventral visual pathway is altered for other visual classes in autism. The majority of existing studies, in both children and adults with autism, have focused on atypical activation in face-related cortex, and in the FG in particular. This has been an obvious first step, especially in light of the behavioral findings of face-processing impairments; however, there are hints in both the behavioral and neuroimaging work that objects may also be processed atypically, and perhaps precociously, in autism. For example, several studies have reported equivalent or even superior performance on building and common object recognition (Boucher and Lewis, 1992; Hauck et al., 1998; Trepagnier et al., 2002; Teunisse and de Gelder, 2003; Scherf et al., 2008a), and faster early latency (100 ms) neural responses to common objects in right extrastriate cortex (Kylliäinen et al., 2006) in children and adolescents with autism. However, one developmental neuroimaging study found smaller volumes of object-related activation in the right FG in children with autism (Pierce and Redcay, 2008). At least two studies with adults have reported normal activation for objects in the lateral FG and lateral occipital area (Schultz et al., 2000; Humphreys et al., 2008) and for places in the parahippocampal place area (PPA) (Bird et al., 2006; Humphreys et al., 2008). However, there are no studies that systematically map out the functional organization in the ventral visual pathway for all three visual categories (faces, places, and objects) in children or adolescents with autism. This is particularly important to do given the findings of striking differences in early brain growth in key areas engaged in visual cognition and object processing, including frontal and temporal lobes and the amygdala (Carper and Courchesne, 2005; Hazlett et al., 2005). The effects of this early abnormal brain growth are also evident in adolescence (Hazlett et al., 2006), particularly in the temporal lobes (Hardan et al., 2009).

To address this issue, we mapped the functional topography of category-selective activation for faces bilaterally in the FG, but also in the OFA, and posterior STS, for objects in the lateral occipital area (LO), and for places in the PPA in the HFA and TD groups. We predicted that if face-related cortex is selectively disrupted in autism, we would observe specific atypicalities, and possibly hypoactivation in face-selective activation in the FG, OFA, and STS, without any group differences in object-related activation in the LO or place-related activation in the PPA. On the other hand, if the development of the topography of the entire ventral visual pathway is disrupted in autism, we expected to find group differences in the location, magnitude of category selectivity, and/or number of active voxels in all five (FG, OFA, STS, LO, PPA) regions. Either pattern has substantial implications for our understanding of the underlying mechanisms contributing to face-processing deficits in autism as well as for our attempts to ameliorate such deficits.

The third goal of the current work was to investigate a potential mechanism underlying any observed atypicalities in category-selective activation in the ventral visual pathway in autism. Although there is an emerging consensus that children and adults with autism exhibit hypoactivation in face-related cortex, there are few investigations of the mechanisms contributing to such hypoactivation. Some evidence suggests that atypical visual attention, gaze patterns, and/or motivation to attend to faces may be related to patterns of hypoactivation in the FG for faces (for review see Klin, 2008).

For example, although several groups have reported that individuals with autism spend less time looking at the eye region of faces (Klin et al., 2002; Spezio et al., 2007; Adolphs et al., 2008), which may reflect atypical visual attention and perhaps motivation to attend to faces (Dawson et al., 2002; Grelotti et al., 2002), only one study suggests that this atypical fixation pattern in children with autism may be directly related to hypoactivation in the FG (Dalton et al., 2005). Related, two studies in adults with autism reported normal face-related activation in the FG when participants were continuously cued to the eye region of a face with a central fixation cross (Hadjikhani et al., 2004, 2007; however, see Dapretto et al., 2006; Humphreys et al., 2008 for contrary results). Another set of studies suggests that the potentially atypical visual attention and motivation to look at faces can be modulated by presenting images of personally familiar faces to adults and children with autism, which results in more normal patterns of face-related activation (Pierce et al., 2004; Pierce and Redcay, 2008; however see Dalton et al., 2005 Study II for contrary results). Critically, it should be noted that the relation between purported atypicalities in the locus of fixations during face processing and cortical activation patterns in children with autism is quite controversial (see Boraston and Blakemore, 2007).

We investigated an alternative mechanism for potential hypoactivation in category-selective activation in adolescents with autism. Based on findings of extensive individual variability in behavioral performance (e.g., Scherf et al., 2008a,b) and in the timecourse of activation in both the FG and LO in adults with autism (Humphreys et al., 2008), we evaluated the role of *within-group individual variability* in the size, location, magnitude of category selectivity, and proportion of participants who exhibit identifiable category-selective activation in *all five regions of interest (ROIs)* bilaterally. This included evaluating individual variability within the ROIs identified in a voxelwise whole brain analysis for each group (HFA, TD) and within individually defined ROIs for each participant. We also investigated whether individual variability in the severity of behavioral symptoms in the HFA group was related to individual variability in any of the neural measures.

MATERIALS AND METHODS

PARTICIPANTS

The participants included 10 high-functioning right-handed male adolescents with autism (mean age 12.2 years: range 10–14 years). The group mean IQ was in the average range (VIQ = 108, PIQ = 97, FSIQ = 102). Each participant with autism was yoked to a TD right-handed control participant based on age, sex, and Full Scale IQ. **Table S1** in Supplementary Material illustrates the demographic and IQ information for each of the 10 autism and control participants. There were no differences between the groups in age, Verbal, Performance, or Full Scale IQ (all $p > 0.13$). Written informed consent was obtained from participants' legal guardians, as well as written assent from the participants themselves, using procedures approved by the Internal Review Boards of the University of Pittsburgh and Carnegie Mellon University.

The diagnosis of autism was established using the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994), the Autism Diagnostic Observation Schedule-G (ADOS) (Lord et al., 2001), and expert clinical diagnosis (Minshew, 1996). The individuals with HFA, recruited from autism conferences and parent support groups, were medically healthy and had no identifiable genetic, metabolic, or infectious etiology for their disorder. Participants were also free of birth or traumatic brain injury, seizures, attention deficit disorder, and depression. Their personal and family health histories were evaluated in the initial screening interview and in the medical review portion of the ADI. IQ was determined for all participants using the Wechsler Abbreviated Scale of Intelligence.

Typically developing adolescents were recruited via advertisements given to them at school to take home to their parents. TD participants were included if they were in good physical health, free of regular medication usage, had good peer relationships as determined by parent or self-report and staff observations during the screening procedures, and did not exhibit behavioral symptoms that could be indicative of autism or any psychiatric diagnosis (as determined via a behavioral checklist completed by a parent).

PROCEDURE

To maximize natural viewing conditions, participants freely viewed a silent, fluid concatenation of short movie vignettes, containing scenes of faces in various situations (e.g., walking through a crowd, in conversation), panoramic and close-up views of buildings, navigation through open fields, and miscellaneous common objects (e.g., kitchen utensils, musical instruments, desk items) in a blocked fMRI paradigm. The vignettes were organized into 32 randomized 15-s blocks containing stimuli from a single category (eight blocks per stimulus category). The task began with a 29-second blank screen followed by a 9-s block of abstract pattern stimuli and ended with a 21-s blank screen (see **Figure S1** in Supplementary Material). This task has been used successfully to map category-selective activation in the ventral visual cortex in typical adults (Hasson et al., 2004) and in adults with autism (Humphreys et al., 2008) as well as in TD children and adolescents (Scherf et al., 2007). It has three clear benefits for a developmental study comparing typical and clinical populations. First, it elicits more natural exploration of the visual environment than the static photographs used in previous developmental neuroimaging studies. Second, this task elicits greater category-selective activation in the ventral temporal lobe than more traditional tasks with static images (Avidan et al., 2005). Third, since there are no specific task demands, group differences in behavioral performance are not a confound for different levels of functional activation. Also, a block, rather than an event-related paradigm was employed to provide a direct comparison with previous robust results with TD adolescents (Scherf et al., 2007) and with high-functioning adults with autism (Humphreys et al., 2008). It also provides stronger signals with better statistical power. The movie vignettes were displayed on a rear-projection screen located inside the MR scanner. Immediately prior to the scanning session, all participants were trained to lie still for 20 min in a mock scanner that simulated the noise and confinement of an actual MR scanner.

DATA ACQUISITION

EPI BOLD images were acquired in 35 AC-PC aligned slices on a Siemens 3T Allegra scanner, covering most of the brain and all of the occipital and temporal lobes (TR = 3000 ms; TE = 35 ms; 64 × 64, 3 mm slice thickness, 3.203 × 3.203 mm in-plane resolution).

Anatomical images were acquired using a three-dimensional volume magnetization prepared rapid gradient echo (3D-MPRAGE) pulse sequence with 192 one-mm, T1-weighted, straight sagittal slices.

DATA ANALYSES

The data were analyzed using Brain Voyager QX (Brain Innovation, Maastricht, Netherlands). Preprocessing of functional images included 3D-motion correction, filtering out of low frequencies, and re-sampling the voxels to 1 mm^3. Participants who moved more than 3.0 mm (1 voxel) were excluded from the analyses. Separate one-way ANOVAs on each of the six motion dimensions revealed no group differences in movement ($F < 1$). Thus, any group differences in the functional profile of the ventral visual pathway cannot be explained by motion differences across the groups.

For each participant, the timeseries images for each brain volume were analyzed for category differences in a fixed-factor GLM. Each of the categories was defined as a separate predictor and was modeled with a box-car function adjusted for the delay in hemodynamic response. The timeseries images were then spatially normalized into Talairach space. Although the brains of individuals with autism and typical individuals may differ in size, volumetric differences are minimized as IQ differences are reduced (for review see Stanfield et al., 2008) and Talairach transformation of functional data is still widely used (e.g., Pierce and Redcay, 2008). There was no spatial smoothing.

In both the group and individual level analyses, the timeseries images for each brain volume for each individual participant were used to define category selectivity. As in previous studies using this movie task, we adopted a conservative definition of category selectivity contrasting the averaged BOLD response amplitude (across blocks within a category) for each category to that of all the others (Hasson et al., 2004; Avidan et al., 2005; Scherf et al., 2007; Humphreys et al., 2008). For example, face-selective activation was defined by the weighted contrast [(faces – (objects + buildings + navigation)]. Because scenes of buildings and navigation both drive PPA activation, place-selective activation was defined as [(buildings + navigation) – (faces)].

Identifying category selectivity within each group

Category selectivity was initially evaluated separately in each group by submitting the individual subject timeseries images to a whole brain voxelwise random effects GLM in which the category was a fixed factor and participant was a random factor. This GLM generates beta weights for each individual participant in each condition (i.e., visual category), which were then submitted to two-tailed t-tests in each voxel to determine the relative magnitude of category selectivity. The group level contrast maps (e.g., [(faces – (objects + buildings + navigation)]) were corrected for multiple comparisons using a Monte Carlo simulation. To achieve $p < 0.05$ significance, the simulation required a minimum of four contiguous 3 mm voxels with a t-value ≥ 2.3. These group level maps of the topography of category-selective activation for each group are shown in **Figure 1**.

Evaluating group differences in target regions of interest

Group-defined ROIs. As a group, the TD adolescents represent the normal state of functional organization in the ventral temporal lobe for this developmental period. To evaluate potential alterations in this organization in HFA adolescents, the TD group-defined ROIs (**Figure 1A**; right and left FFA, PPA, LO, and right OFA and right STS) provided the initial candidate regions for examining atypical activation patterns. This analysis uncovers the functional profile of activation in HFA adolescents in the ROIs that are category-selective in TD adolescents. To this end, we computed a ROI-based GLM separately in each TD-defined ROI (see **Table 1**). This involved computing a random effects, two-factorial ANOVA across all participants on the timeseries data from the entire experiment within each ROI, with participant as the random factor, visual category as the within-subjects factor, and group as the between-subjects factor. This generated beta weights for all main conditions for each participant (faces, objects, buildings, navigational scenes), which were then submitted to a two-tailed t-test selectivity contrast [e.g., in the TD FFA: TD (faces – objects) versus HFA (faces – objects)] to evaluate group differences in the magnitude of selectivity within each ROI. Although the investigation of the profile of activation in the TD group within these ROIs is not independent from the selection of the ROIs (and we are fully aware of potential statistical biases when defining the ROI and then using the timeseries from the same voxels for the condition comparison), the important and independent contribution of this analysis is in evaluating how aberrant or similar the pattern of activation is within these TD-defined ROIs in the *HFA group*. To emphasize our focus on the analysis of the HFA group within these TD-defined ROIs, we computed the simple two-tailed t-test between visual categories (e.g. faces versus objects) within each ROI for the HFA group, whose timeseries are completely independent of those used to define the ROIs.

To evaluate the possibility that HFA adolescents exhibit category selectivity in different ROI locations compared to the TD adolescents, the same analyses described above were performed on the profile of activation for each group within the *HFA group-defined* ROIs. Again, we acknowledge that the investigation of the profile of activation in the HFA group within these ROIs is not independent from the selection of the ROIs. However, investigating potential differences in the profile of activation in the *TD group* within these HFA-defined ROIs is an independent analysis and provides meaningful information about the functioning of these regions in typical individuals. Again, to emphasize our focus on the analysis of the TD group within these HFA-defined ROIs, we computed the simple two-tailed t-test between visual categories (e.g. faces versus objects) within each ROI for the TD group, whose timeseries are completely independent of those used to define the ROIs.

Individually defined ROIs. To evaluate whether individual variability in the location, size, and/or magnitude of selectivity within the HFA group contributed to group differences in the functional organization within the ventral visual pathway, the functional profile of category-selective activation was determined in *individually defined* ROIs for each participant in each group. These ROIs were extracted from separate contrast maps (face, place, object-selective ROIs as defined above) in each participant that were corrected for multiple comparisons using the False Discovery Rate Procedure to ensure that fewer than 10% of the significantly active voxels were false positive activations (Genovese et al., 2002). Composite maps were generated for each visual category (faces, places, objects) of each individual participant's category-selective activation mapped onto a single inflated brain, illustrating the relative size and extent of overlap in each participant's individually defined category-selective activation. To evaluate individual variability in the loci of these ROIs, following Müller et al. (2003), we calculated the distance in stereotactic space between the midpoint of each individually defined ROI and the midpoint of the appropriate group-defined ROI in each individual of the respective group. For example, the midpoint of the HFA group-defined face-selective right FG occurred at the coordinates (43, −65, −17). For HFA Participant 3, the midpoint of his individually defined face-selective right FG ROI occurred at the coordinates (41, −50, −15), resulting in a distance of 15.3 mm, calculated as follows:

$$\sqrt{\left(2^2+15^2+2^2\right)}=15.3$$

This computation is roughly equivalent to calculating the geometric mean of the differences; however, it preserves the pooled distance in the original units (mm). To compare group differences in these scores, they were submitted to separate independent-samples t-tests for each ROI in each hemisphere and only individuals with definable ROIs were included in this analysis. This was only computed for the right and left FG, PPA, and LO ROIs. A similar analysis could not be computed in the OFA since only the TD group exhibited a definable right OFA and only the HFA group exhibited a definable left OFA.

To evaluate group differences in the magnitude of within-subject category-selective activation within each of these ROIs (FG, OFA, PPA, LO) in each hemisphere, separate ROI-based GLMs were conducted for each subject who exhibited identifiable category-selective activation in each ROI. This generated beta weights for all main conditions for each participant (faces, objects, buildings, navigational scenes), which were then submitted to separate repeated-measures ANOVAs with visual category as the within-subjects variable and group as the between-subjects variable. To evaluate the extent of the category-selective activation within these individually defined ROIs, the number of significantly active voxels was extracted for each ROI in each hemisphere and compared for group differences in separate two-tailed t-tests. If a participant had no identifiable category-selective activation, the mean beta weight for each condition from the appropriate group (TD, HFA) and ROI was submitted to the analysis of category selectivity and a value of 0 was entered in the analyses of the extent of activation, so that all participants contributed to the analysis. In all analyses (magnitude, extent, locus), there was equal variance across the two groups as determined by the Levene's test of equality of variances.

Correlation analyses

To examine associations between patterns of brain activation and participant demographics in the autism group, Pearson correlation coefficients between the size (number of significantly active voxels) and category selectivity (difference in beta weights of respective visual categories in individual ROI GLMs) of each of the individually defined ROIs and ADOS and IQ scores were computed.

RESULTS

CATEGORY-SELECTIVE TOPOGRAPHY WITHIN EACH AGE GROUP

Figure 1 shows the average face-, object-, and place-related activation maps generated from the whole brain analyses for each group projected onto the inflated cortical surface of a representative individual. Category selectivity was defined by the weighted contrast of the category of interest (e.g., faces) versus all other visual categories (e.g., objects, navigation, buildings). All regions of significant activity for each contrast in each group are reported in **Table S2** in Supplementary Material, but the focus of the results described here is on patterns of significant activity in the target ROIs including the FFA, OFA, STS, LO, and PPA (see **Table 1**).

Consistent with previous findings from this task, TD adolescents (**Figure 1A**) exhibited significant face-related activation bilaterally in the FFA, and in the right OFA and STS. TD adolescents also activated bilaterally the medial portion of the FG and the ventral LO when viewing common objects and the PPA when viewing places.

HFA adolescents showed typical patterns of activation when viewing objects and places, with object-selective activation bilaterally in similar medial portions of the FG and the ventral LO and place-selective activation in the PPA (**Figure 1B**). However, unlike TD adolescents, the only face-related region that the HFA adolescents activated as a group was a very posterior region of the FG bilaterally (right: 43, −65, −17; left: −38, −75, −14), which was so ventral that it did not project onto the inflated surface in **Figure 1B**. The HFA adolescents also activated a portion of the left OFA (Talairach coordinates of the midpoint: −46, −74, −9), which was not present in the TD adolescents. To evaluate whether any face-selective activation is present in more dorsal and anterior portions of the FG in the HFA adolescents as a group, the threshold on the group level face contrast was reduced to a corrected $p < 0.10$ (**Figure 1C**). At this reduced threshold, a larger face-selective ROI emerged only in the right FG (Talairach coordinates of the midpoint: 42, −61, −18; 1079 voxels). This appears to be a dorsal and anterior extension of the posterior FG activation that was significant at $p < 0.05$. However, the region is still more ventral and posterior than the TD FFA activation and only overlaps with the TD group ROI in a small portion of the most anterior part of the HFA ROI and posterior part of the TD ROI (Talairach coordinates of the midpoint: 40, −46, −22; 22 voxels). A region of right posterior STS face-selective activation also emerged at this more lax threshold in the HFA group (Talairach coordinates of the midpoint: 48, −51, 9; 477 voxels). No such face-selective regions emerged in the right OFA or the left STS even at this more lax threshold.

GROUP DIFFERENCES IN TARGET REGIONS OF INTEREST

Figure 2 shows the cortical activation profiles for each group, as measured by the difference in relevant beta weights from the separate ROI GLMS contrasting category selectivity across groups within each ROI, in both the TD-defined (**Figures 2A–C**) and HFA-defined (**Figures 2D–F**) group level ROIs.

Group-defined ROI analyses

TD-defined ROIs. To evaluate potential alterations in the normal state of functional organization in the ventral temporal lobe, the TD group-defined ROIs provided the initial candidate regions for examining atypical activation patterns in the HFA group. The HFA group exhibited comparable magnitude of category selectivity for places in the right and left TD-defined PPA (**Figure 2A**) and for objects in the right and left TD-defined LO (**Figures 2B,C**) as did the TD group [all $t(18) \leq 1.0$]. However, within the face-selective ROIs, the HFA group exhibited less selectivity for faces compared

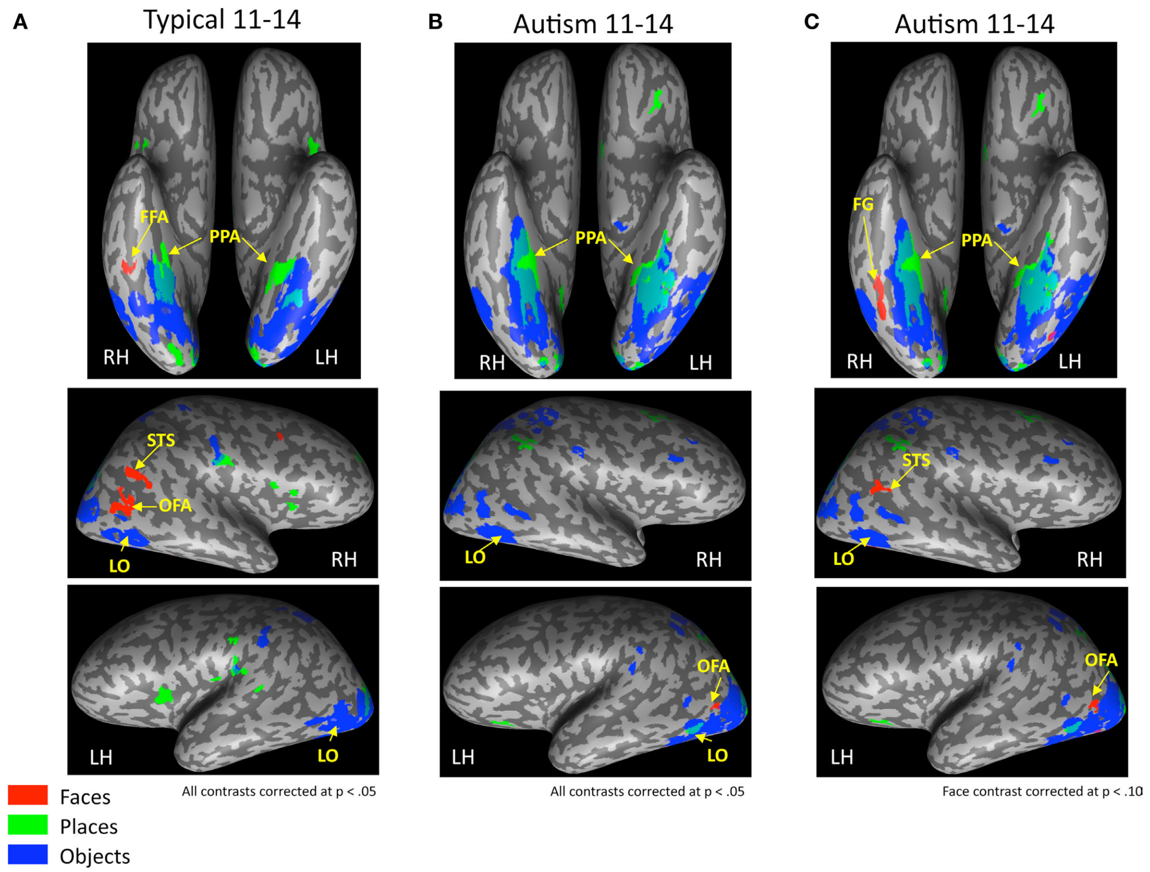

FIGURE 1 | Category-selective topography within each group. Contrast maps for each object category from the group level random effects GLM showing average face-, object-, and place-related activation maps generated from the whole brain analyses for each group. Activation is mapped onto the ventral projection and the lateral hemispheres of a single representative inflated brain for the **(A)** typically developing adolescents and **(B)** adolescents with autism, when all contrasts are corrected at $p < 0.05$. Although there were small loci of face-selective activation in the right and left FG in the HFA group, these ROIs are so ventral that they do not project onto the inflated brain (see **Table 1** for coordinates and size of ROIs) **(C)** To evaluate whether face-selective activation is present in more dorsal and/or anterior portions of the fusiform gyrus in the HFA adolescents as a group, the threshold on just the group level *face contrast* was reduced to a corrected $p < 0.10$. A larger face-selective ROI emerged in the right fusiform gyrus and the right STS. FFA, fusiform face area; FG, fusiform gyrus; OFA, occipital face area; STS, superior temporal sulcus; LO, lateral occipital object area; PPA, parahippocampal place area.

to objects than did the TD group in the TD-defined right FFA, $t(18) = 2.2, p < 0.05$, left FFA, $t(18) = 3.8, p < 0.005$, and right OFA, $t(18) = 2.1, p < 0.05$ (see **Figures 2A,B**). In fact, the HFA group failed to exhibit selectivity for either faces or objects in both the right FFA, $t(9) = 0.3, p =$ ns, and OFA, $t(9) = 0.1, p =$ ns. However, in the case of the left FFA, the HFA group exhibited more selectivity for *objects* than faces, $t(9) = 3.4, p < 0.01$. Interestingly, the groups showed equal selectivity for faces in the TD-defined right STS, $t(18) = 0.5, p =$ ns.

HFA-defined ROIs. To evaluate the possibility that HFA adolescents exhibit category selectivity, and face selectivity in particular, in different ROI locations compared to the TD adolescents, similar analyses were performed on the profile of activation for each group within the *HFA group-defined* ROIs. Unlike in the TD-defined place-selective ROIs, the HFA group tended to exhibit *more* selectivity for buildings compared to faces in the HFA-defined right PPA, $t(18) = 1.9, p = 0.10$, and left PPA, $t(18) = 2.8, p < 0.01$, than did the TD group (**Figure 2D**). However, there were no group differences [all $t(18) \leq 1.0$] in category selectivity for objects bilaterally in the HFA-defined LO (**Figures 2E,F**). Finally, in the HFA-defined face-selective ROIs (at the $p < 0.05$ corrected threshold), as expected the HFA group exhibited more activation to faces than to objects in the right and left FG, and in the left OFA. However, in the right FG, there was a significant difference between the groups in face selectivity, $t(18) = 3.1, p < 0.01$. (**Figure 2D**). While the HFA group exhibited selectivity for faces in the ROI, the TD group exhibited selectivity for objects in this ROI, $t(9) = 2.2, p = 0.05$, presumably because of its posterior location. The groups did not differ in the magnitude of selectivity in the left FG, $t(18) = 1.0, p =$ ns, or in the left OFA, $t(18) = 1.2, p =$ ns. Interestingly, the TD group did exhibit more selectivity for objects than faces in the HFA-defined face-selective left OFA when it was defined at the reduced threshold ($p < 0.10$ corrected), $t(18) = 2.4, p < 0.05$.

In summary, the group-defined ROI analyses revealed an important double dissociation in face-selective regions in the FG. Those regions defined by the TD group were more object-selective in the HFA group, and the reverse was true for the face-selective ROIs

Table 1 | Target regions of interest for face, place, and object-selective activation identified in whole brain analysis within each group.

ROI	Hemisphere	Autism group				Typically developing group			
		Talairach coordinates			Size	Talairach coordinates			Size
		X	*Y*	*Z*		*X*	*Y*	*Z*	
FG ($p < 0.05$)	Right	43	–65	–17	109	40	–41	–23	510
	Left	–38	–75	–14	128	–39	–51	–19	361
FG ($p < 0.10$)	Right	42	–61	–18	1083				
	Left	–38	–75	–15	303				
OFA ($p < 0.05$)	Right					50	–61	9	2625
	Left	–46	–74	–9	108				
OFA ($p < 0.10$)	Right								
	Left	–45	–73	–7	294				
STS ($p < 0.05$)	Right					55	–50	12	564
STS ($p < 0.10$)	Right	48	–51	9	477				
	Left	–45	–67	6	924				
PPA ($p < 0.05$)	Right	26	–41	–11	3687	26	–42	–8	3531
	Left	–24	–45	–12	4636	–24	–45	–8	3601
LO ($p < 0.05$)	Right	45	–60	–3	7087	41	–61	–11	5934
	Left	–42	–59	–11	8751	–41	–66	–7	10075

Coordinates represent centroid of activation within each ROI. All ROIs significant at a corrected p-value. Size represented in terms of number of 1 mm voxels. Face-selective activation in FG, OFA, and STS defined as the weighted contrast of faces versus objects and places. Place-selective activation in the PPA was defined as the weighted contrast of places (buildings and navigation) versus faces. Object-selective activation in LO was defined as the weighted contrast of objects versus faces and places. When no coordinates are listed for a particular ROI, it indicates that this ROI was not identifiable for the respective group. Face selective activation at the reduced p < .10 threshold was only evaluated in the autism group.

defined by the HFA group, they were more object-selective in the TD group. There were no group differences in object-related ROIs defined by either group, and the place-selective ROIs were slightly more selective for places in the HFA group, but only in the HFA-defined PPA.

Individually defined ROI analyses

Face-related ROIs. To illustrate individual variability within the HFA and TD groups in the location and size/extent of face-related ROIs, composite maps were created that show face-related activation defined uniquely for each individual in the FG (**Figures 3A,B**) and OFA (**Figures 3E,F**). In the composite maps, each participant's individually defined face-selective activation is shown in a unique color with the group-defined face-selective FG and OFA activation illustrated in white (see **Table S3** in Supplementary Material for coordinates and size of individual ROIs). Ninety percent of the individuals in the TD group exhibited face-selective activation in the right FG, with 60% exhibiting face-selective activation in the TD group-defined right FFA ROI. In contrast, 70% of the individuals in the HFA group exhibited face-selective activation in the right FG, however, only 30% of the HFA individuals exhibited face-selective activation in the right FFA, as defined by the TD group. Similarly, 80% of the TD individuals exhibited face-selective activation in the right OFA, with 70% overlapping in the TD group-defined OFA ROI. Although 100% of the HFA participants exhibited face-selective activation in the right OFA, only 30% of these individuals exhibited overlapping activation in the OFA as defined by the TD group. These results indicate that 20% fewer individuals within the HFA group exhibited identifiable face-selective activation in the FG, even at the somewhat lenient statistical threshold of FDR $q < 0.10$, and 50% fewer exhibited such activation in the FFA region of the FG. Furthermore, when this face-selective activation can be identified, there is considerably more variability in the location of both the FG and OFA face-selective ROIs among the individuals with autism.

To quantitatively compare differences in variability in the loci of activation in these individually defined face-related regions, we computed the distance in stereotactic space between the midpoint of each individual ROI and the midpoint of the respective group ROI. As expected, individual spatial variability was higher in the HFA individuals than in the TD individuals in the right FG, $t(14) = 2.9$, $p < 0.01$, and in the left FG, $t(12) = 2.9$, $p < 0.025$. **Figure 4** illustrates the mean distance in millimeters of the individually defined ROIs from the location of the respective group-defined ROI for both the HFA and TD groups.

Surprisingly, despite the increased variability in the locus of face-related activation in the HFA group, particularly in the FG, HFA individuals with identifiable face-related activation exhibited comparably sized ROIs as did the TD group (**Figures 3C–H**). There were no group differences in extent of activation, as measured by the total number of face-selective voxels, in either the FG or the OFA in either hemisphere [all $t(18) < 1.7$].

Finally, the repeated-measures ANOVAs on the magnitude of face-selective activation, as measured by the difference in beta weights for faces and objects from the individual subject ROI GLMs in the respective regions, only revealed subtle group differences.

In the right FG, there was a main effect of visual category, $F(1, 18) = 77.3$, $p < 0.001$, with higher activation to faces compared to objects in both groups and a trend for a main effect of group, $F(1, 18) = 77.3$, $p = 0.064$, with the TD group showing more activation in the right FG than the HFA group across both visual categories.

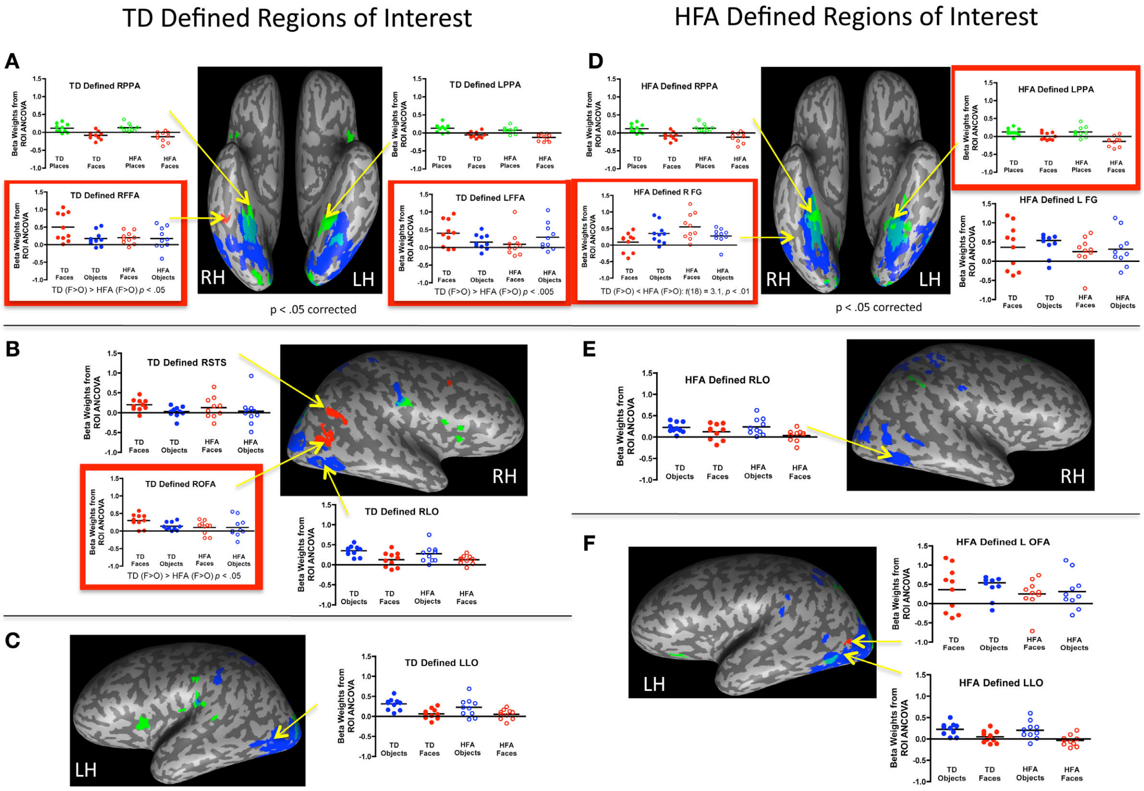

FIGURE 2 | Group differences in profile of activation within target regions of interest (ROI). Group differences in the magnitude of category selectivity were directly evaluated within each of the target ROIs (FFA, OFA, STS, LO, PPA) identified in the typically developing (TD) adolescent group map **(A–C)** and also in the group map of the high-functioning adolescents with autism (HFA: **(D–F)**) at a corrected $p < 0.05$ threshold. Significant differences in beta weights derived from the ANOVA are outlined in red. There were no group differences in selectivity for objects in any of the object-selective ROIs, regardless of how they were defined (by the TD or HFA group). Similarly, in the TD-defined PPA ROIs, the groups were equally selective for places compared to faces. In the HFA-defined PPA ROIs, the HFA group exhibited more place-selectivity than did the TD group, which was related to lower beta values for faces, as opposed to higher beta values for places. The most prominent group differences were evident in the fusiform gyrus and OFA face-related ROIs, as defined by each group. In the right TD-defined FFA and OFA, HFA participants exhibited *object,* as opposed to face, selectivity. In the HFA-defined right fusiform gyrus, the TD group exhibited *object,* as opposed to face, selectivity. This set of findings represents a double dissociation such that face-related ROIs in the TD group were more object selective in the HFA group, whereas face-related ROIs in the HFA group were more object selective in the TD group.

However, there was no visual category × group interaction, $F(1, 18) = 1.7$, p = ns, indicating that both groups showed comparable selectivity for faces compared to objects in spite of the generally lower level of activation in this region in the HFA group. Similarly, in the left FG, there were also main effects of visual category, $F(1, 18) = 296.4$, $p < 0.001$, with higher activation to faces compared to objects in both groups, and of group, $F(1, 18) = 10.5$, $p < 0.01$, with TD individuals exhibiting more activation across both faces and objects than the HFA group. However, as in the right FG there was no visual category × group interaction, $F(1, 18) = 1.7$, p = ns, indicating similar selectivity for faces in both groups. The same pattern of results emerged in the right and left OFA. There were main effects of visual category in both the right, $F(1, 18) = 109.4$, $p < 0.001$, and left, $F(1, 18) = 291.7$, $p < 0.001$, OFA ROIs with faces eliciting more activation than objects across both groups. There was a main effect of group in the left OFA, $F(1, 18) = 5.9$, $p < 0.05$, and a trend for the main effect of group in the right OFA, $F(1, 18) = 3.4$, $p = 0.08$, with TD individuals exhibiting more activation across both faces and objects than did the HFA group. However, there were no visual category × group interactions in either the right, $F(1, 18) = 0.7$, p = ns, or the left, $F(1, 18) = 0.9$, p = ns, OFA ROIs, indicating similar profiles of category selectivity for faces in both groups. There were too few individuals in each group to perform similar analyses in either the right or left STS.

Place-related ROIs. **Figure 5** illustrates the composite maps of place-related activation defined uniquely for each individual within each group in the parahippocampal gyrus (**Figures 5A,B**). Eighty percent of the individuals in the TD group exhibited place-selective activation in the right parahippocampal gyrus, with 50% exhibiting place-selective activation in the TD group-defined right PPA ROI. Similarly, 70% of the individuals in the HFA group exhibited place-selective activation in the right parahippocampal gyrus and all of these HFA individuals exhibited place-selective activation in the right PPA as defined by the TD group. In contrast, although 70% of the TD individuals exhibited place-selective activation in the left PPA, only 20% of individuals showed overlapping activation in the TD group-defined left PPA, whereas 70% of the HFA individuals

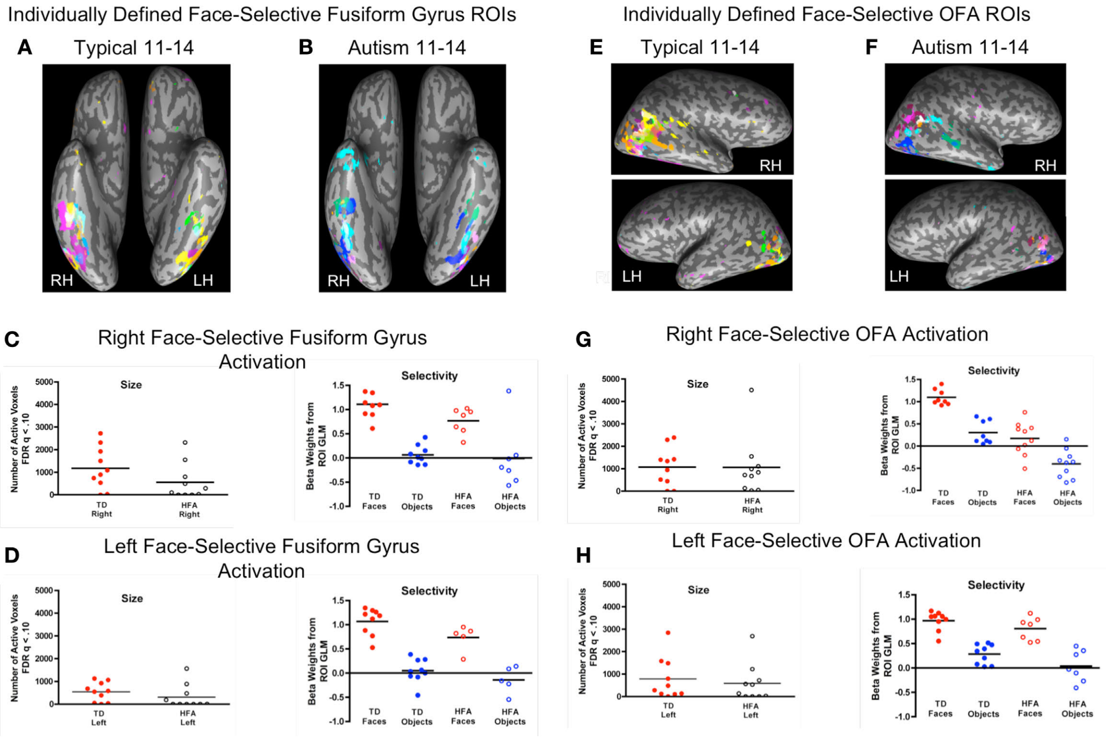

FIGURE 3 | Individual subject variability in location, size, and magnitude of face-selective activation in the fusiform gyrus (A–D) and OFA (E–H). The individually defined face contrast map for each participant in each group, *represented in a unique color*, was thresholded using the FDR procedure ($q < 0.10$) and overlaid onto a single inflated brain. The respective group-defined ROIs are illustrated in white. **(A)** Ninety percent of the TD individuals exhibited face-selective activation in the right fusiform gyrus, with 60% in the TD group-defined right FFA ROI. **(B)** In contrast, 70% of the HFA exhibited face-selective activation in the right fusiform gyrus, however, only 30% exhibited face-selective activation in the right FFA, as defined by the TD group. **(C,D)** There were no group differences in the extent (total number of active voxels) or magnitude of face selectivity (difference in beta weights for faces and objects from the individual subject ROI GLMs) in the right or left face-related fusiform gyrus ROIs. **(E)** Eighty percent of the TD individuals exhibited face-selective activation in the right OFA, with 70% overlapping in the TD group-defined OFA ROI. **(F)** Although 100% of the HFA participants exhibited face-selective activation in the right OFA, only 30% exhibited overlapping activation in the OFA as defined by the TD group. **(G,H)** There were no group differences in the size or magnitude of face selectivity in the individually defined OFA ROIs, however, the TD individuals tended to exhibit more activation across both visual categories in the right and left OFA.

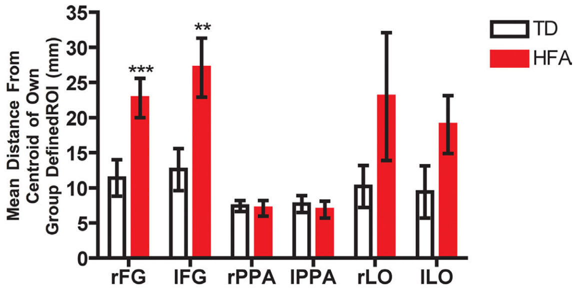

FIGURE 4 | Inter-subject spatial variability in the centroid of activation in face-, place-, and object-related regions. The spatial distance from the respective group mean was computed in each individual participant in three-dimensional normalized space in each region separately. In the right and left fusiform gyrus (FG) the HFA group exhibited more variability than did the TD group in the locus of face-related activation; however, both groups exhibited similar spatial variability in the locus of place- and object-related activation. $^{**}p < 0.01$, $^{*}p < 0.025$.

exhibited place-selective activation in the left PPA and 60% of these individuals showed overlapping activation in the TD group-defined left PPA. Furthermore, there were no group differences in the individual variability of the locus of activation in either the right PPA, $t(13) = 0.3$, $p =$ ns, or the left PPA, $t(12) = 0.5$, $p =$ ns (see **Figure 5**).

Similarly, the extent and profile of activation in the individually defined PPA ROIs was also comparable across the groups. **Figures 5C,D** show the extent, as measured by the total number of active voxels, and magnitude of place-selectivity, as measured by the difference in beta weights for places and faces from the individual subject ROI GLMs in the individually defined right and left PPA for each group. There were no group differences in the extent of place-selective activation in either the right or left PPA [all $t(18) < 1.0$]. Both groups exhibited strong category selectivity for places compared to faces bilaterally in the PPA as indicated by a main effect of visual category in the right, $F(1, 18) = 514.9$, $p < 0.001$, and left, $F(1, 18) = 1138.6$, $p < 0.001$, PPA. In the left PPA, there was a main effect of group, $F(1, 18) = 19.2$, $p < 0.001$, such that the TD individuals exhibited more activation across both visual categories compared to the HFA individuals. However, there was no such effect of group in the right PPA, $F(1, 18) = 0.3$, $p =$ ns. Interestingly, although there was no interaction between visual category and group in the left PPA, $F(1, 18) = 0.3$, $p =$ ns, indicating similar degrees of category selectivity for buildings compared to

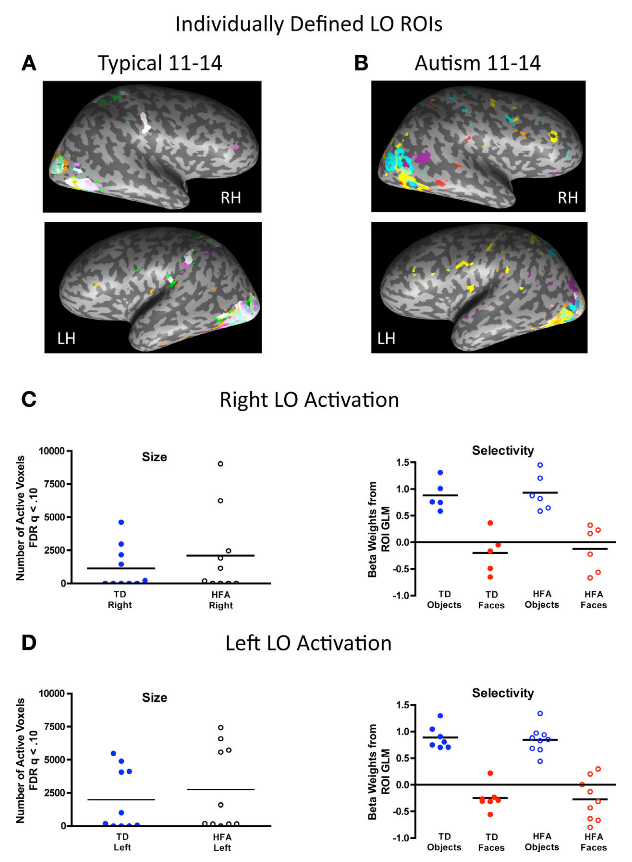

FIGURE 5 | Inter-subject variability in location, size, and magnitude of place-selective activation in the parahippocampal place area (PPA). The individually defined place contrast map for each participant in each group, *represented in a unique color*, was thresholded using the FDR procedure ($q < 0.10$) and overlaid onto a single inflated brain. The respective group-defined ROIs are illustrated in white. There was high consistency in the location of place-selective activation across the groups. **(A)** Eighty percent of the TD individuals exhibited place-selective activation in the right PPA, with 50% in the TD group-defined right PPA ROI. **(B)** Similarly, 70% of the HFA individuals exhibited place-selective activation in the right PPA and all of these individuals exhibited place-selective activation in the right PPA as defined by the TD group. In contrast, although 70% of the TD individuals exhibited place-selective activation in the left PPA, only 20% of individuals showed overlapping activation in the TD group-defined left PPA, whereas 70% of the HFA individuals exhibited place-selective activation in the left PPA and 60% of these individuals showed overlapping activation in the TD group-defined left PPA. **(C,D)** There were no group differences in the extent (total number of active voxels) or magnitude of place-selectivity (difference in beta weights for places and faces from the individual subject ROI GLMs) in the right or left PPA ROIs.

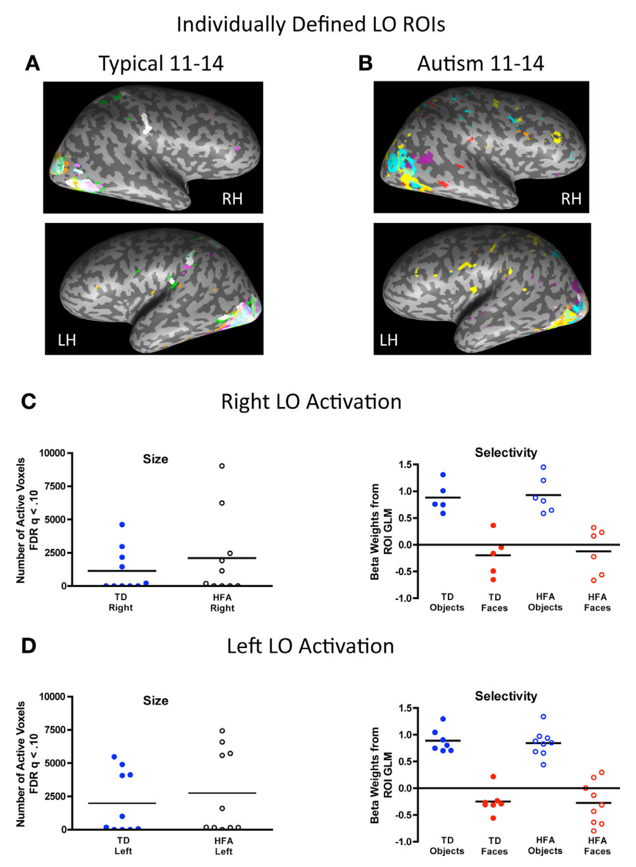

FIGURE 6 | Inter-subject variability in location, size, and magnitude of object-selective activation in the lateral occipital (LO). The individually defined object contrast map for each participant in each group, *represented in a unique color*, was thresholded using the FDR procedure ($q < 0.10$) and overlaid onto a single inflated brain. The respective group-defined ROIs are illustrated in white. There was high consistency in the location of object-selective activation across the groups, particularly in the left LO. **(A)** Fifty percent of the TD individuals exhibited object-selective activation in the right LO, with 40% in the TD group-defined right LO ROI, and 70% of the TD individuals exhibited object-selective activation in the left LO, all of whom showed overlapping activation in the TD group-defined left LO. **(B)** Similarly, 60% of the HFA individuals exhibited object-selective activation in the right LO and all of these individuals exhibited object-selective activation in the right LO, as defined by the TD group, and 90% of the HFA individuals exhibited object-selective activation in the left LO with 70% of these individuals showed overlapping activation in the TD group-defined left LO. **(C,D)** There were no group differences in the extent (total number of active voxels) or magnitude of object-selectivity (difference in beta weights for objects and faces from the individual subject ROI GLMs) in the right or left LO ROIs.

faces, there was a significant visual category × group interaction in the right PPA, $F(1, 18) = 5.6$, $p < 0.05$. Separate independent-samples two-tailed t-tests revealed that the difference in selectivity in the left PPA was driven by more negative responses to faces in the HFA than TD group, $t(18) = 2.62$, $p < 0.025$. The groups evinced comparable activation to buildings in the left PPA, $t(18) = 0.6$, $p =$ ns.

Object-related ROIs. **Figure 6** illustrates the composite maps of object-related activation defined uniquely for each individual within each group in the lateral occipital complex (**Figures 6A,B**). Only 50% of the individuals in the TD group exhibited object-selective activation in the right LO, with 40% exhibiting object-selective activation in the TD group-defined right LO ROI. Similarly, 60% of the individuals in the HFA group exhibited object-selective activation in the right LO and all of these HFA individuals exhibited object-selective activation in the right LO as defined by the TD group. In the left LO, 70% of the TD individuals exhibited object-selective activation and all of these individuals showed overlapping activation in the TD group-defined left LO. Ninety percent of the HFA individuals exhibited object-selective activation in the left LO and 70% of these individuals showed overlapping activation in the TD group-defined left LO. Furthermore, there were no group differences in the individual variability of the locus of activation in either the right LO, $t(9) = 1.2$, $p =$ ns, or the left LO $t(14) = 1.7$, $p =$ ns (see **Figure 6**).

Similarly, the extent and profile of activation in the individually defined LO ROIs was also comparable across the groups. **Figures 6C,D** show the extent and magnitude of object-selectivity, as measured by the difference in beta weights for objects and faces from the individual subject ROI GLMs in the respective regions, for the individually defined right and left LO in each group. There were no group differences in the extent of object-selective activation in either the right or left LO [all $t(18) < 1.0$]. Both groups exhibited strong category selectivity for objects compared to faces bilaterally in the LO as indicated by a main effect of visual category in the right, $F(1, 18) = 598.6$, $p < 0.001$, and left, $F(1, 18) = 450.6$, $p < 0.001$, LO ROIs. Unlike in the face- and place-related ROIs, there were no main effects of group or interactions between group and visual category in either the right of left LO, indicating a similar profile of category selectivity for objects compared to faces in the LO bilaterally in both groups.

CORRELATIONS

There were no significant correlations between any of the individually defined ROI measures and ADOS (social, communication, total tested separately) or IQ (VIQ, PIQ, FSIQ tested separately) scores in the autism group.

DISCUSSION

The goals of this study were to investigate alterations in the functional topography of category-selective cortical activation for faces, places, and objects in the ventral visual pathway in high-functioning adolescents with autism and to evaluate whether and how individual variability in the location, size, and magnitude of selectivity contribute to any observed atypicalities. The largest group differences were obtained in the patterns of face-selective activation, primarily in terms of the *location* of such activation within the network of core face-processing regions (FG, OFA, STS), but also in terms of the lack of face-selective activation in the expansive network of frontal regions that was observed in the TD adolescents (see **Table S2** in Supplementary Material). HFA adolescents exhibited object-related activation bilaterally in the LO and place-related activation in the PPA in both the group and individually defined ROI analyses that was comparable to that of the TDs. The number of active voxels, location, and magnitude of category-selective activation was also similar in the HFA and TD groups for object-selective LO and place-selective PPA. Together, these findings indicate that atypicalities in the functional topography of the ventral visual pathway in autism are primarily related to the emergence of face-related cortex and are already evident in early adolescence. These findings lend further support to the notion that adolescence is a particularly vulnerable period of visuoperceptual development, particularly for face processing, in autism (see Scherf et al., 2008a,b).

DEVELOPMENTAL DIFFERENCES IN FACE-SELECTIVE ACTIVATION

Using dynamic, naturalistic, and rich stimuli, we found the classic pattern of hypoactivation of face-selective neural responses bilaterally in the FG during adolescence, a sensitive developmental period for the maturation of such regions in TD individuals. These findings are consistent with studies that reported similar hypoactivation in adults (Critchley et al., 2000; Schultz et al., 2000; Pierce et al., 2001; Hall et al., 2003; Hubl et al., 2003; Ogai et al., 2003; Piggot et al., 2004; Wang et al., 2004; Dalton et al., 2005; Deeley et al., 2007; Humphreys et al., 2008) and in adolescents (Wang et al., 2004; Dalton et al., 2005; Grelotti et al., 2005) with autism. Importantly, we also observed face-related hypoactivation in the OFA and STS, suggesting that the entire network of face-related regions in the ventral visual pathway is altered in autism. This finding was derived from the within-group whole brain analysis (**Figure 1**). However, direct group comparisons across all participants (and within the HFA group independently) in the magnitude of face selectivity within the typical face-related ROIs (as defined by the TD group map), also indicate that HFA adolescents do not exhibit selectivity for faces in either the right or left FFA, or right OFA. Surprisingly, there were no group differences in the magnitude of category selectivity for faces in the TD-defined right STS.

It is important to acknowledge that despite our findings of hypoactivation in the right OFA and FFA region of the FG in the HFA adolescents, as a group they did exhibit face-selective activation in a small very ventral and posterior region of the right and left fusiform gyri at the standard statistical threshold. Furthermore, when the threshold was relaxed, the group-defined face-selective activation hinted at a shadow of the typical profile in the right FG and in the right STS. However, the midpoint of this face-related FG activation was extremely posterior and only exhibited a small amount of overlap with the TD group-defined FFA (approximately 22 voxels). Importantly, although this region was selective for faces in the HFA group, it was selective for *objects* in the TD group (see **Figure 2D**).

Together, converging results from the group average maps and the analyses of differences in the profile of activation across individuals within these group-defined ROIs suggest that the difference between the HFA and TD groups is in the *location* of face-selective activation, particularly in the FG, the pre-eminent face-processing region. In the TD group, face-selective activation in the FG is centered on a region that corresponds to the "fusiform face area" (Puce et al., 1995; Kanwisher et al., 1997). This same region is not face-selective in the HFA group, nor is the typical right OFA region. Instead, face-selective activation in the HFA group is much more ventral and posterior and located in a region that is object-selective in the TD adolescents. Importantly, these FG face-selective regions in the HFA group in both hemispheres were more posterior and ventral than the range of FFA coordinates reported in a meta-analysis of a wide range of face processing neuroimaging studies in typical adults (Joseph, 2001). However, the TD adolescent FFA ROIs were well within the range of coordinates reported in the same meta-analysis.

We recognize that the results derived from these group-defined analyses are not completely independent and unbiased; therefore, we recommend exercising caution in their interpretation. However, for the following reasons, we are re-assured that the pattern of results derived from these analyses is, in fact, real and bolsters our overall interpretation of group differences in face-related cortex. First, the pattern of results from the group-defined ROI analyses is robust and consistent with the results from the independently defined ROI analyses. Second, we do not find group differences in all regions of cortex where the ROI is defined by one or the other group (i.e. the group difference between TD and HFA individuals is only present in the face-related ROIs but in not in either the TD- or HFA-defined LO ROIs, or the TD-defined PPA ROIs). This result provides us with some confidence (although not totally foolproof on its own) that selecting the ROI from a single group and then using their timeseries necessarily leads to group differences. Third, the follow-up *t*-tests comparing the beta weights for visual categories (e.g. faces versus objects) within each ROI in the non-defined group (e.g., the HFA group when evaluating TD-defined ROIs), are completely independent of the timeseries data used to define the ROIs. As a result, we argue that our interpretations of differences in the location of face-selective activation are not likely to be related to artifactual differences, biases in the analyses, or in the extent of motion in the scanner since the groups were matched on motion parameters.

Our finding that adolescents with autism use object-related regions (as defined by the TD group) of the ventral visual pathway when processing faces is consistent with two previous studies of adults with autism (Schultz et al., 2000; Humphreys et al., 2008) and supports the hypothesis that perceptual processing of faces in autism may be more like perceptual processing of common objects in TD individuals. This hypothesis provides a specific explanation for the etiology of face-processing deficits in autism, namely that such deficits result from atypical perceptual processing more generally. Several hypotheses have been advanced to account for the differences in perceptual processing in autism with some researchers providing evidence for enhanced processing of local features (Mottron et al., 2006) and others for reduced processing of global or holistic information (Behrmann et al., 2006b; Happé and Frith, 2006). On the basis of a more fundamental perceptual difficulty, individuals with autism may be limited in the ability to develop expertise with *any class* of visual objects that are perceptually homogenous, like faces (Behrmann et al., 2006a,b). By some accounts, fine-grained discrimination and representation of the *configural properties* of these stimuli is required to differentiate similar objects (Diamond and Carey, 1986). A failure to encode the face in a typical fashion may hamper the ability of individuals with autism to master visuoperceptual expertise that is critical for face recognition. Such a failure would then require that they rely more on featural than on configural analyses. Several behavioral findings support the notion that children and adolescents with autism lack visual perceptual expertise for faces (Joseph and Tanaka, 2003; Lahaie et al., 2006), as well as other perceptually homogenous novel objects (Ashworth et al., 2008; Scherf et al., 2008a), and rely more on featural than configural analyses of these stimuli.

This last finding is particularly important for constraining interpretations about the aberrant functional topography of face-related activation in autism. Although the OFA, FFA, and STS are critical regions in the broader face-processing network, there is evidence that these regions are not exclusive to face processing. For example, in TD adults, the acquisition of visuoperceptual expertise for classes of perceptually homogenous novel objects (e.g., Greebles) and objects of expertise (e.g., cars, birds) produces increased activation in the FFA region (e.g., Gauthier et al., 1999). This finding has lead to the hypothesis that the FFA does not mediate face perception *per se*, but, rather, serves the computational process of individuating perceptually similar exemplars within a visual class (Tar and Gauthier, 2000). Based on these behavioral and neuroimaging findings, a plausible interpretation of the atypical face-related activation in autism is that it reflects a generalized deficit in visuoperceptual processing that interferes with the ability to develop expert configural processing. This deficit adversely impacts recognition of any within-class perceptually homogenous objects, and especially faces (Behrmann et al., 2006a; Scherf et al., 2008a).

INDIVIDUAL VARIABILITY IN FACE-SELECTIVE ACTIVATION IN HFA ADOLESCENTS

We also evaluated whether and how individual variability in the location, size, and magnitude of selectivity contributes to alterations in the functional topography of the ventral visual pathway in autism. We addressed this question by investigating group differences in the profile of activation in the set of individually defined ROIs. Importantly, as in the group level analyses, there were no group differences in the size, location, or magnitude of category-selective activation in either the right or left LO or PPA. The only differences emerged in the analyses of the face-related ROIs.

In the autism group, face-related activation at the standard statistical threshold was smaller and more ventral and posterior than in the TD individuals. At a more lenient threshold, this group-defined face-selective activation extended to become larger and more dorsal and anterior, hinting at a shadow of the typical profile of activation. Surprisingly, 70% of the HFA individuals exhibited category-selective activation for faces in the right FG and 100% did so in the right OFA. In both regions, activation was generally weaker but was equally face-selective and of comparable size as the regions defined in the TD individuals. However, as was also revealed in the group-defined ROI analyses, the key difference between the TD and HFA groups was the *location* of the individually defined face-related ROIs. In fact, there was more spatial variability among HFA individuals in the locus of the individually defined face-selective regions than in TD individuals. These results indicate that in early adolescence, there is *some degree of consistency* in face-related activation in the right FG and OFA in autism, but the specific locus of activation within these regions is largely variable across individuals.

Note that the relatively greater individual variability in the location of face-selective activation in the HFA individuals is not likely to be related to more variability in brain volume among these participants. Although we did not analyze the structural images for morphometric differences, we are confident that any such differences were minimized by yolking the groups on IQ (see Stanfield et al., 2008) and by normalizing the brain volumes. Furthermore, any such potential differences in brain volume did not affect the location of the place- or object-related activation across the groups. Unless there is evidence of selective abnormalities in the morphology of the lateral (and not medial) FG bilaterally, it is difficult to argue that differences in brain volume selectively influenced the location of face-selective activation.

This lack of consistency in the location of face-selective activation across HFA participants is likely a root cause for the findings of hypoactivation in the group average maps (**Figure 1B**). Our results provide clear evidence that individual variability in the location of face-selective activation is a primary mechanism of the hypoactivation observed at the group level. The finding that abnormal individual variability in the location of functional activation contributes to reduced or abnormal recruitment of cortical circuits in autism has also been reported in the context of both simple finger tapping (Müller et al., 2001) and more complex visuomotor learning paradigms (Müller et al., 2003). In more related domains, there are reports that adults with autism exhibit idiosyncratic patterns of activation during face perception (Pierce et al., 2001) and when watching a popular audio-visual movie (Hasson et al., 2009). For example, Pierce and colleagues reported that in every autistic adult tested, faces maximally activated "aberrant and individual-specific neural sites" (with only one adult exhibiting maximal activation to faces in the FG). They interpreted these findings to suggest that individuals with autism "see" faces utilizing different neural systems than do typical individuals. Although our findings of atypical individual variability in the locus of activation of face-related regions are generally consistent with these previous studies, our results of comparable place- and object-related activation across HFA and TD adolescents suggest that this variability is not a general property of brain function in autism. Also, our findings that 70% of HFA adolescents exhibited face-selective activation within the FG that was of comparable extent and magnitude of category selectivity indicate that there may be more consistency than previously thought in the neural circuitry utilized to perceive faces in *some* individuals with autism.

We also note that a significant proportion of HFA participants did not have identifiable FG face-selective activation, particularly in the left hemisphere, suggesting that the hypoactivation of face-related cortex observed in the group level map may also be driven by an inability for some individuals with autism to develop populations of neurons that are relatively selective for faces. Of note, for those HFA participants who do evince face-selective activation in FG, it is of equal volume and selectivity as the same regions identified in the TD individuals.

This marked individual variability in the number of individuals with identifiable face-selective activation and in the location of such activation in the HFA participants leads to natural questions about whether and how demographic measures can predict such variability. Such predictability could have substantial implications for identifying individuals who might benefit from a behavioral intervention designed to improve face processing and/or more general visuoperceptual processing, such as configural processing. Unfortunately, neither symptom severity, as measured by subcomponents of the ADOS, nor IQ measures, predicted which of the HFA individuals demonstrated face-related activation in the FG, OFA, or STS. For example, the individual with the largest combined ADOS score (19), where the higher the score the more severe the autism characteristics, exhibited the largest volume of face-selective activation in the FG that largely overlapped with the TD group-defined FFA region. In contrast, the HFA participant with the lowest combined ADOS score (11) exhibited one of the smallest volumes of face-selective activation in the FG, which failed to overlap with the TD group-defined right FFA region.

Although we could not identify a relation between symptom severity and the profile of face-related activation in this study, caution should be used in interpreting these null results. The ADOS is a standard diagnostic tool in autism research and its goal is to provide standardized contexts in which to observe the social-communicative behavior of individuals across the life span in order to aid in the diagnosis of autism. As stated in the ADOS manual, "For this reason, it may not be a good measure of response to treatment or developmental gains especially in later modules (that are administered to adolescents and adults)" (Lord et al., 1994). Future work including a more specific measure of symptom severity and/or a broader range of levels of functioning within the spectrum (PDD, Asperger's, higher, and lower functioning individuals with autism) could reveal a more systematic relation between symptom severity and the degree of alteration to face-related activation.

Also, we used a passive viewing paradigm and did not employ a behavioral measure that could be used for evaluating potential atypicalities in brain-behavior correspondences in these adolescents with autism. Future studies investigating the relation between face-processing behavior and alterations in the functional organization of the ventral visual pathway in autism will be critical. Unfortunately, this has proven to be a difficult endeavor even in TD populations and very few studies have been able to report developmental changes in such brain-behavior correspondences (for review see Scherf et al., 2009). Relating atypical developmental changes in face-processing behavior and functional brain organization in autism may require (1) the use of fine-grained tests of face-processing behavior that are sensitive enough to observe differences through adolescence, when face-related activation continues to change in TD individuals (e.g., Scherf et al., 2007), and (2) an investigation of face-related regions beyond the "core" ventral visual regions to include regions in the extended face-processing network, including the amygdala, insula, anterior temporal lobe, and medial prefrontal regions (Gobbini and Haxby, 2007).

MECHANISMS OF ALTERED FUNCTIONAL TOPOGRAPHY FOR FACE-RELATED ACTIVATION

We have emphasized the contribution of variability in the number of individuals with identifiable face-selective activation and in the location of such activation as factors contributing to the classic pattern of hypoactivation in face-related cortex in autism. Note that this interpretation highlights our finding that there is a subset of high-functioning adolescents with autism who do show face-selective activation in the FG, albeit in a traditionally object-related region. For these individuals, the magnitude of face selectivity is comparable to that observed in TD individuals as is the volume of these regions. This pattern of results is quite similar to those observed in younger TD children (ages 5–8 years), in whom activation in *place* and *object* areas is adult-like even in early childhood, whereas the development of the *face*-related regions (FFA, OFA, STS) is much more protracted, becoming more selective and responsive to faces with age, but not reaching adult-like activation until adolescence (Golarai et al., 2007; Scherf et al., 2007). In these same studies, approximately 80–85% of the children have identifiable face-selective activation in the FG, although the locus of such activation is quite variable and not centered on the FFA. One rather obvious possibility then, is that visual category-selective cortex in autism may reflect an early plateau in development, rather than deviance, with the category selectivity resembling that of younger TD children (for similar argument see Humphreys et al., 2008).

Alternatively, a number of authors propose abnormalities of neural connectivity in autism (Frith, 2003; Belmonte et al., 2004; Courchesne and Pierce, 2005a,b; Rippon et al., 2007), specifically under-connectivity between different functional regions (long-range) and over-connectivity at a more local level (short-range) (Markram et al., 2007). Abnormalities in neural connectivity specifically related to the FG and other regions in the broad face-processing network (e.g., amygdala, STS, posterior cingulate) have been identified anatomically in post-mortem studies (Van Kooten et al., 2008) and functionally in neuroimaging studies of adults with autism (Kleinhans et al., 2008). Along with previous authors (e.g. Johnson et al., 2002), we speculate that the longer a particular function takes to mature, the greater the cascading effects of differences in the development of neurons and synapses. This may explain how differences in face-related cortex are much more pronounced in autism than are those affecting the earlier maturing place- or object-related cortices. The refinement in selectivity of later-developing cortex may be impeded as a result of over-connectivity at a local level, giving rise to a face-processing system prone to crosstalk and noise, resulting in reduced functional specificity (see Rippon et al., 2007). This framework would also predict that greater TD/autism group differences would be evident in other, later-developing brain functions. For example, we would expect to find greater differences on tasks that engage secondary and tertiary visual cortex compared to those relying on primary visual cortices (Hasson et al., 2009). These predictions are also consistent with findings of greater divergence between the groups on tasks tapping frontal functions (e.g., Takarae et al., 2007).

ALTERNATIVE EXPLANATIONS

An alternative hypothesis for the face-processing deficits in autism is that decreased motivation to attend to social stimuli limits the ability to gain expertise in face processing (Dawson et al., 2002; Grelotti et al., 2002). In support of this hypothesis, several groups have reported that individuals with autism spend less time looking at the eye region of faces (Klin et al., 2002; Spezio et al., 2007; Adolphs et al., 2008), and one study suggests that this atypical fixation pattern may be related to aberrant activation in the FG (Dalton et al., 2005). Based on these findings, one possible interpretation of the hypoactivation in face-related regions in our study is that the adolescents with autism were simply not looking at the faces, and therefore, were paying less attention to the faces. We did not collect eye-tracking data in this experiment and so cannot evaluate definitively this alternative explanation; however, it should be noted that the relation between purported atypicalities in the locus of fixations during face processing and cortical activation patterns in children with autism is controversial (see Boraston and Blakemore, 2007). For example, one study of young adolescents with autism found no differences from TD controls in fixation patterns when observing facial expressions, despite finding impressive differences in the patterns of neural activation under these same conditions (Dapretto et al., 2006). Also, at least one study in adults with autism found similar patterns of face-related hypoactivation in the FG when participants were required to fixate a central dot overlaid on the center of each stimulus and under free viewing conditions (Humphreys et al., 2008). Additional studies evaluating the relation between fixation, BOLD activation patterns, and behavioral measures of attention to faces, particularly in a developmental context, are clearly necessary.

A second alternative explanation for our pattern of results is related to our use of unfamiliar faces in the movie task. Although this is consistent with the majority of previous studies reporting atypical face-related activation in autism, some evidence suggests that more normal patterns of activation can be observed, particularly in the FG, in children and adults with autism when they view personally familiar faces (Pierce et al., 2004; Pierce and Redcay, 2008). Importantly, in these studies the authors reported that both the magnitude of signal change in response to faces and the number of voxels exhibiting this signal change was more comparable to controls in the FG during observation of familiar faces. They did not investigate differences in the locus of such activation across the groups. Consistent with these previous findings, when we identified the face-selective activation in the FG for each participant, we also observed comparable volume and magnitude of face selectivity in the FG in the TD and HFA groups (see **Figures 3C–H**), even under conditions in which they observed *unfamiliar* faces. Our central finding is that fewer of the HFA adolescents exhibit face-selective activation and when they do, there is more variability in the locus of such activation within the FG (and OFA). One way to reconcile these results is to suggest that, as in TD individuals, familiarity can modulate face-related activation in the FG in individuals with autism; however, given the differences in the locus of such activation, even familiar faces may be processed more like objects. Future behavioral and neuoimaging studies evaluating the computational mechanisms that individuals with autism use to process both familiar and unfamiliar faces are essential to evaluate this interpretation.

CONCLUSIONS

This is the first study to map the functional topography of category-selective activation for faces, places, and objects in the ventral visual pathway in high-functioning adolescents with autism and to evaluate whether and how individual variability in the location, size, and magnitude of selectivity contribute to such alterations in the topography. Our findings indicate that there is not a generalized disruption in the development of the functional topography of the entire ventral visual pathway in autism. Instead, our results suggest that the functional topography of face-related cortex is selectively disrupted in autism and that this alteration is already present in early adolescence, an important stage of cortical specialization for faces in TD adolescents. In particular, for HFA individuals who do exhibit face-selective activation, it tends to be located in traditionally object-related regions, which supports the hypothesis that the perceptual processing of faces in autism may be more like perceptual processing of common objects in TD individuals. Such alterations could result from direct pathology to regions within the face-processing network, like the FG (Van Kooten et al., 2008), and/or to the structural and functional connections between such regions. Also, alterations in the visual experiences that individuals with autism have with faces as a result of social aversion and/or excessive focus on features may configure these regions in the face-processing network atypically.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/neuroscience/humanneuroscience/paper/10.3389/fnhum.2010.00026/

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Social functioning in children with brain insult

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Social dysfunction is commonly reported by survivors of brain insult, and is often rated as the most debilitating of all sequelae, impacting on many areas of daily life, as well as overall quality of life. Within the early brain insult (EBI) literature, physical and cognitive domains have been of primary interest and social skills have received scant attention. As a result it remains unclear how common these problems are, and whether factors predictive of recovery (insult severity, lesion location, age at insult, environment) in other functional domains (motor, speech, cognition) also contribute to social outcome. This study compared social outcomes for children sustaining EBI at different times from gestation to late childhood to determine whether EBI was associated with an increased risk of problems. Children with focal brain insults were categorized according to timing of brain insult: (i) Congenital (n = 38): EBI: first–second trimester; (ii) Perinatal (n = 33); EBI: third trimester to 1-month post-natal; (iii) Infancy (n = 23): EBI: 2 months–2 years post-birth; (iv) Preschool (n = 19): EBI: 3–6 years; (v) Middle Childhood (n = 31): EBI: 7–9 years; and (vi) Late Childhood (n = 19): EBI: after age 10. Children's teachers completed questionnaires measuring social function (Strengths and Difficulties Questionnaire, Walker–McConnell Scale of Social Competence and School Adjustment). Results showed that children with EBI were at increased risk for social impairment compared to normative expectations. EBI before age 2 years was associated with most significant social impairment, while children with EBI in the preschool years and in late childhood recorded scores closer to normal. Lesion location and laterality were not predictive of social outcome, and nor was social risk. In contrast, presence of disability (seizures) and family function were shown to contribute to aspects of social function.

Keywords: social skills, childhood, infant, brain insult, plasticity, vulnerability

INTRODUCTION

Children, and indeed all humans, live in a world in which they are in constant interaction with other people. These social interactions are based on understanding and storing knowledge about other persons, and about the self, as well as possessing knowledge about interpersonal norms and scripts required to navigate the social world (Adolphs, 2009; Van Overwalle, 2009). Recent advances in the social neurosciences illustrate the close association between these social skills, and underlying brain function (Adolphs, 2009). Further, the development of this knowledge is critical for normal social development, and disruptions to these processes has negative implications for a range of domains including mental health, school progress, and child and family quality of life (Yeates et al., 2007).

Social functioning, and a child's ability to interact appropriately with peers, is an important goal of childhood. Social skills play an integral role in development, and emerge gradually through infancy and childhood. In the first few months of life, the child begins to smile and engage with others, and imitate the actions of those around them in an interactive manner. By 5–8 months, infants display evidence of goal-directed social behavior (Woodward, 1998). At 3–4 years children can describe the mental states or beliefs of others, distinct from their own (Saxe et al., 2004), and begin to be able to suppress inappropriate responses (Diamond and Taylor, 1996) and participate in pretend play (Goncu et al., 2002). Through this period the child's interest in social relationships emerges with more and more time spent in peer interactions (Howes and Phillipsen, 1998). By 7–8 years children begin to predict the behavior of others based on past experiences (Rholes et al., 1990), and are able to understand and employ non-literal language, such as sarcasm and irony (Demorest et al., 1984). Social decision making and judgments emerge later, in early adolescence (Dodge et al., 2002), and adolescents spend almost a third of their waking hours in social interactions with their peers (Csikszentmihaly and Larson, 1984). During this protracted developmental process, any disruption that impacts on normal maturation processes will have the capacity to impair future progress.

It is likely that social function will be dependent on many factors – both environmental and biological. The influences of family and environmental factors for social development are well established (Ladd and Pettit, 2002). Findings emerging from the social neurosciences suggest that brain-related factors also need to be considered. An example of such a 'disruption' in childhood is brain insult which can result in serious and permanent consequences, in areas such as cognition and academic performance (Ewing-Cobbs et al., 1997; Taylor et al., 2002; Anderson et al., 2005, 2009). Social problems have also been identified (Janusz et al., 2002; Yeates et al., 2004; Ganesalingam et al., 2006) including poor self-esteem, reduced adaptive behaviors, loneliness (Andrews et al. 1998; Yeates et al., 2004), and difficulties in peer relationships (Bohnert et al., 1997).

While there is some evidence that insult severity may be related to degree of social problems (Andrews et al., 1998; Yeates et al., 2004), this dose–response relationship does not appear to be as clear cut for social skills as it is for physical and cognitive function, where it is well established that more severe insult leads to greater deficits (Anderson et al., 2005). Similarly, there has been little research to date to investigate whether other established predictors of physical and cognitive outcomes following childhood brain insult (for example, location of lesion, age at insult, disability and environmental factors) make a significant contribution to social outcomes after childhood brain insult. In the adult literature, location of lesion has been shown to contribute to social outcome, with the frontal lobes and the right hemisphere designated as playing key roles. These relationships have been less well evaluated in the child-based literature. Anderson et al. (2000) and Eslinger and Biddle (2008) have reported on serial evaluations of two young adult patients who sustained right-sided prefrontal cortex damage prior to 16 months of age. Despite largely intact cognitive abilities, both cases displayed severe and increasing social problems through childhood, including few friendships, rejection by peers, and lack of guilt, empathy, remorse or fear. Spencer-Smith and Anderson (2009) have also examined a small sample of children with subcortical band heterotopia, a congenital disorder of the CNS occurring during gestation and impacting the brain quite globally. They reported that half of their sample were rated by their caregiver as demonstrating impaired social skills, suggesting that, either frontal connections may be disrupted in this condition, or that total brain integrity is important for intact social function.

The objective of this study was to address the gaps in the literature and investigate social functioning in a sample of children with brain insult. We focused on children for whom recovery was expected to be largely complete (>12-months post-insult), and for whom social skill should be well established (10–16 years of age). Our primary aims were to examine the incidence of social difficulties in the context of EBI, and to determine the role of age at insult for outcome. Our secondary aim was to explore factors which might contribute to the emergence of social problems after EBI. Based on the available literature we predicted that: (1) children with EBI would have poorer than expected social functioning; (2) Earlier age at brain insult would be associated with poorer social functioning; (3) lesion characteristics, including location, laterality, presence of seizures would predict social function. Specifically, based on adult literature, we expected poorer social outcome after EBI would be associated with both focal frontal and right hemisphere lesions and presence of seizures; and (4) environmental factors, including higher social risk and poorer family function, would be linked to poor social outcome after EBI.

MATERIALS AND METHODS

SAMPLE

The sample comprised 147 children, including 84 (57.1%) males, aged between 10–16 years at recruitment ($M = 12.99$, SD = 1.88), with a history of early brain insult (EBI). This group was part of a larger study looking at a broad range of outcomes from EBI (Anderson et al., 2009). Participants were recruited from the Royal Children's Hospital, Melbourne, Australia between 2005 – 2007. Potential participants were identified via medical record review and referrals to neuroscience outpatient clinics. Inclusion criteria were: (1) 10–16 years at assessment; (2) English speaking; (3) evidence of focal brain insult documented via MRI scan; and (4) brain insult sustained at least 12 months prior to assessment, to allow for stabilization of recovery processes. Approaches were made to 215 families, with 50 families declining to participate (76.7% participation rate) due to time burden ($n = 18$), lack of interest ($n = 29$), or distance ($n = 3$). Eleven children were excluded from the study based on study criteria. Eighteen cases were excluded due to missing questionnaire data.

Children were then categorized into six 'age at insult' groups, with these chosen based on developmental spurts in neurological and cognitive processes: (1) Congenital ($n = 34$): EBI during the 1st and 2nd trimester of gestation; (2) Perinatal ($n = 29$): 3rd trimester of gestation to 1-month post-birth; (3) Infancy ($n = 21$): 2 months to 2 years; (4) Preschool ($n = 18$); 3–6 years; (5) Middle Childhood ($n = 30$): 7–9 years; and (6) Late Childhood ($n = 15$): 10–16 years.

Mechanism of EBI was diverse and included developmental (dysplasia, heterotopia, polymicrogyria), ischemic (periventricular leukomalacia, infarct, stroke), neuroplastic (tumor, cyst, abscess), traumatic (penetrating head injury) and infective (encephalitis, meningitis) (see **Table 1**).

MATERIALS

Descriptive information

Demographic information. Parents completed a questionnaire providing information on their child's medical and developmental history.

Social risk. The Social Risk Index – SRI (Roberts et al., 2008): rates a number of family factors, such as maternal age at the child's birth and primary caregiver's level of education and income. High scores indicate high social risk. Median split was used to determine high social risk and low social risk.

Family functioning. The General Family Functioning scale from the Family Assessment Device (Miller et al., 1985) provided a measure of family functioning. Low scores indicate better family functioning. Median split was used to determine high family functioning and low family functioning.

General intelligence. The four-subtest version of the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999) was administered. A Full Scale Intelligence Quotient (FSIQ) ($M = 100$), SD = 15) was derived.

Brain insult characteristics

MRI scans. i) *Acquisition* MRI scans were conducted, via standard protocol, as part of routine clinical practice prior to recruitment. For those who had not undergone scanning, or whose scans were unavailable, scans were conducted simultaneously with neurobehavioral evaluation. Scans were conducted on a 1.5 Tesla scanner, and axial and coronal slices were obtained. ii) *Coding protocol* A coding protocol developed by Leventer et al. (1999) was modified and employed to describe brain insult characteristics including: brain regions affected (lobes, subcortical structures), laterality

Table 1 | Characteristics for the sample across early brain insult groups.

	Congenital	**Perinatal**	**Infancy**	**Preschool**	**Middle childhood**	**Late childhood**
N	**34**	**29**	**21**	**18**	**30**	**15**
Males: *n* (%)	18 (52.9)	20 (69.0)	12 (57.1)	11 (61.1)	16 (53.3)	7 (46.7)
Age at test (SD)*	12.8 (1.8)	13.3 (1.9)	12.4 (1.0)	12.6 (1.8)	12.9 (1.8)	14.5 (1.6)
FSIQ: *M* (SD)*	83.5 (20.2)	84.2 (18.2)	79.7 (19.2)	95.7 (16.9)	97.8 (20.0)	94.9 (22.0)
SRI: *M* (SD)	3.23 (2.03)	2.76 (1.51)	2.83 (1.62)	2.80 (1.90)	3.04 (1.77)	4.00 (2.30)
Family funct. *M* (SD)	1.73 (0.41)	1.74 (0.41)	1.76 (0.44)	1.69 (0.38)	1.88 (0.40)	1.93 (0.36)
Seizures: *n* (%)*	21 (61.8)	14 (48.3)	14 (66.7)	5 (27.8)	11 (36.7)	4 (26.7)
MECHANISM**						
Developmental *n* (%)	26 (79.4)	5 (17.2)	0 (0)	0 (0)	0 (0)	0 (0)
Infective *n* (%)	0 (0)	0 (0)	1 (4.8)	1 (5.6)	1 (3.3)	1 (6.7)
Ischemic *n* (%)	2 (5.9)	22 (75.9)	4 (19.0)	6 (33.3)	11 (36.7)	6 (40.0)
Neuroplastic *n* (%)	5 (14.7)	2 (6.9)	14 (66.7)	7 (38.9)	10 (33.3)	5 (33.3)
Traumatic *n* (%)	0 (0)	0 (0)	2 (9.5)	4 (22.2)	8 (26.7)	3 (20.0)
REGION						
Frontal *n* (%)	19 (55.9)	20 (69.0)	7 (33.3)	9 (50.0)	14 (46.7)	8 (53.3)
Extra-frontal *n* (%)	25 (73.5)	21 (72.4)	17 (81.0)	11 (61.1)	14 (46.7)	9 (60.0)
Subcortical *n* (%)	19 (55.9)	21 (72.4)	11 (52.4)	10 (55.6)	15 (50.0)	5 (33.3)
LATERALITY						
Left *n* (%)	7 (20.6)	10 (34.5)	8 (38.1)	7 (38.9)	14 (46.7)	4 (26.7)
Right *n* (%)	9 (26.5)	5 (17.2)	7 (33.3)	5 (27.8)	7 (23.2)	7 (46.7)
Bilateral *n* (%)	18 (52.9)	14 (48.3)	6 (28.6)	6 (33.3)	9 (30.0)	4 (26.7)
NATURE						
Focal *n* (%)	17 (50)	15 (51.7)	14 (66.7)	13 (72.2)	18 (60.0)	11 (73.3)
Multifocal *n* (%)	17 (50)	14 (48.3)	7 (33.3)	5 (27.8)	12 (40.0)	4 (26.7)

$*p < 0.05$; $**p < 0.001$.

(left, right, bilateral), extent of insult (focal, multifocal), volume of brain affected (number of regions) (**Table 2**). Scans were coded by an experienced paediatric neuroradiologist (L.C) and neuropsychologist (M.S.S), blind to group membership. A randomly selected subset of 10 scans was re-coded independently by L.C and M.S.S, with inter-rater reliability of 0.97.

Timing of brain insult was determined via a combination of MRI, brain biopsy, and medical record (clinical history, medical investigations). For pre and perinatal insults this information was reviewed by an experienced paediatric neurologist (R.L) and a neuropsychologist (M.S.S), and rated according to the coding established by Leventer et al. (1999). Ten cases were double-rated, with 100% consistency. *Mechanism of insult* was coded as: developmental, infective, ischemic, neuroplastic, or traumatic. Presence of seizure history was recorded.

Outcome measures

Social functioning was measured by questionnaires completed by the child's teacher, who was asked to rate their student's behavior over the previous 6 months.

The strengths and difficulties questionnaire – SDQ. Goodman (1997) provided a measure of social, behavioral and emotional functioning. While this study specifically addressed social function, all SDQ subscales were included in analyses to provide information on areas of function (behavior, emotions) closely related to social function. The questionnaire consists of 25 items, providing five subscales: Emotional Symptoms (ES) (e.g., often unhappy, often seems worried, nervous or clingy in new situations), Conduct

Table 2 | Definitions for classification of lesion location, laterality and extent, based on MRI scan (adapted from Leventer et al., 1999).

Variable	**Definition**
REGION	
Frontal	Brain pathology involves the frontal lobe.
Extra-frontal	Brain pathology involves the parietal, occipital or temporal lobe only.
Subcortical	Brain pathology involves the corpus callosum, thalamus or basal ganglia.
Posterior fossa	Brain pathology involves the brain stem or cerebellum.
LATERALITY	
Left hemisphere	Brain pathology confined to left hemisphere.
Right hemisphere	Brain pathology confined to right hemisphere.
Bilateral	Brain pathology located in both left and right hemispheres.
EXTENT	
Focal	Brain pathology is confined to one area.
Multifocal	Brain pathology involves two or more areas.

Problems (CP) (e.g., often has temper tantrums, is disobedient, often lies or cheats), Hyperactivity-Inattention (HYP) (e.g., easily distracted, restless, overactive), Peer Problems (PP) (e.g., tend to play alone, bullied by other children), and Prosocial Behaviors (PB) (e.g., considerate of others, often volunteers to help others). A Total Difficulties (SDQ:TD) score was obtained by combining the scores for all but the PB subscale, consistent with manual instructions. Higher scores on PB indicate better social functioning, while, higher scores on all other subscales and SDQ:TD indicate poorer social functioning. Scaled scores were derived based on published Australian norms (Mellor, 2005) and scores were ranked as Normal, Borderline or Abnormal consistent with manual instructions.

The Walker–McConnell scale of social competence and school adjustment: adolescent version – WMS. Walker and McConnell (1995) was used to identify social skills deficits. It consists of 53 items, from which four subscales are obtained: Self Control (SC) (e.g., reacts to situations without becoming violet or destructive, can accept not getting his/her own way, gains peers' attention in an appropriate manner); Peer Relations (PR) (e.g., making friends, interacting with peers, engaging in conversation with peers); School Adjustment (SA) (e.g., displays independent study skills, listens while others are speaking); and Empathy (EM) (e.g., sensitive to the need of others, compliments others regarding personal attributes). These subscales combine to provide a Total score (WMS:TOT). Scores were converted to scaled scores ($M = 100$, SD = 15 for TOT; $M = 10$, SD = 3 for subscales) with a lower score indicating poorer social skills.

PROCEDURE

This study represents a subset of a larger study of children with EBI (Anderson et al., 2009). The study was approved by the Human Research Ethics Committee, Royal Children's Hospital, Melbourne, Australia. Eligible families were initially identified via medical records, neuroradiology meetings or outpatient clinics. Families were mailed details of the study and requests for written consent. Consenting families were first screened and then attended for interview at an outpatient clinic, with a small number of children assessed at home, for family convenience. Parents completed the demographic questionnaire, FAD and SRI in a single session, while their child underwent intellectual evaluation with a trained child psychologist. Following consent from families, the children's teachers were contacted via mail and asked to complete the SDQ and the WMS, based on observations over the previous 6 months. Completed questionnaires were returned directly to researchers in the envelope provided.

STATISTICAL ANALYSIS

Quantitative analyses were conducted using SPSS (version 17.0).

To address research question 1, that children with EBI would have poorer than expected social functioning, total group mean ratings on subscales of the SDQ and WMS were compared to normative expectations using single sample *t*-tests. Research question 2, that earlier age at brain insult would be associated with poorer social functioning, was examined using one-way ANOVA to identify differences in mean ratings between the six EBI groups, effect size determined by η^2, and *post hoc* analyses were performed using Turkey's honestly significant differences test. Age at assessment was not included as a covariate because age-scaled scores were examined. Chi Square test-for-Independence analyses investigated differences in rates of impairment between the EBI groups, effect size determined by Cramer's *V*. Research question 3 was examined by using ANCOVA to identify group differences with respect to lesion characteristics (location, laterality, presence of seizures) and environmental factors (social risk, family function). These analyses also included effect size, determined by η^2, and *post hoc* analyses using Turkey's honestly significant differences test.

RESULTS

Sample characteristics

No group differences were identified for gender, social risk, or family functioning (see **Table 1**). A significant age at test difference was identified, $F(5, 146) = 3.09, p = 0.01, \eta^2 = 0.10$, with the Late Childhood group older at the time of assessment than the Congenital ($p = 0.02$), Infancy ($p = 0.007$), Preschool ($p = 0.04$), and Middle Childhood ($p = 0.05$) groups. General intelligence differed between groups, $F(5, 139) = 3.67, p = 0.004, \eta^2 = 0.21$, with the Middle Childhood group performing better than the Congenital ($p = 0.05$) and Infancy ($p = 0.02$) groups. Significant group differences were also detected for presence of seizures χ^2 $(5, n = 143) = 11.09, p = 0.04, V = 0.29$, with a larger proportion of children with a history of seizures in the Congenital and Infancy groups and a smaller proportion in the Preschool and Later Childhood groups.

Mechanism of EBI differed between the groups, χ^2 $(20, n = 146) = 130.10$, $p = < 0.001$, $V = 0.47$, consistent with the heterogeneity of the sample (see **Table 1**). However, there were no statistically significant group differences for: (1) region of insult, frontal, χ^2 $(5, n = 147) = 6.86, p = 0.23, V = 0.22$, extra-frontal, χ^2 $(5, n = 147) = 8.91, p = 0.11, V = 0.25$, subcortical, χ^2 $(5, n = 147) = 6.89, p = 0.24, V = 0.22$; (2) Laterality (left, right, bilateral), χ^2 $(5, n = 147) = 11.59, p = 0.31, V = 0.20$; or (3) extent of insult (unifocal, multifocal), χ^2 $(5, n = 147) = 4.86, p = 0.43, V = 0.18$, suggesting groups did not differ greatly with respect to these brain insult characteristics.

DO CHILDREN WITH EBI HAVE POORER SOCIAL FUNCTIONING THAN POPULATION DATA WOULD EXPECT?

The total group of children with EBI performed poorer than normative expectations on most measures of social functioning (see **Table 3**). For the SDQ, poorer results were identified for most subscales: ES, $t(147) = 7.73, p < 0.001$; HYP, $t(147) = 7.68, p < 0.001$; PP, $t(147) = 5.43, p < 0.001$; PB, $t(147) = -5.43, p < 0.001$; SDQ: TD, $t(147) = 8.21$, $p < 0.001$, with the difference between the total group and normative expectations approaching significance on CP, $t(147) = 1.87, p = 0.06$. For the WMS, there was a significant discrepancy from normative expectations only for PR, $t(144) = -3.17, p < 0.001$, suggestive of difficulties with peer relations. The total group performed close to normative expectations on SC, $t(144) = 0.36, p = 0.72$; SA, $t(144) = 0.28, p = 0.78$; EM, $t(144) = 1.10, p = 0.27$; WMS:TOT, $t(144) = -0.20, p = 0.84$.

DOES AGE AT BRAIN INSULT IMPACT ON SOCIAL FUNCTIONING?

Differences in social functioning across EBI groups

On the SDQ, there was a significant group difference for ES, $F(5, 146) = 2.45, p = 0.04, \eta^2 = 0.08$, with the Congenital group performing poorer than the Preschool group ($p = 0.04$) (see **Table 4**). There

Table 3 | Comparison of EBI groups and normative data for the SDQ and WMS: mean differences and confidence intervals.

	Congenital	Perinatal	Infancy	Preschool	Middle childhood	Late childhood	Total group
SDQ							
ES	2.07	0.88	2.03	0.27	1.37	1.27	1.38
	(1.35, 2.80)**	(0.14, 1.62)*	(0.91, 3.15)**	(–0.42, 0.95)	(0.42, 2.31)*	(0.27, 2.26)*	(1.03, 1.74)**
CP	0.47	0.69	0.14	–0.28	0.33	–0.13	0.29
	(–0.27, 1.22)	(–0.12, 1.50)	(–0.65, 0.94)	(–0.72, 0.17)	(–0.34, 1.01)	(–1.07, 0.80)	(–0.02,0.59)
HYP	1.85	1.60	1.93	0.94	1.73	0.97	1.59
	(0.94, 2.33)**	(0.62, 2.59)*	(1.02, 2.83)**	(–0.33, 2.22)	(0.80, 2.66)**	(–0.61, 2.55)	(1.18, 2.00)**
PP	1.49	1.54	0.40	1.07	0.67	0.27	1.00
	(0.65, 2.33)**	(0.59, 2.48)*	(–0.60, 1.40)	(0.07, 2.06)*	(–0.12, 1.45)	(–0.59, 1.13)	(0.63, 1.36)**
PB	1.51	–1.63	–1.04	–0.24	–1.27	0.00	–1.15
	(–2.49, –0.52)*	(–2.74, –0.51)*	(–1.91, –0.16)*	(–1.23, 0.74)	(–2.52, –0.41)*	(–0.87, 0.87)	(–1.56, –0.73)**
SDQ:TD	5.87	4.70	4.49	2.05	4.42	2.36	4.32
	(0.3.18, 8.57)**	(2.16, 7.23)**	(1.94, 7.04)**	(–0.21, 4.30)	(2.12, 6.73)**	(–0.61, 5.32)	(3.28, 5.36)**
WMS							
SC	–0.50	–2.07	–0.57	1.71	0.29	0.67	0.08
	(–1.62, 0.62)	(–1.34, 0.92)	(–1.56, 0.42)	(0.94, 2.47)**	(–0.82, 1.39)	(–0.94, 2.27)	(–0.38, 0.55)
PR	–1.97	–1.48	–0.81	–0.29	0.36	0.13	–0.83
	(–3.17, –0.77)*	(–2.78, –0.19)*	(–2.03, 0.41)	(–1.98, 1.39)	(–0.69, 1.40)	(–1.22, 1.49)	(–1.35, –0.31)*
SA	–0.41	–0.24	–0.52	1.29	0.25	0.80	0.06
	(–1.45, 0.63)	(–1.29, 0.81)	(–1.5, 0.45)	(0.27, 2.32)*	(–0.82, 1.32)	(–0.71, 2.31)	(–0.38, 0.50)
EM	0.00	–0.38	–0.38	1.41	0.75	0.60	0.24
	(–1.06, 1.06)	(–1.50, 0.74)	(–1.40, 0.64)	(0.42, 2.41)*	(–0.21, 1.71)	(–0.75, 1.95)	(–0.19, 0.68)
WMS:TOT	0.29	–4.62	1.33	7.71	0.36	5.47	–0.29
	(–8.71, 2.65)	(–12.88, 3.64)	(–6.60, 3.93)	(2.01, 13.40)*	(–7.30, 8.02)	(–2.14, 13.08)	(–3.12, 2.54)

$p < 0.05$; $**p < 0.001$.

Table 4 | Differences in social functioning ratings across EBI groups.

	Congenital M (SD)	Perinatal M (SD)	Infancy M (SD)	Preschool M (SD)	Middle childhood M (SD)	Late childhood M (SD)	Total group M (SD)
SDQ							
ES*	3.47 (2.08)	2.28 (1.94)	3.43 (2.46)	1.67 (1.37)	2.77 (2.53)	2.67 (1.80)	2.78 (0.18)
CP	1.47 (2.14)	1.69 (2.14)	1.14 (1.74)	0.74 (0.90)	1.33 (1.81)	0.87 (1.69)	1.29 (1.85)
HYP	4.35 (2.63)	4.10 (2.58)	4.43 (1.99)	3.44 (2.57)	4.23 (2.49)	3.47 (2.85)	4.09 (2.51)
PP	3.09 (2.42)	3.14 (2.49)	2.00 (2.19)	2.67 (2.00)	2.27 (2.10)	1.87 (1.55)	2.60 (2.23)
PB	6.29 (2.82)	6.17 (2.93)	6.76 (1.92)	7.56 (1.98)	6.33 (2.82)	7.80 (2.56)	6.65 (2.56)
TD	12.38 (7.73)	11.21 (6.67)	11.00 (5.60)	8.56 (4.53)	10.93 (6.81)	8.87 (5.36)	10.83 (6.38)
WMS							
SC	9.50 (3.20)	9.79 (2.70)	9.43 (2.18)	11.71 (1.49)	10.29 (2.84)	10.67 (2.90)	10.08 (2.81)
PR*	8.03 (3.43)	8.52 (3.40)	9.19 (2.68)	9.71 (3.27)	10.36 (2.70)	10.13 (2.45)	9.17 (3.15)
SA	9.59 (2.98)	9.76 (2.76)	9.48 (2.14)	11.29 (1.99)	10.25 (2.76)	10.80 (2.73)	10.06 (2.67)
EM	10 (3.04)	9.62 (2.95)	9.62 (2.25)	11.41 (1.94)	10.75 (2.47)	10.60 (2.44)	10.24 (2.66)
TOT	96.97 (16.29)	95.38 (21.72)	98.67 (11.57)	107.71 (11.07)	100.36 (19.75)	105.47 (13.74)	99.71 (17.18)

$p < 0.05$.

were no significant group differences for the remaining subscales: CP, $F(5, 146) = 0.85$, $p = 0.51$, $\eta^2 = 0.03$; HYP, $F(5, 146) = 0.59$ $p = 0.71$, $\eta^2 = 0.02$; PP, $F(5,146) = 1.45$, $p = 0.21$, $\eta^2 = 0.05$; PB, $F(5,146) = 1.51$, $p = 0.19$, $\eta^2 = 0.05$; TD, $F(5, 146) = 1.18$, $p = 0.32$, $\eta^2 = 0.04$.

On the WMS, there was a significant group difference for PR, $F(5, 143) = 2.43$, $p = 0.04$, $\eta^2 = 0.08$, with the Congenital group performing poorer than the Middle Childhood group ($p = 0.04$) (see **Table 3**). There were no significant group differences for the remaining subscales: SC, $F(5, 143) = 1.94$, $p = 0.09$, $\eta^2 = 0.07$; SA, $F(5, 143) = 1.49$, $p = 0.20$, $\eta^2 = 0.05$; EM, $F(5, 143) = 1.60$, $p = 0.18$, $\eta^2 = 0.05$; TOT, $F(5, 143) = 1.68$, $p = 0.14$, $\eta^2 = 0.06$.

Differences in impairment rates across EBI groups

For the SDQ, the difference in impairment ratings between groups did not reach significance for: ES, χ^2 (5, 147) = 7.04, $p = 0.22$, $V = 0.22$; CP, χ^2 (5, 147) = 6.95, $p = 0.22$, $V = 0.22$, HYP, χ^2 (5, 147) = 3.38, $p = 0.64$, $V = 0.15$; PP, χ^2 (5, 147) = 1.99, $p = 0.85$, $V = 0.12$; PB, χ^2 (5, 147) = 7.62, $p = 0.18$, $V = 0.23$; TD, χ^2 (5, 147) = 5.29, $p = 0.38$, $V = 0.19$ (see **Figure 1A**).

There was a significant difference on the WMS between groups for PR, χ^2 (5, 144) = 13.74, $p = 0.02$, $V = 0.31$, with a higher proportion of children impaired in the Congenital groups (47.1%) and a smaller proportion of children impaired in the Late Childhood group (6.7%). The difference between groups approached significance for SC, χ^2 (5, 144) = 9.60, $p = 0.09$, $V = 0.26$, and EM, χ^2 (5, 144) = 9.60, $p = 0.09$, $V = 0.26$. The Perinatal group had a higher proportion of children impaired on the SC (31%) and EM (31%) subscales while the Preschool group had no children with impairments on these subscales. There were no significant differences between groups for: SA, χ^2 (5, 144) = 7.96, $p = 0.16$, $V = 0.24$; or TOT, χ^2 (5, 144) = 9.68, $p = 0.09$, $V = 0.26$ (see **Figure 1B**).

DO LESION CHARACTERISTICS (LOCATION, LATERALITY, PRESENCE OF SEIZURES) IMPACT ON SOCIAL OUTCOMES FOLLOWING EBI?

Lesion location

On the SDQ and WMS, ANCOVA (covarying for age at insult and seizures) identified no significant differences in outcomes between children with frontal brain pathology and those with lesions to extra-frontal regions only on any subscales (see **Table 5**). Similarly, no significant differences were identified on the SDQ or WMS for children with EBI confined to the left hemisphere, right hemisphere, or bilateral lesions on any subscales.

History of seizures

Group differences between children with and without seizure history approached significance on the SDQ for PP, $F(1, 142) = 0.3.61$, $\mathrm{p} = 0.06$, $\eta^2 = 0.03$, and children with presence of seizure history had poorer ratings. No differences between groups were found on any other subscales. On the WMS there was a significant difference in outcome between children with and without presence of seizure history on SC, $F(1, 139) = 4.17$, $p = 0.04$, $\eta^2 = 0.03$; PR, $F(1, 139) = 5.14$, $p = 0.03$, $\eta^2 = 0.04$; and SA, $F(1, 139) = 5.62$, $p = 0.02$, $\eta^2 = 0.04$. For these subscales children with presence of seizure history had poorer ratings than children without presence of seizure history. Differences between groups approached significance for EM, $F(1, 139) = 3.66$, $p = 0.06$, $\eta^2 = 0.03$ and TOT, $F(1, 139) = 3.60$, $p = 0.06$, $\eta^2 = 0.03$, suggesting that children with presence of seizure history had poorer ratings than children without presence of seizure history.

DO ENVIRONMENTAL FACTORS (SOCIAL RISK, FAMILY FUNCTION) IMPACT ON SOCIAL OUTCOMES FOLLOWING EBI?

There were no significant differences between children with high social risk and low social risk on any subscales on the SDQ or WMS (see **Table 6**). On the SDQ, there was a significant difference between children with low family functioning and children with high family functioning on ES, $F(1, 146) = 5.04$, $p = 0.03$, $\eta^2 = 0.03$, and PB, $F(1, 146) = 5.65$, $p = 0.02$, $\eta^2 = 0.04$, showing that children with low family functioning had poorer outcomes than children with high family functioning. There were no significant differences between groups on the remaining subscales. No significant differences between groups were identified on subscales of the WMS.

DISCUSSION

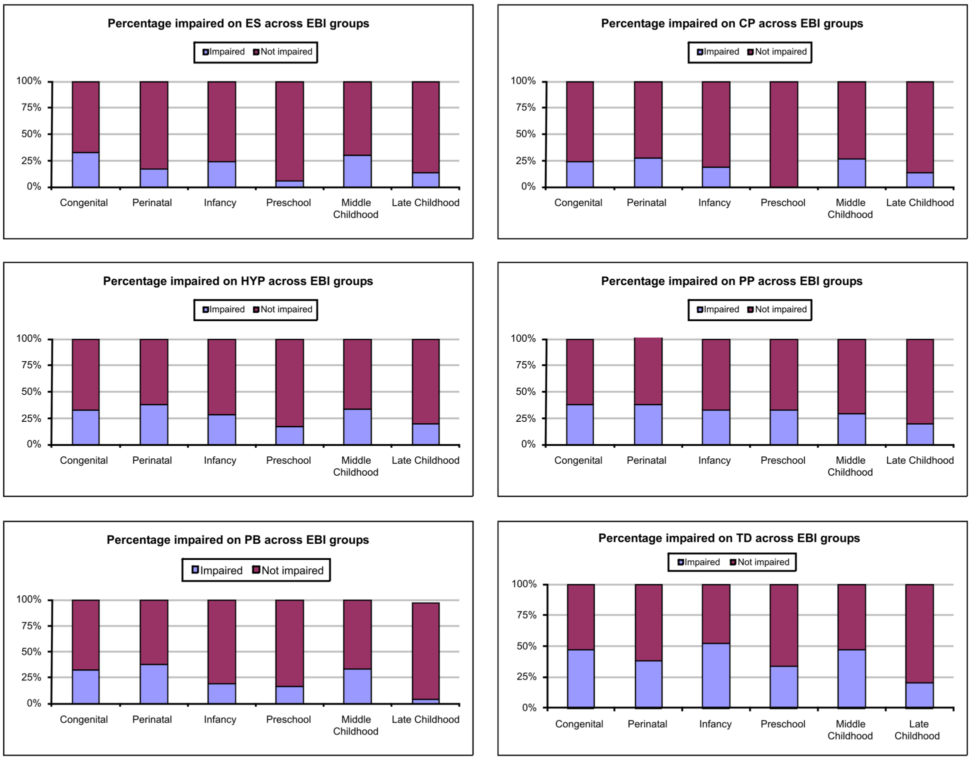
The primary purpose of this study was, first, to determine whether children with EBI were at increased risk of social dysfunction, and, if so, to examine whether the impact of age at insult on social function mimics that documented for cognitive skills. Our secondary aim was to explore the impact of lesion characteristics, seizure history and environmental factors on social functioning. Based on adult literature we predicted that, for the biological dimension, frontal pathology, right hemisphere involvement and presence of seizures would be associated with poorer social outcomes. From an environmental perspective, high social risk and family dysfunction were expected to be related to social dysfunction. Our results suggest that, as a group, children with EBI were at increased risk of social dysfunction, with elevated scores across a broad range of measures. EBI before age 3 years conferred particular risk, but significant effects were confined to the domains of peer relations and emotional symptoms. Presence of seizures and family dysfunction also contributed to social outcomes, while lesion location and social risk did not.

DO CHILDREN WITH EBI HAVE POORER SOCIAL FUNCTIONING THAN POPULATION DATA WOULD EXPECT?

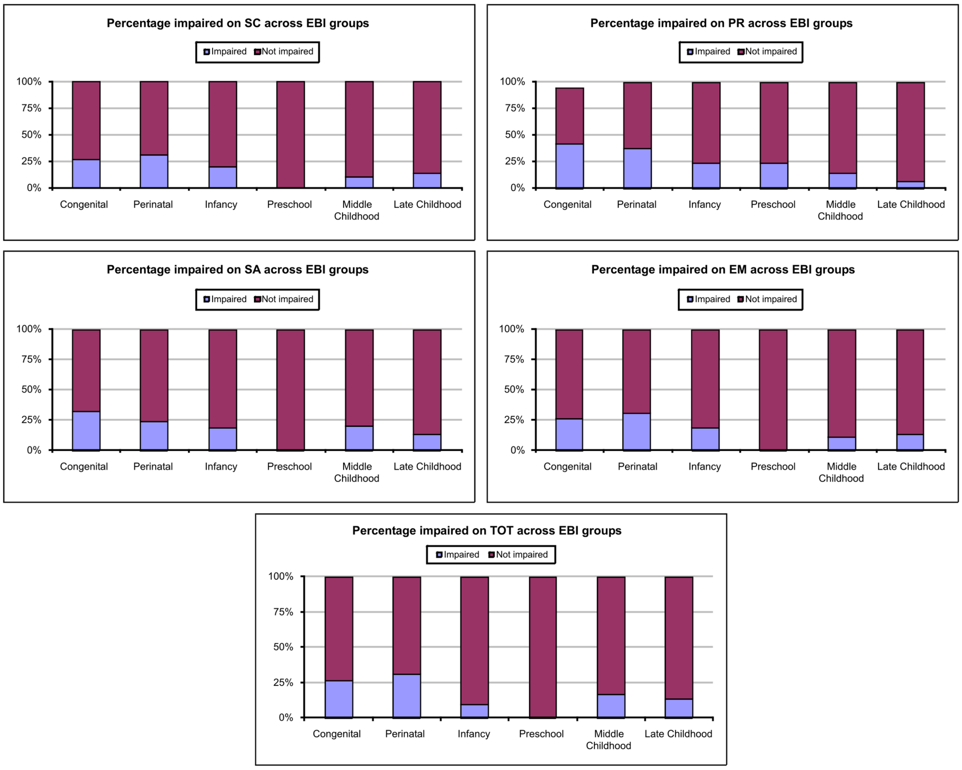
Children with brain insult, as a group, performed poorer than normative expectations on most measures (5/6 subscales on the Strengths and Difficulties Questionnaire, 1/5 on the Walker–McConnell Scale). While mean ratings for the EBI group were not severely impaired (that is, generally falling within 1 SD of expectations), the group were rated to have fewer prosocial behaviors than typically developing peers, with difficulties in peer relationships identified on both the WMS and the SDQ. Children with EBI also experienced significantly more emotional symptoms and hyperactivity than population expectations. Somewhat surprisingly, children with EBI did not demonstrate significant deviations from normal for self-control, school adjustment or empathy. Our findings are in line with previous group-based studies examining children with brain injury (Bohnert et al., 1997; Andrews et al., 1998; Janusz et al., 2002; Ganesalingam et al., 2006; Yeates et al., 2007) and case reports (Anderson et al., 2000; Eslinger and Biddle, 2008; Spencer-Smith and Anderson, 2009) which have consistently identified persisting social and behavioral problems in children with a variety of diagnoses (e.g., traumatic brain injury, stroke, tumor).

DOES DEVELOPMENTAL AGE OF EBI IMPACT ON SOCIAL FUNCTIONING?

Our results provided limited evidence for the role of age of insult in predicting social difficulties, especially when considering group mean performances. Group differences were identified only for emotional symptoms and peer relations, with the Congenital group recording poorest scores in each case. Examination of impairment ratings yielded additional findings. On the SDQ (Total score) approximately half of the Congenital, Infancy and Middle Childhood



A % impaired across EBI groups on the SDQ



B % impaired across EBI groups on the WMS

FIGURE 1 | (A) Percentages of children across EBI groups with impairment on the SDQ. **(B)** Percentage of children across groups identified as impaired on the WCS.

Table 5 | Differences in social functioning ratings for lesion characteristics and seizure history.

	Lesion location		Lesion laterality			Seizures	
	Frontal M (SD)	**No frontal M (SD)**	**Left M (SD)**	**Right M (SD)**	**Bilateral M (SD)**	**Yes M (SD)**	**No M (SD)**
SDQ							
ES	2.82 (2.16)	2.74 (2.20)	2.78 (2.03)	2.60 (2.02)	2.91 (2.40)	2.65 (2.04)	2.99(2.34)
CP	1.21 (1.85)	1.37 (1.87)	1.04 (1.64)	1.58 (2.02)	1.30 (1.91)	1.11 (1.59)	1.45 (2.01)
HYP	4.08 (2.46)	4.10 (2.57)	3.74 (2.52)	4.50 (2.72)	4.11 (2.34)	3.85 (2.37)	4.33 (2.66)
PP	2.52 (2.28)	2.69 (2.20)	2.46 (2.24)	2.85 (2.24)	2.54 (2.25)	2.26 (2.22)	2.96 (2.18)
PB	6.65 (2.66)	6.66 (2.46)	6.56 (2.61)	6.75 (2.99)	6.67 (2.21)	6.99 (2.39)	6.30 (2.66)
TD	10.62 (6.38)	11.06 (6.41)	10.22 (6.00)	11.55 (6.56)	10.86 (6.62)	10.01 (5.61)	11.72 (6.94)
WMS							
SC	10.28 (2.63)	9.87 (2.99)	10.65 (2.65)	10.05 (2.94)	9.61 (2.80)	10.57 (2.52)*	9.62 (2.98)*
PR	9.39 (2.89)	8.93 (3.42)	9.31 (3.13)	9.23 (3.10)	9.00 (3.26)	9.75 (3.16)*	8.54 (3.13)*
SA	10.21 (2.60)	9.90 (2.77)	10.39 (2.44)	9.90 (2.79)	9.89 (2.81)	10.60 (2.52)*	9.54 (2.73)*
EM	10.32 (2.39)	10.16 (2.94)	10.08 (2.82)	10.36 (2.70	10.30 (2.52)	10.67 (2.50)	9.81 (2.81)
Total	100.83 (16.64)	98.49 (17.79)	98.35 (21.10)	101.18 (14.66)	99.87 (15.07)	102.90 (16.08)	97.93 (14.91)

*$p < 0.05$.

Table 6 | Differences in social functioning ratings for environmental factors.

	Social risk		Family function	
	High M (SD)	**Low M (SD)**	**Poor M (SD)**	**Good M (SD)**
SDQ				
ES	2.75 (2.29)	2.86 (1.86)	3.18 (2.27)*	2.38 (2.00)*
CP	1.29 (1.83)	1.28 (1.93)	1.45 (2.04)	1.12 (1.65)
HYP	4.10 (2.62)	4.07 (2.25)	4.28 (2.59)	3.89 (2.41)
PP	2.54 (2.27)	2.74 (2.16)	2.53 (2.17)	2.67 (2.30)
PB	6.56 (2.74)	6.88 (2.08)	6.16 (2.54)*	7.15 (2.50)*
TD	10.78 (6.59)	10.95 (5.90)	11.43 (6.67)	10.22 (6.05)
WMS				
SC	10.10 (2.82)	10.05 (2.80)	10.28 (2.83)	9.89 (2.78)
PR	9.35 (3.26)	8.74 (2.88)	9.17 (2.90)	9.16 (3.40)
SA	10.14 (2.85)	9.88 (2.24)	9.94 (2.63)	10.18 (2.73)
EM	10.33 (2.71)	10.05 (2.55)	10.15 (2.59)	10.33 (2.74)
Total	99.65 (18.82)	99.84 (12.70)	100.90 (13.63	98.55 (20.07)

*$p < 0.05$.

groups (47.1%, 52.4%, 48.7% respectively), and one-third of the Perinatal and Preschool groups (37.9%, 33.3% respectively) fell in the impaired range. While still elevated, impairment was less common in the Late Childhood group (20.0%). Inspection of the data illustrated in **Figure 1A** indicates fairly consistent levels of dysfunction across subscales for children sustaining EBI before 3 years of age. In children with later EBI, insults between 7 and 9 years appear to result in similar levels of impairment to those of younger children. Overall, outcome was best for children with insults in the preschool period or late in childhood. Preschool insults were associated with low rates of emotional symptoms and conduct problems, while late childhood insults led to relatively few problems with emotional symptoms, conduct disorder or prosocial behaviors.

For the WMS, impairment rates were lower overall. As for the SDQ, Preschool insults were the most benign, with no child falling in the impaired range in any area with the exception of peer relationships. The Congenital (26.5%) and Perinatal (31.0%) groups recorded highest rates of social impairment overall. In contrast, the Late Childhood, Middle Childhood and Infancy groups recorded low rates of impairment (13.3%, 16.7%, 9.5% respectively) and none of the Preschool group fell in the impaired range.

Our findings indicate that, in contrast to the age-related cognitive deficits documented following EBI (Jacobs et al., 2007; Anderson et al., 2009), social functions appear to be less clearly linked to neurodevelopmental processes. Children sustaining EBI prior to age 3 years do demonstrate a tendency to poorer social and behavioral function, however outcomes appear less linked to age at insult for later insults. Consistent with these results, emerging research has begun to explore behavioral consequences of EBI and likely risk and resilience factors. Findings suggest that, for domains such as social and behavioral function, environmental influences, child disability and associated levels of child adjustment may play a greater role (Janusz et al., 2002; Anderson et al., 2006) than injury-related factors, especially in the long-term post-insult.

DO LESION CHARACTERISTICS, PRESENCE OF SEIZURES OR ENVIRONMENTAL FACTORS IMPACT ON SOCIAL OUTCOMES?

Research investigating cognitive functioning in children with EBI has established a strong relationship between outcomes and lesion characteristics (e.g., severity, location), seizure history and environmental factors (Anderson et al., 2002, 2005). We observed a somewhat different pattern when examining social function in children with EBI. Findings indicated no significant relationship between lesion characteristics and social function. Specifically, children's social outcomes did not appear to be dependent upon whether lesions involved the frontal lobes or extra-frontal areas. Similarly, laterality of lesion was not predictive of social outcomes or behavioral skills.

With respect to disability, as has been previously reported for cognition (Hartel et al., 2004; Chilosi et al., 2005; Ballantyne et al., 2007), presence of seizures was found to impact social functioning. Children with seizure history were rated as having more peer problems and more difficulties with school adjustment than children who had not experienced seizures. Lower self-control was also identified.

For environmental factors, social risk, as measured in this study, did not impact social functioning in children with EBI. Social function was not significantly related to high or low social risk (determined by family structure, maternal education, employment status and occupation of primary income earner, language spoken at home and maternal age at birth of the child). In contrast, family functioning was found to impact on the prosocial behavior and emotional subscales of the SDQ, with children from more dysfunctional families at higher risk for impairment in these domains.

LIMITATIONS AND FUTURE DIRECTIONS

When interpreting study findings, a number of potential limitations should be considered. First, use of normative data rather than an appropriately constructed healthy comparison group is a study limitation. Of importance, our results are consistent with previous research documenting the detrimental effects of brain insult sustained early in life (Ewing-Cobbs et al., 1997; Anderson et al., 2005; Chilosi et al., 2005; Jacobs et al., 2007) and provide little evidence to corroborate early plasticity notions, which argue for good outcome from EBI. Use of normative data does have an advantage over use of small, unrepresentative control samples (e.g., with inflated IQ scores) commonly reported in this field, which increase the risk of inaccurate characterisation of the study results (Ballantyne et al., 2008). Second, this study chose to recruit children based on developmental age at insult rather than the traditional 'condition-based' approach. In doing so, the sample necessarily included children for whom mechanism of insult varied, creating the risk that findings might reflect differences in brain pathology rather than age at insult. In order to minimize this risk, we confined our recruitment to children with focal brain pathologies and collected detailed information on brain pathology (e.g., location, laterality), allowing us to control for these potential confounds. We believe that this approach has provided important data to assist in understanding the impact of EBI from an empirical perspective. Of note, we employed a categorical approach to quantifying developmental stage. While these categories reflect central nervous system growth spurts, they are necessarily inexact and may mask specific critical developmental periods. Third, the lack of association between social risk and social outcome is somewhat surprising, and may have been due to choice of social risk measure. Thus, this finding should be interpreted with caution. Finally, while the total sample size provided sufficient power to investigate our primary and secondary aims, it was insufficient to conduct sub-analyses, which may have provided further information about the roles of sub-regions of the brain for social outcome. To extend these findings, prospective, multi-centre research facilitating larger sample sizes is required.

CONCLUSIONS

This study has explored social outcomes in children with EBI. Our findings demonstrate that these children are at elevated risk of problems in social functioning. While the relationship between development age at insult and outcomes is not as clear as has been identified in studies investigating cognitive outcomes, our results do suggest that children with congenital and perinatal brain insults are most at risk, and children with brain insult during the preschool-age and in late childhood demonstrate relatively good social function. Presence of seizures and family dysfunction was also predictive of aspects of social function following EBI. Finally, although previous research has indicated that lesion characteristics (location, laterality) contribute to cognitive outcome in children, this did not appear to be the case for social outcomes. Surprisingly, social risk also contributed little to social outcome in our sample.

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Adolescent development of the reward system

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Adolescence is a developmental period characterized by increased reward-seeking behavior. Investigators have used functional magnetic resonance imaging (fMRI) in conjunction with reward paradigms to test two opposing hypotheses about adolescent developmental changes in the striatum, a region implicated in reward processing. One hypothesis posits that the striatum is relatively hypo-responsive to rewards during adolescence, such that heightened reward-seeking behavior is necessary to achieve the same activation as adults. Another view suggests that during adolescence the striatal reward system is hyper-responsive, which subsequently results in greater reward-seeking. While evidence for both hypotheses has been reported, the field has generally converged on this latter hypothesis based on compelling evidence. In this review, I describe the evidence to support this notion, speculate on the disparate fMRI findings and conclude with future areas of inquiry to this fascinating question.

Keywords: adolescence, brain development, striatum, reward

INTRODUCTION

Adolescence is a developmental period characterized by increased reward-seeking behavior. This anecdotal and empirical observation has motivated the field of developmental cognitive neuroscience to identify the neural substrates of this phenomenon. As extensive animal and human work has identified the dopamine-rich striatum as the seat of reward sensitivity in the brain (e.g., Schultz, 1998; Montague et al., 2004), this region has been the focus of intense study in the adolescent reward literature and is the focus of this review. Findings on other regions (e.g., the orbitofrontal cortex) that also receive rich dopamine innervation and that are implicated in reward sensitivity are not discussed here. While studies to date agree that the striatum is the neural region most responsive to reward across development, from children to adults, the relative engagement of this neural system during adolescence is a topic of debate. In this review, I begin by briefly reviewing insights from the animal literature regarding reward-related striatal development. A subsequent section reviews developmental neuroimaging findings and outlines plausible explanations and speculations for the discrepancies across studies. Finally, I conclude with caveats and future directions of this captivating area of research.

THEORIES OF REWARD-DRIVEN BEHAVIOR IN ADOLESCENCE

To assert that dramatic behavioral changes occur during adolescence is an understatement (Dahl, 2004; Steinberg, 2005; Somerville et al., 2009). The field has generally assumed and agreed upon the notion that these behavioral changes are largely driven by rewards, including monetary, novel and social rewards, and by extension, the reward-sensitive dopamine system. Less understood is *how* the reward system changes across development to encourage the reward-driven behaviors adolescents often exhibit.

There are two primary theories about adolescent reward behavior that center around two opposing possibilities: is the striatal system hypo- or hyper-responsive to rewards during adolescence? Some theorists have proposed that adolescent reward-seeking and risk taking might result from a relative deficit in the activity of motivational circuitry (Blum et al., 1996, 2000; Bjork et al., 2004) such that more intense or more frequent rewarding stimuli are necessary to achieve the same activation as adults. This view is likely an extension of a theory of adolescent anhedonia, which is the inability to feel pleasure (Larson and Asmussen, 1991). Support for this theory comes from data showing differences between adolescents and other ages in the perception of pleasure. For instance, human adolescents exhibit an increase in negative affect and depressed mood relative to older and younger adults (Rutter et al., 1976; Larson and Asmussen, 1991) and also appear to experience the same positive situations as less pleasurable than adults (as based on self-reports) (Watson and Clark, 1984). Adolescents also find sweetness (sugar) less pleasant than children (DeGraff and Zandstra, 1999). Based on these data, some speculate that adolescents may generally attain less positive feelings from rewarding stimuli, which drives them to purse new appetitive reinforcers through increases in reward-seeking that increase activity in dopamine-related circuitry (Spear, 2000). An opposing theory postulates that disproportionately increased activation of the ventral striatal dopamine circuit (that is, the increased dopaminergic release in response to rewarding events during adolescence) underlies adolescent reward-related behavior (Chambers et al., 2003). This view arises from extensive work on dopamine and its principal role in the translation of encoded motivational drives into action (Panksepp, 1998). This theory posits that adolescent behavior is driven by reward-related appetitive systems. Based on a majority of the work reviewed below, the field has generally converged on this latter theory; that is, that adolescents are, in part, motivated to engage in high reward behaviors because of developmental changes in the striatum that confer hypersensitivity to reward (e.g., Ernst et al., 2009). However, data in support of the hypo-responsiveness hypothesis are reviewed as well.

STRIATAL DOPAMINE DEVELOPMENT

Investigations in humans can only examine *in vivo* striatal development at a systems level using neuroimaging methods. This methodological limitation precludes precise identification of how the dopamine system changes developmentally at the neural level. Rather, the link between striatal response to reward is only an index of presumed dopamine activity. These assumptions are based on insights gleaned from animal models of striatal circuitry and the dopamine system (e.g., Berridge and Robinson, 1998). As such, they are reviewed briefly here.

Available evidence suggests that there are significant alterations in the dopamine system across development, and in particular, during adolescence. Dopamine levels increase in the striatum during adolescence (Teicher et al., 1993; Andersen et al., 1997). However, other reports have shown that young adolescent rats also display lower estimates of dopamine synthesis in the nucleus accumbens (NAcc) relative to older adolescent animals and lower NAcc dopamine turnover rates relative to adults. Stamford (1989) work showed an apparent resolution to these different results by reporting a reduced basal rate of dopamine release but a larger dopamine storage pool in periadolescent, relative to adult, rats (Stamford, 1989). In fact, dopaminergic neurons in the adolescent, despite reduced dopamine release in basal conditions (Stamford, 1989; Andersen and Gazzara, 1993), are actually able to release more dopamine, if stimulated by environmental and/or pharmacological challenges (Laviola et al., 2001). Bolanos et al. (1998) showed that striatal slices from adolescent rats were more sensitive to the dopamine uptake inhibitors cocaine and nomifensine than adults, which is in contrast to the diminished behavioral responsivity to these dopamine agonists during adolescence that the same group has reported. Together, these data suggest that, during adolescence, rewarding events may result in larger dopamine release, when compared to adults (Laviola et al., 2003). So, if indeed it is the case that adolescent animals have lower basal rates of dopamine release, then perhaps adolescents initially seek out more stimulation (rewards) that will increase dopamine release; once stimulated, however, the adolescent will show greater dopamine release that subsequently contributes to a reinforcing feedback cycle that motivates additional reward-seeking behavior.

DEVELOPMENTAL CHANGE IN DOPAMINE RECEPTORS

Several reports have noted that there is dopamine receptor overproduction followed by pruning during adolescence (Teicher et al., 1995). Striatal and NAcc dopamine receptor binding of D_1 and D_2 receptors peaks in adolescence (P40) at levels that are about 30–45% greater than those seen in adulthood (Teicher et al., 1995; Tarazi et al., 1998, 1999). Using autoradiography in male and female rats, Andersen et al. (1997) showed a sexual dimorphism of this effect, such that adolescent males had greater overproduction (approximately 4.6-fold) and elimination of striatal D_1 and D_2 striatal receptors than adolescent females. Interestingly, these effects are not mediated by gonadal hormone surges (Andersen et al., 2002) but do appear to have functional consequences (Andersen and Teicher, 1999) that may correspond with behavior. A similar pattern is observed in prefrontal cortex, albeit with a more protracted elimination period (Andersen and Teicher, 2000). Confocal microscopy has revealed that retrogradely traced cortical output neurons in the prefrontal cortex express higher levels of D_1 receptors during adolescence than older or younger rodents (Brenhouse et al., 2008). These rodent findings coincide with human postmortem work. Seeman et al. (1987) reported notable changes in dopamine receptor populations in human striatum during the juvenile-to-adult period, with one-third to one-half or more of the dopamine D_1-like and D_2-like receptors present in the striatum of juveniles being lost by adulthood. Developmental declines in D_1 receptors from infancy to adulthood in humans have been also been reported by others (Palacios et al., 1988; Montague et al., 1999). Together, these animal and postmortem findings suggest that the dopamine system in adolescence may predispose individuals in this age group to greater reward sensitivity. In subsequent sections, I describe neuroimaging data that has built on these findings to show similar developmental patterns of change at the systems level.

INSIGHTS FROM NEUROIMAGING

Magnetic resonance imaging (MRI) methods introduced a new set of noninvasive tools for capturing brain development in humans. MRI is particularly useful in the study of children and adolescents as it provides precise, high-resolution anatomical images without the use of ionizing radiation (Kennedy et al., 2003). Although total brain size is approximately 90% of its adult size by age 6 (Casey et al., 2005), the gray and white matter subcomponents continue to undergo changes throughout adolescence (Giedd et al., 1999; Sowell et al., 2003; Gogtay et al., 2004). Specifically, there is a significant decrease in cortical gray matter by 12 years (Giedd et al., 1999) and an increase in cerebral white matter throughout childhood and young adulthood (Caviness et al., 1996). Recent data suggest that gray matter volume has an inverted U-shape pattern, with greater regional variation than white matter (Sowell et al., 1999, 2003; Gogtay et al., 2004). Particularly relevant to a review about the development of the dopamine system is evidence showing that the dopamine-rich frontal and striatal regions undergo significant maturational changes through adolescence (Giedd et al., 1996; Sowell et al., 1999), with a volumetric decrease in regions of the basal ganglia (Giedd et al., 1996, 1999). Similar to rodent findings, brain regions show sexual dimorphism across regions. Caudate volumes decrease during the adolescent years and are relatively larger in females (Giedd, 2004). In contrast to parietal, temporal and occipital lobes, large anatomical differences emerge between adolescents and adults in the frontal lobes and in the striatum (Sowell et al., 1999), suggesting that these two regions are relatively immature in adolescence compared to adulthood. Further, these findings suggest continued plasticity in these regions that may mediate dopamine-related behaviors and learning.

Functional MRI (fMRI) provides a measurement of brain activation that captures changes in blood oxygenation in the brain that are assumed to reflect changes in neural activity (Bandettini and Ungerleider, 2001; Logothetis et al., 2001). To study development of the dopamine system in humans, investigators have examined neurodevelopment in neural regions known to be rich in dopamine cell bodies and projections, mainly midbrain, striatal and prefrontal regions (Koob and Swerdlow, 1988). As fMRI is simply a presumed index of neuronal activity, studies that utilize this tool cannot definitely conclude changes in dopamine expression and/or activity. However, by using converging methods and insights from animal models, work in humans can begin to further probe the development of dopamine-rich circuitry. To do so, initial studies have used reward paradigms as a way to tap into this circuitry, given reports in adult humans showing the robust effect of reward on eliciting striatal activity (e.g., Knutson et al., 2001; Montague and Berns, 2002). Developmental studies have shown that, indeed, children and adolescents recruit the same neural circuitry that adults do when presented with monetary and nonmonetary rewards (e.g., Bjork et al., 2004; Ernst et al., 2005; Galván et al., 2006; van Leijenhorst et al., 2009). However, *how* adolescents differ from adults in neural recruitment has been the subject of debate in the cognitive developmental neuroscience literature.

DISPARATE FMRI FINDINGS OF REWARD SENSITIVITY IN ADOLESCENTS

Developmental fMRI studies of reward have yielded two main findings that directly map onto the two hypotheses outlined above. The first suggests that adolescents, relative to adults, show less engagement of the ventral striatum in anticipation of reward (Bjork et al., 2004). Bjork and colleagues compared early and mid-adolescents to a group of adults on the monetary incentive delay (MID) task, which was designed for and has been widely used in adult samples (e.g., Knutson et al., 2001). In the MID task, participants were first presented with one of seven cues. After a delay, they were asked to press the target and finally, feedback was presented to notify participants whether they had won or lost money during the trial. Despite similar behavioral performance, the authors found significant neural differences between age groups, such that adolescents showed less ventral striatal activation in anticipation of reward compared to adults. There were no group differences in response to feedback. Bjork and colleagues interpreted these data as support for the hypothesis that adolescents have a ventral striatal activation deficit. That is, that adolescents engage in extreme incentives (e.g., risky behaviors) ‘as a way of compensating for low ventral striatal activity (Spear, 2000; Bjork et al., 2004)’.

Although Bjork and colleagues have more recently replicated these findings in a doubled sample size and using an improved headcoil (Bjork et al., in preparation, personal communication), numerous papers have reported the opposite results (May et al., 2004; Ernst et al., 2005; Galván et al., 2006; van Leijenhorst et al., 2009). These studies have shown that, relative to other age groups, adolescents show greater activation in the ventral striatum in response to reward. For instance, in our work, children, adolescents and adults were asked to perform a simple, youth-friendly task in the scanner in which different reward values were delivered following correct responses (Galván et al., 2006). Relative to children and adults, the adolescent group showed heightened ventral striatal activation in anticipation of reward. In another example, Ernst et al. (2005) used a probabilistic monetary reward task to show that adolescents recruited significantly greater left NAcc activity than adults during winning trials. These findings directly contrast the Bjork paper and lend support for the hypothesis that disproportionately increased activation of the ventral striatal motivational circuit characterizes adolescent neurodevelopment and behavior (Chambers et al., 2003). A recent paper by van Leijenhorst et al. (2009) supports the hyper-responsive view as well. In contrast to the majority of similar work, they used an fMRI paradigm that was not dependent on behavior. That is, participants passively viewed stimuli that either certainly or uncertainly predicted subsequent reward. This approach is particularly important because previous studies may have been confounded by the behavioral component of the tasks. Their main finding is that adolescents show greater striatal activation than children or adults in response to reward receipt (van Leijenhorst et al., 2009), suggesting that even when reward is not contingent on behavior and thus there are no differences in motivation, adolescents show a hyper-active striatal response to reward.

These opposing findings further fuel the debate about how the dopamine system is altered during adolescence and mirror the seemingly contrasting findings of basal versus stimulated dopamine release in rodents. Given that there is relatively more evidence in support of the latter view, recent reviews on this topic suggest that the field has converged on the notion that, during adolescence, the striatal system is hyper-responsive to rewards and incentives (Ernst et al., 2009; Somerville et al., 2009). However, it is important to consider some plausible explanations for the disparate results.

POSSIBLE EXPLANATIONS FOR THE DISCREPANCIES

There are several possible explanations for the striking differences between studies. **Table 1** summarizes the major areas of divergence in the most commonly cited papers on this topic. This table is not intended to be exhaustive and only includes work conducted in typically developing youth; data from clinical populations are not discussed. First, the studies differ greatly in the developmental stages and ages of participants. Second, studies differ in the comparison groups. Last, differences in task design, analysis and baseline conditions can lead to significant differences in interpretation. Where appropriate, suggestions and possible strategies to minimize these methodological differences in future work are described.

What is adolescence?

A significant issue that is underappreciated across and between studies is the problem of defining adolescence in humans. Adolescence can and is defined by a myriad ways, including age, sexual maturation, puberty, educational grade, the law, and/or financial independence, by a multitude of experts including educators, scientists, policy makers and parents. Given the seemingly endless possible definitions, adolescent researchers face a daunting task in deciding which individuals to include in their “adolescent” sample. Some scientists have identified adolescence as ‘the gradual period of transition from childhood to adulthood (Spear, 2000; Dahl, 2004)’. While this broad definition is useful when describing heterogeneous bodies of work, as in literature reviews, it is not the most suitable way to define participant samples to be included in developmental studies. The reason this is inappropriate for empirical work is because of the vast heterogeneity that characterizes adolescence biologically and socially.

Table 1 | Developmental fMRI reward studies.

Study	Main findings	Age range of adolescent group	Comparison group	Task design	Analysis focus	Baseline
Bjork et al. (2004)	Adolescents show *hypo-responsive* striatal activity relative to adults	12–17	Adults (ages 21–28)	Reward magnitude	Anticipation of reward	Entire trial
May et al. (2004)	No comparison group	9–16	None	Reward probability	Entire trial	First timepoint of each trial
Ernst et al. (2005)	Adolescents show *hyper-responsive* striatal activity relative to adults	9–17	Adults (ages 20–40)	Reward magnitude and probability	Feedback (outcome)	Subset of fixation trials
Galván et al. (2006)	Adolescents show *hyper-responsive* striatal activity relative to children and adults	13–17	Children (ages 7–11) and adults (ages 23–29)	Reward magnitude	Anticipation	Intertrial interval
van Leijenhorst et al. (2009)	Adolescents show *hyper-responsive* striatal activity relative to children and adults	14–15	Children (ages 10–12) and adults (ages 18–23)	Reward probability	Anticipation and feedback	No baseline
Geier et al. (2009)	Adolescents show *hypo-responsive* striatal activity to reward cues and *hyper-responsive* activity in anticipation of reward relative to adults	13–17	Adults (ages 18–30)	Reward probability	Cue, anticipation and feedback	Implicit baseline (e.g., non-task activation)

While some groups restricted inclusion of the adolescent sample to high school students (Galván et al., 2006; Geier et al., 2009) and one group included an age-restricted adolescent group that inarguably captured adolescence (van Leijenhorst et al., 2009), the age range of the adolescent group in the remaining studies listed in **Table 1** varies widely. For instance, the Bjork et al. (2004), May et al. (2004) and Ernst et al. (2005) studies included 12-year-old children (the latter studies included even younger children, at 9 years old) in their "adolescent" sample. While a 12-year-old might be considered an early adolescent in some academic circles, it would be hard to make the same claim for a 9-year-old. Besides, even if a 12-year-old can be considered an early or pre-adolescent, that individual is a very different adolescent than say, a 17-year-old, who presumably has more independence, has a greater likelihood of having engaged in risky and reward-seeking behavior, and has a different appreciation of money (the most commonly used reward in these studies). As such, it is time for the field to set standards on how adolescents are classified; this is particularly crucial now that we have evidence that developmental changes follow a non-linear pattern in many brain regions that peaks in mid-adolescence (Shaw et al., 2008). At the very least, investigators should make a more concerted effort to report how the age groups were defined. These definitions might include a particular age, puberty, or year in school (e.g., only high school students). While obtaining a broad age range is typically the ideal standard in developmental work to examine *developmental change*, this approach is only useful if the analyses are conducted in such a way as to appreciate the age and developmental continuum. That is, a broad age range that includes early-, middle- and late-adolescence is only developmentally informative if age is included as a regressor to examine individual variability across development. Instead, all studies described above group the "adolescent" sample and compare it to the comparison group, without taking advantage of the developmental distribution. By the time the study is whittled down to a summary, the generalized message neglects to highlight the significant variability in age.

Comparison groups

Identifying the appropriate comparison group for adolescents is almost as difficulty as defining adolescence. This identification is challenging because the boundaries between child and adolescent and adolescent and adult are often murky. While some investigators would classify a 12-year-old as a child (van Leijenhorst et al., 2009), others would include that same child in the adolescent group (Bjork et al., 2004; May et al., 2004; Ernst et al., 2005). Similarly, most neuroimaging studies, including developmental and adult studies, include 18- and 19-year-olds as the adult group. This practice has likely arisen for two primary reasons: (1) in the United States, 18-year-olds are defined by law as adults and (2) college students are an easy subject pool for recruitment purposes. This inclusion persists despite the fact that numerous studies have documented the protracted development of the brain through the mid- to late-twenties (Giedd, 2004) and the questionably mature disposition of individuals in this late adolescent age range. As such, it is quite possible that individuals who are only a few months apart in age (e.g., a 17-year-old and an 18-year-old) are classified as an adolescent and adult, respectively (Geier et al., 2009) which begs the question of whether the adult comparison group is truly an accurate comparison group.

Task design

Despite asking basically the same question (what is the developmental trajectory of dopamine-rich striatal circuitry in response to reward?), no two experimental paradigms described here are alike. While some focused on reward magnitude (Bjork et al., 2004; Galván et al., 2006), others manipulated reward probability (May et al., 2004; van Leijenhorst et al., 2009) or both (Ernst et al., 2005; Eshel et al., 2007). Further, in all but one study (van Leijenhorst et al., 2009), rewards were dependent on participants' behavioral response including reaction time (e.g., Bjork et al., 2004) and response accuracy (Ernst et al., 2005; Galván et al., 2006; Eshel et al., 2007). Given known developmental differences in reaction time speed and accuracy ability, the difficulty of the task could have had large influence on neural activation patterns.

Another obvious difference between the studies listed in **Table 1** is the wide range of tasks used and the degree to which they were developmentally appropriate. Task choice is not a trivial issue, as differences in task engagement and comprehension can have significant effects on neural activation. While some studies designed the tasks to maximize the probability that developmental populations would find them engaging (Galván et al., 2006; van Leijenhorst et al., 2009), such as through the use of cartoon-like stimuli and by describing the task as a video game (e.g., "your goal is to help the pirate in this video game earn as much money as possible"), others simply implemented tasks that were designed for adults (e.g., Bjork et al., 2004; May et al., 2004). This latter approach is problematic for several reasons. First, the use of fMRI tasks designed for adults is done under the assumption that youth will find the adult-appropriate tasks as engaging as adults do. Second, this also assumes that children and adolescents will comprehend the tasks as well as adults. Third, this approach may be an unfortunate illustration of a broader negligence of making special considerations when studying children and adolescents. For instance, if the investigators are comfortable using tasks that will likely be uninteresting to children and adolescents, one might wonder if the investigators similarly neglected to implement special child-friendly scanning practices (e.g., ensuring the child is comfortable and that the experience is as anxiety-reducing as possible). To ensure making the tasks as youth-friendly as possible, some suggestions include using cartoon or otherwise animated stimuli, ensuring proper response time for children (as a plethora of studies have shown that children have longer reaction times than adults), and making the task as simple as possible without multiple conditions and rules that the child needs to hold online. For example, while seven predictive cues might be reasonable for an adult to keep in mind in the MID task (Knutson et al., 2001), adolescents may find this task demand more difficult (Bjork et al., 2004) and subsequently become less engaged in the task. This could eventually lead to less neural activation, as compared to the relatively more engaged adults.

Task analyses

An additional consideration that almost certainly contributed to differences in results is the stage of reward processing that was analyzed. All of these fMRI tasks included three basic stages: cue presentation, anticipation of reward following behavioral response, and feedback. Of the studies reviewed here, three studies examined anticipation of reward (Bjork et al., 2004; Galván et al., 2006; Eshel et al., 2007), three studies analyzed responses to feedback (Bjork et al., 2004; Ernst et al., 2005; van Leijenhorst et al., 2009) and one study did not distinguish between stages and instead analyzed the entire trial (May et al., 2004). The difficulty in analyzing these different stages of reward processing is that temporally proximal events (e.g., the cue and anticipation phase) are difficult to parse out in fMRI analyses. In practice, this means that while only one phase was of interest, MR signal from the other stages may have bled into activation. In other words, while researchers may have intended to examine one aspect of the task, they may have been measuring (and reporting) another aspect of the task. Without the raw data, it is impossible to glean from the papers if this was the case. This possibility may explain the different results reported even when the focus of analysis was the same. For instance, while Bjork et al. (2004) and Galván et al. (2006) both examined the anticipation phase, their data are completely opposite. Also, while Ernst et al. (2005) and van Leijenhorst et al. (2009) report greater ventral striatal activation in adolescents compared to adults during feedback, Bjork et al. (2004) failed to detect any activation differences between groups in any of the feedback contrasts.

A recent study by Geier et al. (2009) illustrates how adolescents may have distinct activation profiles during different stages of the task. These authors cleverly designed the task precisely to be able to deconvolve the distinct stages of the task. During the cue component, adolescents showed an attenuated response in the ventral striatum compared to adults. However, during reward anticipation, the same adolescents showed heightened activity in the same region, as compared to adults. Collectively, these data suggest that temporally distinct aspects of reward tasks may yield significantly different results and should be carefully considered when making sweeping generalizations about the adolescent striatum and reward sensitivity.

Baseline issues

The interpretation of functional imaging studies of development is dependent on the sensitivity and accuracy of the imaging methods used to detect these changes (Kotsoni et al., 2006). As the blood oxygen level dependent (BOLD) signal is used as a measure of brain activity in most fMRI studies, a variety of variables including heart rate, heart rate variability and respiration can influence the hemodynamic response. For instance, heart rate and respiratory rate in children are nearly twice those in adults (Kotsoni et al., 2006). These physiological differences across development are a significant concern in developmental neuroimaging studies because they can introduce greater noise in echo planar and spiral imaging due to movement of lungs and diaphragm (van de Moortele et al., 2002). As such, these developmental differences should be taken into consideration when identifying an appropriate baseline. Thomason et al. (2005) examined how developmental differences in respiration influenced the fMRI signal while participants breathed normally in the scanner without engaging in a task. They found that in addition to greater noise in the children's data, this noise contributed to increased "baseline" activation in children's relative to adult's percent signal change. As passive rest in the scanner (similar to the instructions Thomason's participants received) is commonly used as the baseline condition by which all cognitive task conditions are compared, these differences can have significant and deleterious influence on fMRI results and interpretations. This broader discussion of baseline issues is not new, as Schlaggar et al. (2002) have raised the problem of appropriate comparison tasks previously. Whether children (and adolescents) display increased or decreased resting baseline states will influence the final outcome and interpretations of results when their data are compared to adults' data if the baseline problem is not taken into consideration and controlled for during task design and data analysis.

At least three types of baselines were used in the studies described here. Bjork et al. (2004) defined the baseline as the mean signal value averaged across the entire time series. In the Ernst et al. (2005) paper, 18 (of 129) trials were fixation trials that served as baseline. That is, all contrasts of interest were compared to trials in which the participant was presumed to be doing nothing but staring at a fixation cross (refer to Thomason et al., 2005 above to note how this may be problematic). Similarly, Galván et al. (2006) used the intertrial interval as the relative baseline, during which the participant was presented with a fixation cross. Finally, van Leijenhorst et al. (2009) and Geier et al. (2009) did not define an implicit baseline and instead generated contrast images between different trial types (e.g., certain versus uncertain reward trial types). All authors presumably had good reason to choose the baseline they did and there is no standard baseline in the field but, clearly, small differences in baseline can have dramatic effects on final results. For instance, if adolescents have a higher (or lower) resting baseline than adults, the subtraction method (e.g., comparing image contrasts) used in fMRI analyses may lead to incorrect interpretations.

While agreeing upon a standard baseline is neither feasible nor optimal, as nuances in questions and experimental tasks warrant individual baseline requirements, there are ways to ensure that the chosen baseline in individual studies is comparable across groups. One way to circumvent these inherent developmental differences in resting fMRI signal is to establish separate baselines for each group and then compare task conditions within-group. Several neuroimaging software packages, such as FSL, allow for this type of analysis without compromising statistical group comparisons. A second way is to first confirm that signal activation differences for the baseline condition do not significantly differ between age groups prior to subsequent cognitive task comparisons. Finally, a different approach would be to compare only youth and adults who show similar baseline activation patterns. This approach would be similar to *post hoc* performance-matching described previously for behavioral data (Schlaggar et al., 2002).

INDIVIDUAL DIFFERENCES IN REWARD SENSITIVITY

Although the work presented thus far suggests that adolescence is a heightened period of reward sensitivity, not all adolescents are reward-seekers. The importance of examining individual differences in behavior and neural activity has been appreciated in adult samples (e.g., Tom et al., 2007) but less work has been conducted in developmental populations. Reward-seeking and risk-taking behaviors (e.g., gambling and illicit drug use) are more frequent in individuals with a particular behavioral trait, such as elevated novelty and sensation-seeking (Willis et al., 1994). Relevant to this review is that anticipatory activation of the ventral striatum predicts reward-related risks at an individual differences level (Montague and Berns, 2002; Matthews et al., 2004; Kuhnen and Knutson, 2005). For instance, individuals who show greater activation in the ventral striatum prior to a gambling choice are more likely to make a risky, rather than safe, choice (Kuhnen and Knutson, 2005). More generally, previous studies have documented striking individual differences in the efficiency of cognitive control (Fan et al., 2002), which is necessary for self-regulation in rewarding situations. In fact, the ability to direct attention away from rewarding stimuli during a delay of gratification task in toddlers predicts cognitive control later in life (Eigsti et al., 2006). Together, these studies underscore the importance of taking into consideration individual differences in experience, behavior and neural activation when examining complex brain-behavior operations such as reward processing in developmental populations. In a recent study (Galván et al., 2007), we examined individual differences to help disentangle the complexities that underlie increased vulnerability in some individuals to reward-driven behaviors and negative outcomes, such as addiction. Our approach was to examine the association between activity in reward-related neural circuitry in anticipation of a large monetary reward with personality trait measures of risk-taking and impulsivity in adolescence. fMRI scans and anonymous self-report rating scales of risky behavior, risk perception and impulsivity were acquired in individuals between the ages of 7 and 29 years. The main finding was that there was a positive association between NAcc activity and the likelihood of engaging in risky behavior across development; that is, individuals more likely to report higher frequency of risky behavior in "real-life" recruited the ventral striatum the most in the lab. These findings suggest that during adolescence, some individuals may be more prone to engage in risky behaviors due to developmental changes dopamine-rich regions in concert with variability in a given individual's predisposition to engage in risky behavior. These studies are a good starting point to investigate the role of individual differences in reward sensitivity. However, future work also needs to examine the neural correlates of reward that incorporates sex, age, pubertal stage and ethnic differences.

WHAT IS REWARDING TO A HUMAN ADOLESCENT?

The majority of studies reviewed above used money as a reward probe, as it is an easy reward to manipulate, elicits robust recruitment of dopamine-rich circuitry, and has been used extensively in adult models of reward. However, adolescents are motivated by more than simply monetary rewards and studies that take advantage of social, novelty and primary reinforcing rewards that also motivate adolescents may shed new light on the reward system. What is rewarding changes with development, so what adolescents deem uniquely rewarding, relative to children and adults, may inform the field about the underlying dopamine system. For instance, while children are most rewarded by primary reinforcers, such as sugar, adolescents find peer interactions more rewarding than children and adults (Csikszentmihalyi et al., 1977). One study showed increased recruitment of the ventral striatum to passive viewing of images of socially desirable, but not undesirable peers (Guyer et al., 2009). Without an appropriate manipulation of the socially desirable peers as rewarding stimuli, it is impossible to know if, indeed, adolescents find socially desirable peers more rewarding than others but this study does implicate dopamine-rich circuitry in adolescent sensitivity to social interactions. As such, *what* is rewarding and the context in which the rewards are presented are important factors to consider when comparing motivation, behavior and underlying reward circuitry in adolescents relative to other groups. This is particularly relevant to the well-characterized risk-taking behavior in adolescents (Steinberg, 2004). Relative to adults or children, adolescents are more likely to categorize risk-taking as "fun" or rewarding (Maggs et al., 1995); this suggests that in response to a risky opportunity, adolescents may be more likely to engage the dopamine system than other age groups, which may contribute to their increased risk-taking tendencies. This phenomenon has been reviewed extensively elsewhere (e.g., Steinberg, 2004; Ernst and Mueller, 2008; Somerville et al., 2009).

FUTURE AREAS OF INQUIRY

This review did not include the extensive literature on hormonal development as they relate to behavioral changes during adolescence as it has been reviewed numerous times elsewhere (Spear, 2000). However, complex interactions between the dopamine system and changes in hormones during adolescence likely contribute to the expressed reward-related behaviors. In future work, designing experiments that can assess how the function of dopamine-rich circuitry is mediated by changes in hormones may provide useful insights into this complex association.

Also, further examination of how changes in sleep patterns influence neural function during adolescence will be a useful area of inquiry. Mounting evidence suggests that sleep is critical for brain function and development (Benca, 2004; Hagenauer et al., 2009). Recent examination of this critical question provides invaluable insight into how normally occurring changes in sleep patterns might exacerbate detrimental adolescent-typical behaviors (Dahl and Lewin, 2002; Holm et al., 2009). Holm et al. (2009) show that poor sleep quality and fewer minutes of sleep were associated with blunted striatal activity during reward anticipation and outcome (Holm et al., 2009). These data highlight the importance of considering contextual effects on reward-related neural sensitivity across development.

Various reports have noted sexual dimorphism in dopamine system development in animal models (Andersen et al., 1997) and structural MRI work (Giedd et al., 2004). However, this area of research has been relatively under-studied in functional MRI studies, probably because of practical constraints imposed by relatively limited sample sizes in these studies. This effect is a critical area of study as there are prominent sex differences in the onset and maintenance of several mental health disorders that may be related to aberrant dopamine functioning (Paus et al., 2008).

CONCLUSIONS

This review began with the following question: is the dopamine system hypo- or hyper-responsive to rewards during adolescence? The investigations described in this review provide unequivocal evidence that the reward system undergoes massive changes during adolescence. Further, they show strong support for the hypothesis that the dopamine system is hyper-responsive, or over-engaged, in response to rewards during adolescence. While initial neuroimaging work (Bjork et al., 2004) seemed to provide support for hypo-responsive reward system hypothesis, numerous studies since have instead yielded data that provide support for an overactive reward system during adolescence. As such, the field seems to be converging on this latter conclusion (Casey et al., 2008; Steinberg, 2008; Ernst et al., 2009; Somerville et al., 2009). However, subtle nuances in experimental manipulation, interpretation and environmental context have significant effects on this generalization. As best illustrated in recent work by Geier et al. (2009), different aspects of reward are paralleled by distinct neural sensitivity in adolescence, such that initial presentation of a reward-predicting cue does not lead to similar hyperactivity as the anticipation of upcoming reward. In our own work, human adolescents showed increased activation, relative to children and adults, in the dopamine-rich NAcc in response to high reward but showed *diminished* activation in this same region in response to low reward (Galván et al., 2006). Thus, *what* is rewarding to an adolescent will influence circuitry implicated in reward and risk-taking and, presumably, subsequent behavior. Reward value is not absolute and rewards are instead appreciated in the context of other available rewards. Adolescents may be particularly sensitive to these changing contexts.

In sum, while there is no doubt that the reward system undergoes dramatic developmental changes during adolescence, the precise features of these maturational events cannot easily be determined and will require further exploration, in both the animal and human literatures. By rooting research on the dopamine system in animal findings, we can begin to constrain interpretations of data from the human work, to better understand what precisely is changing in the striatal dopamine system that predisposes adolescents to engage in high reward-seeking behaviors.

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Better than expected or as bad as you thought? The neurocognitive development of probabilistic feedback processing

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Learning from feedback lies at the foundation of adaptive behavior. Two prior neuroimaging studies have suggested that there are qualitative differences in how children and adults use feedback by demonstrating that dorsolateral prefrontal cortex (DLPFC) and parietal cortex were more active after negative feedback for adults, but after positive feedback for children. In the current study we used functional magnetic resonance imaging (fMRI) to test whether this difference is related to valence or informative value of the feedback by examining neural responses to negative and positive feedback while applying probabilistic rules. In total, 67 healthy volunteers between ages 8 and 22 participated in the study (8–11 years, *n* = 18; 13–16 years, *n* = 27; 18–22 years, *n* = 22). Behavioral comparisons showed that all participants were able to learn probabilistic rules equally well. DLPFC and dorsal anterior cingulate cortex were more active in younger children following positive feedback and in adults following negative feedback, but only when exploring alternative rules, not when applying the most advantageous rules. These findings suggest that developmental differences in neural responses to feedback are not related to valence *per se*, but that there is an age-related change in processing learning signals with different informative value.

Keywords: development, fMRI, feedback, decision-making, learning, cognitive control

INTRODUCTION

Learning to correctly adapt your behavior in a changing environment is an essential feature of human cognition and has been studied extensively over the past decades (for reviews, see Ridderinkhof and van den Wildenberg, 2005; Rushworth and Behrens, 2008). When adapting behavior, individuals often make use of feedback signals, which can be positive, encouraging the continuation of behavior, or negative, discouraging the continuation of behavior and signaling the need for adjustment. Prior studies have indicated that adaptive learning based on feedback signals undergoes pronounced developmental improvements between late childhood and early adulthood, as is evident from tasks in which participants need to switch between multiple rules (Crone and van der Molen, 2004; Somsen, 2007) or in which they need to infer sorting rules based on positive and negative signals (van Duijvenvoorde et al., 2008).

Early developmental improvements in adaptive behavior are observed when feedback has a direct mapping to deterministic rules (Somsen, 2007), however, when the feedback is probabilistic, changes in adaptive learning are observed until late adolescence (Hooper et al., 2004). In these situations, individuals must learn the statistical regularities between actions and outcomes, and use that information to interpret current feedback signals (see also Rangel et al., 2008). Feedback which is not directly mapped to behavior is often more complex because it requires individuals to attend to long term consequences and override the tendency to respond directly to local environmental change.

Neuroimaging studies have shown that regions previously associated with cognitive control and response selection (Miller and Cohen, 2001; Toni et al., 2002) are also active when adults receive negative performance feedback, including the dorsal anterior cingulate cortex (dACC) and the dorsolateral prefrontal cortex (DLPFC) (Klein et al., 2007; Taylor et al., 2007). The dACC is thought to monitor action outcome regularities and is important for signaling adjustment (Botvinick et al., 2001; Yeung et al., 2004). In addition, the dACC may exercise behavioral control via the engagement of the DLPFC (Kerns et al., 2004; Zanolie et al., 2008), which in turn is important for trial-to-trial adjustments of behavior (Dosenbach et al., 2008). Similar to the DLPFC, the parietal cortex is also involved in feedback processing, in particular negative feedback (Crone et al., 2008; van Duijvenvoorde et al., 2008). Finally, these regions are thought to work in close concert with the basal ganglia, specifically the caudate nucleus, which is thought to be engaged when learning action- outcome regularities (for a review see Cools, 2008).

In two prior developmental studies we have identified the developmental time course of these regions during adaptive feedback processing. In the first study (Crone et al., 2008), participants were instructed to infer rules based on positive and negative feedback which could change without warning. Following Somsen (2007), we were interested in the way children, adolescents, and adults processed negative feedback indicating a rule shift. As anticipated, adults engaged DLPFC, dACC, and the parietal cortex when processing negative feedback indicating a rule shift. A similar pattern was observed in 14- to 15-year-old adolescents, but 8- to 11-year-old children engaged these regions less following negative feedback in comparison to positive feedback or a low-level fixation baseline. In the second study (van Duijvenvoorde et al., 2008), participants were instructed to guess a correct rule. Because there were two possible rules, there was a 50% chances of receiving positive feedback, and therefore both feedback signals (negative and positive) were similarly salient and probable. Again, adults engaged DLPFC, dACC, and the parietal cortex following negative feedback, but in this study 8-year-old children engaged DLPFC and the parietal cortex more following positive feedback relative to negative feedback. The developmental trajectory of the dACC followed a different pattern, as it slowly emerged in response to negative feedback at the age of 12, but it was not more active following negative compared to positive feedback at a younger age (see also Velanova et al., 2008). Although the caudate nucleus was involved in these tasks, these studies revealed that there were no developmental differences in activation patterns.

Together, these findings indicate that the possible meaning of positive and negative feedback signals, and the role of the associated neural circuits, changes during development. However, prior studies could not dissociate between neural activation as a result of valence versus informative value, given that negative feedback always signaled response adjustment and therefore had different informative value than positive feedback. Thus, it remains to be determined how the involvement of DLPFC and the parietal cortex is dependent on valence versus informative value of the feedback.

Prior research suggests that differences in positive and negative feedback adjustment are the result of differences in attention regulation (Somsen, 2007). Following this hypothesis, it is argued that children are less able to update the relevant feedback information and therefore they are less flexible in selecting alternative actions. We therefore reasoned that the brain regions implicated in prior feedback studies may be sensitive to the informative value of feedback, and that activation in these brain regions is indicative of feedback attendance. Furthermore, we predicted that attention to feedback may also underlie the developmental differences in brain activation. We hypothesized that DLPFC and parietal cortex would be more active following positive feedback in children and following negative feedback in adults, but only when the feedback has informative value for learning and response adjustment. Thus, we sought to test how neural responses are sensitive to informative value for learning versus valence of feedback, and the developmental trajectory of feedback processing.

We reasoned that feedback valence versus informative value could be disentangled after participants learned probabilistic feedback rules. In the probabilistic learning paradigm, participants need to learn from positive and negative feedback under different levels of probability, and therefore not all positive feedback signals response continuation and not all negative feedback signals response adjustment. The probabilistic learning (i.e., trial-and-error) task employed in this study was based on a prior study by Frank et al. (2004), but was simplified for use with children. In our version of the probabilistic learning task, two different stimulus pairs (AB or CD) were presented in random order, and participants had to learn over trials that one stimulus was more likely to result in positive feedback (70–80%) (see **Figure 1**). Over the course of the experiment participants had to learn the statistical regularities and thus had to learn to choose the stimuli with a high probability of positive feedback (A and C) more often than those with a low probability of positive feedback (B and D).

When participants have gained knowledge of the statistical regularities, they were expected to more often apply the correct rule. Notably, in probabilistic learning tasks individuals generally do not consistently apply the correct rule but show matching behavior; i.e., they choose the correct stimulus with a frequency that is proportional to the probability of positive feedback associated with that stimulus (Estes, 1961; Herrnstein, 1961; Shanks et al., 2002; Frank and Kong, 2008). Thus, we anticipated that participants would apply the correct rule (in this study, choosing the high probability stimuli A and C) more often, but we also anticipated that they would remain exploring the alternative rule (choosing the low probability stimuli B and D). Therefore, this paradigm allowed us to investigate the processing of positive and negative feedback that carries different informative value. In particular, receiving negative feedback when choosing the correct rule should not be interpreted as a signal to switch to the alternative rule because the probability of positive feedback remains higher than for the alternative rule. In contrast, receiving negative feedback when choosing the alternative rule should lead to a switch to the correct rule. To be able to address the question how neural responses are sensitive to feedback signals in the context of learned rules, we only analyzed neural responses after participants had reached a learning plateau.

Based on prior studies, we expected that DLPFC and the parietal cortex would be sensitive to whether feedback signals required greater attention, and would contain greater informative value for performance adjustment on subsequent trials. Therefore, we expected that these regions would be engaged mostly after choosing the alternative rule (B or D), because this feedback contained learning signals for performance adjustment, independent of valence. We also examined the role of the dACC and the caudate as these regions have previously been implicated in feedback processing (Schultz, 2007; Cools, 2008; Rushworth and Behrens, 2008). We expected that the dACC would be most sensitive to negative feedback signals, particularly when indicating the need for behavioral adjustment (Kerns et al., 2004), whereas we expected that the caudate would be most sensitive to positive feedback which signals response continuation (Cools, 2008).

The second question concerned developmental differences in performance and neural activation. In prior research, developmental differences were observed between childhood and mid-adolescence, but differences between adolescence and adulthood remain unclear (Crone et al., 2008; van Duijvenvoorde et al., 2008). For this purpose, we compared behavioral and neural responses of three age groups; children (8–11 years), adolescents (13–16 years), and adults (18–22 years). Behaviorally, we predicted that differences in adaptive learning would be largest between childhood and adolescence, with refinement of learning between adolescence and adulthood (Luna and Sweeney, 2001; Crone and van der Molen, 2004; Somsen, 2007). In addition, we expected to find that these behavioral changes would be paralleled by changes in the areas involved in adaptive control (dACC, DLPFC, parietal cortex and caudate nucleus). For the fMRI analyses, we had three specific

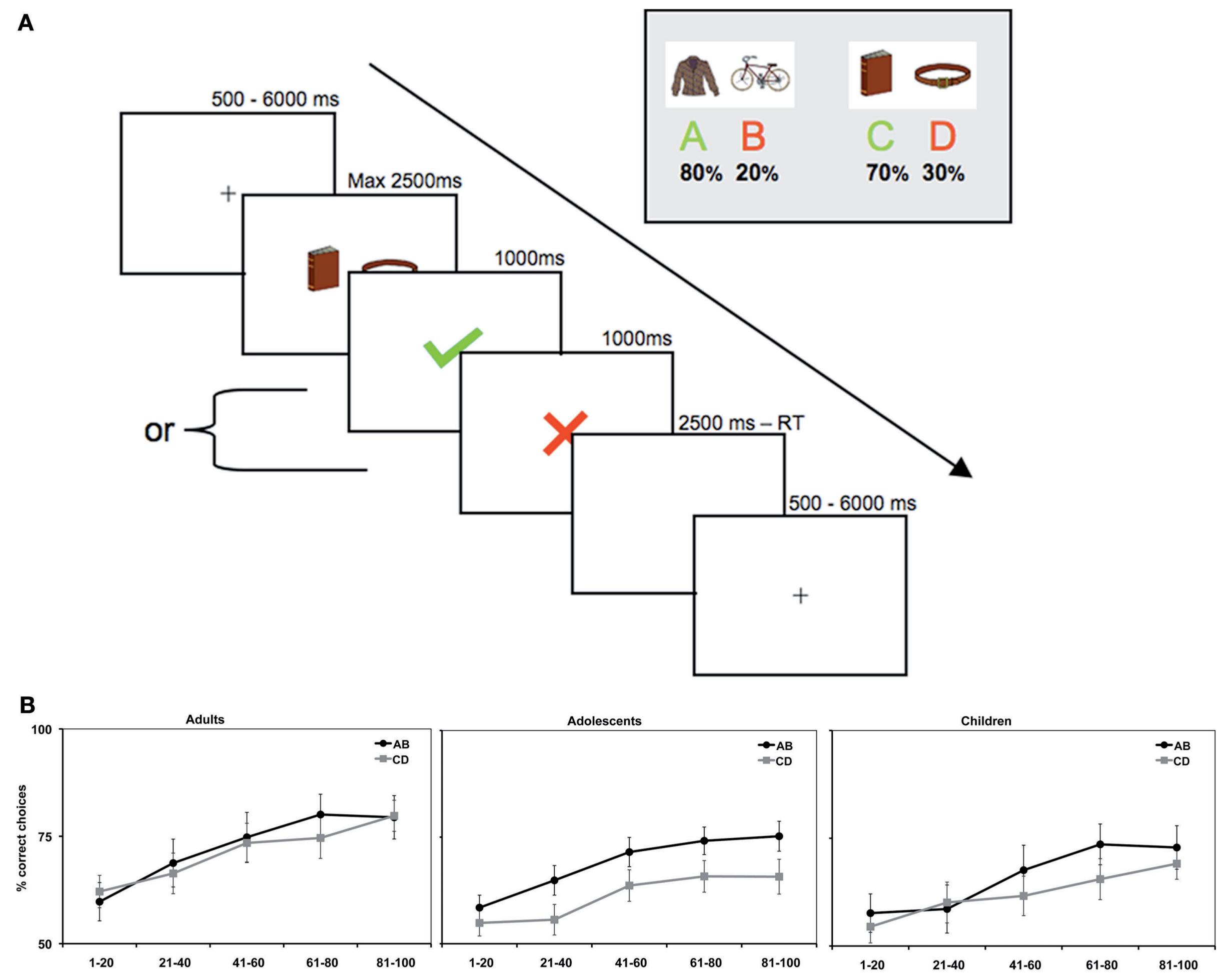

FIGURE 1 | (A) At the beginning of each trial a centrally located cue was presented with a jittered interval between 500 and 6000 ms, followed by a combined presentation of a stimulus pair and a response window of max. 2500 ms, after which feedback was presented for 1000 ms. After the feedback a short filler was presented, in the form of a blank screen, in order to compensate for different reaction times between trials and between participants (filler duration = 2500 ms – reaction time). **(B)** Average accuracy on AB and CD trials per age group.

age-related hypotheses based on prior studies. First, we expected an increase in differentiation in the dACC for positive and negative feedback processing with increasing age (van Duijvenvoorde et al., 2008; Velanova et al., 2008). Second, we expected an attention-based shift in recruitment of DLPFC and the parietal cortex from positive to negative performance feedback with age. Third, we expected age differences in how learned probabilities would be associated with neural changes in feedback processing; in particular we predicted that feedback after exploring the alternative rule would be associated with developmental differences. Because of the children's putative focus on positive feedback, we expected that with increasing age there would be a decrease in activity related to processing positive feedback and an increase in activity related to processing negative feedback following selection of the alternative rule.

Finally, our paradigm allowed us to investigate age differences in adaptive behavior, that is, whether participants stay or shift on subsequent trials based on the received feedback. Besides behavioral analyses of sequential effects, we also employed exploratory sequential condition analyses to further understand the relation between neural activation and subsequent adjustment of behavior (see also Kerns et al., 2004).

MATERIALS AND METHODS

PARTICIPANTS

Sixty-seven healthy right-handed paid volunteers (35 female, 32 male; ages 8–22 participated in the fMRI experiment. Age groups were based on adolescent development stage, resulting in three age groups: children (8- to 11-year-olds, $n = 18$; 9 female), mid-adolescents (13- to 16-year-olds, $n = 27$; 13 female) and young adults (18- to 22-year-olds, $n = 22$; 13 female). A chi square analysis indicated that the gender distribution was similar across age groups, $\chi^2(2) = 0.79, p = 0.67$. All participants reported normal or corrected-to-normal vision and participants or their caregivers indicated an absence of neurological or psychiatric impairments. Participants and their caregivers (for minors) gave informed consent for the study and all procedures were approved by the medical ethical committee of the Leiden University Medical Center. In accordance with Leiden University Medical Center policy, all anatomical scans were reviewed and cleared by the radiology department following each scan. No anomalous findings were reported.

BEHAVIORAL ASSESSMENT

Parents filled out the Child Behavior Check List (CBCL, Achenbach, 1991) for participants younger than 18 years, in order to screen for psychiatric conditions. All participants scored below clinical levels on all subscales of the CBCL, and had scores within 1 SD of the mean of a normative standardized sample.

Participants completed two subscales (similarities and block design) of either the Wechsler Adult Intelligence Scale (WAIS) or the Wechsler Intelligence Scale for Children (WISC) in order to obtain an estimate of their intelligence quotient (Wechsler, 1991, 1997). There were no significant differences in estimated IQ scores between the different age groups, $F(2, 66) = 1.63$, $p = 0.20$ (see **Table 1**).

TASK PROCEDURE

Probabilistic learning task

The procedure for the probabilistic learning task (Frank et al., 2004) was as follows: The task consisted of two stimulus pairs (called AB and CD). The stimulus pairs consisted of pictures of everyday objects (e.g., a chair and a clock). Each trial started with the display of one of the two stimulus pairs and subsequently the participant had to choose one of the two stimuli (e.g., A or B), which were presented on the left or the right side of the screen. The stimulus pairs were presented in random order. Participants were instructed to choose either the left or the right stimulus by pressing a button with the index or middle finger of the right hand within a 2500 ms window, which was followed by a 1000 ms feedback display. The feedback display consisted of a green V-signal for positive feedback and a red cross for negative feedback. If no response was given within 2500 ms, the text “too slow” was presented on the screen. This occurred on less than 2% of the trials.

The feedback displayed was probabilistic. Choosing stimulus A led to positive feedback on 80% of AB trials, whereas choosing stimulus B led to positive feedback on 20% of these trials. The CD pair procedure was similar, but probability for positive feedback was lower; choosing stimulus C led to positive feedback on 70% of CD trials, whereas choosing stimulus D led to positive feedback on 30% in these trials. Thus, the correct choice in order to obtain most positive feedback was A or C, whereas the incorrect choice was B or D.

Table 1 | Group measures.

	IQ	Reaction times (ms)	Points	Head motion avg (mm)	Max (mm)
Adults	107(2.4)	811(44)	118(3)	0.08(0.01)	1.56
Adolescents	108(2.0)	773(39)	114(3)	0.08(0.01)	2.96
Children	111(2.6)	804(42)	107(6)	0.09(0.01)	2.85

Displays means per age group, standard errors between brackets. Final column represents the maximum head motion between two time points in each age group.

Participants were instructed to earn as many points as possible (as indicated by receiving a positive feedback signal), but were also informed that it would not be possible to receive positive feedback on every trial. Further, participants were informed that although stimuli sometimes appeared on the right side and sometimes on the left side, that laterality was an irrelevant dimension. After the instructions and right before the scanning session, the participants played 40 practice rounds on a computer in a quiet laboratory to ensure proficiency on the task.

In total, the task in the scanner consisted of two blocks of 100 trials each: 50 AB trials and 50 CD trials per block. To ensure that participants had to learn a new mapping in both task blocks, the first and the second block consisted of different sets of pictures. The duration of each block was approximately 8.5 min. The stimuli were presented in pseudo-random order with a jittered interstimulus interval (min = 1000 ms, max = 6000 ms) optimized with OptSeq2 (surfer.nmr.mgh.harvard.edu/optseq/, Dale, 1999). During inter trial intervals, a central fixation cross was shown.

DATA ACQUISITION

Participants were familiarized with the scanner environment on the day of the fMRI session through the use of a mock scanner, which simulated the sounds and environment of a real MRI scanner. Data were acquired using a 3.0T Philips Achieva scanner at the Leiden University Medical Center. Stimuli were projected onto a screen located at the head of the scanner bore and viewed by participants by means of a mirror mounted to the head coil assembly. First, a localizer scan was obtained for each participant. Subsequently, T2*-weighted Echo-Planar Images (EPI) (TR = 2.2 s, TE = 30 ms, 80 × 80 matrix, FOV = 220, 35 2.75 mm transverse slices with 0.28 mm gap) were obtained during two functional runs of 232 volumes each. The first two scans were discarded to allow for equilibration of T1 saturation effects. A high-resolution T1-weighted anatomical scan and a high-resolution T2-weighted matched-bandwidth high-resolution anatomical scan, with the same slice prescription as the EPIs, were obtained from each participant after the functional runs. Stimulus presentation and the timing of all stimuli and response events were acquired using E-Prime software. Head motion was restricted by using pillow and foam inserts that surrounded the head.

fMRI DATA ANALYSIS

Data were preprocessed using SPM5 (Wellcome Department of Cognitive Neurology, London). The functional time series were realigned to compensate for small head movements. Translational movement parameters never exceeded 1 voxel (<3 mm) in any direction for any subject or scan. There were no significant differences in movement parameters between age groups $F(2, 65) = 0.152$, $p = 0.85$, (see **Table 1**). Functional volumes were spatially smoothed using a 6 mm full-width half-maximum Gaussian kernel. Functional volumes were spatially normalized to EPI templates. The normalization algorithm used a 12 parameter affine transformation together with a nonlinear transformation involving cosine basis functions and resampled the volumes to 3 mm cubic voxels. The MNI305 template was used for visualization and all results are reported in the MNI305 stereotaxic space (Cosoco et al., 1997), an approximation of Talairach space (Talairach and Tourneaux, 1988).

Statistical analyses were performed on individual participants' data using the general linear model in SPM5. The fMRI time series data were modeled by a series of events convolved with a canonical haemodynamic response function (HRF). The presentation of the feedback screen was modeled as 0-duration events. The stimuli and responses were not modeled separately as these occurred in one prior or overlapping EPI images as feedback presentation.

In the model, feedback was further subdivided into correct vs. alternative rule and positive vs. negative feedback. These trial functions were used as covariates in a general linear model, along with a basic set of cosine functions that high-pass filtered the data, and a covariate for run effects. The least-squares parameter estimates of height of the best-fitting canonical HRF for each condition were used in pair-wise contrasts. The resulting contrast images, computed on a participant-by-participant basis, were submitted to group analyses. At the group level, contrasts between conditions were computed by performing one-tailed *t*-tests on these images, treating participants as a random effect. We further performed voxelwise ANOVAs to identify regions that showed age-related differences in relation to feedback processing. We tested for linear increases (−1 0 1) and decreases (1 0 −1) in the contrasts specified below.

We applied AlphaSim (Ward, 2000) to calculate the appropriate threshold significance level and cluster size for the whole-brain analyses. A significance threshold of $p < 0.05$, corrected for multiple comparisons was calculated by performing 10.000 Monte Carlo simulations in AlphaSim resulting in an uncorrected threshold of $p < 0.001$, requiring a minimum of 24 voxels in a cluster. This threshold was used for all whole-brain analyses.

REGION OF INTEREST ANALYSES

We used the Marsbar toolbox for use with SPM5 (http://marsbar.sourceforge.net, Brett et al., 2002) to perform Region of Interest (ROI) analyses to further characterize patterns of activation. We created ROIs of the regions that were identified in the functional mask of whole-brain analyses. The masks used to generate functional ROIs was based on the general (positive vs. negative feedback) contrasts ($p < 0.001$, > 24 voxels) across all participants, which was unbiased for effects of probability rule or age. Because this statistical image spanned several distinct functional brain regions in the striatum, we used Marsbar anatomical masks for the caudate nucleus to further specify our ROIs.

For all ROI analyses, effects were considered significant at an α of 0.0125, based on Bonferroni correction for multiple comparisons, $p = 0.05/4$ ROIs (caudate, DLPFC, parietal cortex and dACC), unless reported otherwise.

RESULTS

BEHAVIOR

Performance

To investigate the age differences in learning performance for the different stimulus pairs we calculated the percentage of correct choices (choosing the high probability stimulus) per block of 20 trials for each participant, resulting in five blocks in total. Because the two runs in the scanner consisted of new stimulus pairs, the two runs were collapsed.

As expected, the age (8–11 years, 13–16 years, 18–22 years) × probability (AB, CD) × task block (5) ANOVA showed that participants learned to make more correct choices over time, as indicated by a main effect of task block, $F(4, 260) = 40.44$, $p < 0.001$, $\eta_p^2 = 0.038$ (See **Figure 1B**). There was a significant difference in accuracy between the two probabilities; participants were more accurate on the AB (80%–20%) trials than the CD (70%–30%) trials, $F(1,65) = 11.58$, $p < 0.001$, $\eta_p^2 = 0.151$ Contrary to predictions, there were no age differences in learning (age × task block interaction, $F(8,260) = 1.38$, $p = 0.11$, $\eta_p^2 = 0.203\eta_p^2 = 0.041$), no age differences in accuracy on the two pairs (age × probability interaction, $F(2, 65) = 0.941$, $p = 0.393$, $\eta_p^2 = 0.028$), and no age × probability × task block interaction ($p > 0.10$). A similar ANOVA for reaction times revealed no differences for age, probability, or task block (all p's > 0.10) (see **Table 1**).

The task block factor allowed us to obtain the point in learning where participants reached a plateau. By selecting the task phase in which there were no longer differences in learning, we could examine how feedback was processed in the context of applying the correct (choosing the stimuli with a high probability of positive feedback) or alternative rule (choosing the stimuli with a low probability of positive feedback). Follow up comparisons showed that the last 60 trials were appropriate for this purpose, as performance stabilized and participants showed probability matching behavior (Shanks et al., 2002). That is, both the AB and the CD pairs showed no effects of block (learning) on accuracy in the last three blocks, $F(2, 130) = 3.47$, $p = 0.08$ and $F(2, 130) = 1.81$, $p = 0.52$, respectively. When we reanalyzed these last 60 trials, we still found a significant effect of stimulus pair, $F(1, 65) = 16.51$, $p < 0.001$, $\eta_p^2 = 0.203$, and again no significant interactions with age (all p's > 0.3).

To summarize, the behavioral results showed that all participants learned to perform more accurately over time and they learned faster on the easier AB trials than the more difficult CD trials. Performance stabilized in the last 60 trials, at which point participants showed probability matching behavior (Shanks et al., 2002).

The fMRI analyses focused on the last 60 trials. In order to have enough trial numbers in each condition, we collapsed across probabilities in the analyses below. Thus, we differentiated between over-learned high probabilities (A and C collapsed) and alternative low probabilities (B and D trials collapsed). These will be referred to as the correct and alternative rules. Each of these rules could result in positive and negative feedback.

fMRI RESULTS POSITIVE VERSUS NEGATIVE FEEDBACK

Whole-brain comparisons across age groups

First, we identified the neural correlates of feedback processing by comparing the (positive feedback vs. negative feedback) contrast across all participants. This analysis revealed increased BOLD responses for positive feedback > negative feedback in several regions including the left and right caudate, left DLPFC and left parietal cortex (see **Figure 2A**). The opposite contrast (negative > positive feedback) resulted in increased activation in the dACC. The coordinates for these comparisons (positive feedback vs. negative feedback) are reported in **Table 2**.

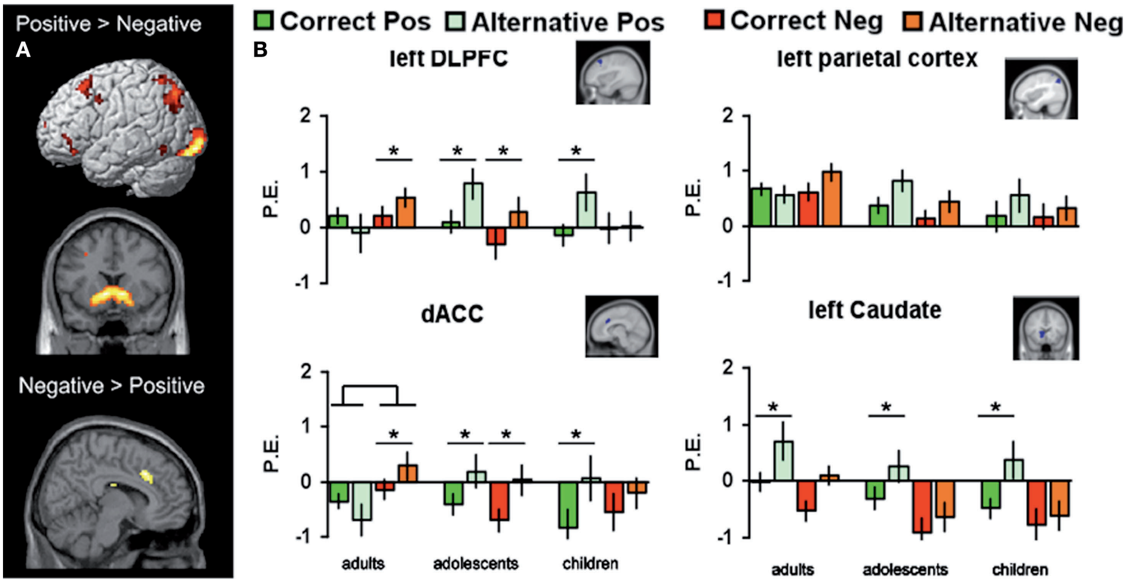

FIGURE 2 | (A) Regions from the (positive vs. negative feedback) contrasts across all participants **(B)** Parameter estimates and standard errors for positive and negative feedback that followed either the correct or the alternative rule displayed for each age group in left DLPFC, left parietal cortex, dACC and left caudate. Significant differences between brain activity in two conditions are indicated with an asterisk (*Bonferroni corrected).

Table 2 | Brain Regions revealed by whole-brain contrasts.

Anatomical region	L/R	voxel volume	Z	MNI coordinates		
				x	y	z
POSITIVE > NEGATIVE						
Striatum (ventral and dorsal)	L/R	774	7.49	–6	12	–3
Dorsolateral prefrontal cortex	L	71	4.61	–27	24	51
Superior parietal cortex	L	170	4.23	–30	–75	48
Precuneus	L/R	137	4.07	–3	–36	33
Ventral medial PFC	L/R	26	4.03	3	54	–12
Visual cortex	L/R	332	4.50	27	–93	–9
NEGATIVE > POSITIVE						
Dorsal anterior cingulate cortex	L/R	63	4.43	9	21	36

MNI coordinators for main effects, peak voxels reported at p < 0.001, at least 24 contiguous voxels (SVC).

fMRI REGION OF INTEREST RESULTS FOR FEEDBACK × RULE × AGE GROUP INTERACTIONS

Next, we tested for age differences and rule sensitivity in these regions by performing region of interest (ROI) analyses. The ROI analyses were restricted to the four *a priori* defined regions which emerged in the (positive vs. negative) contrast across participants: bilateral caudate, left DLPFC, left parietal cortex and dACC. In order to investigate whether there were age differences in how the statistical regularities learned by the participants had an effect on how feedback was processed we performed 3 × 2 × 2 ANOVAs testing for the interaction between valence (positive vs. negative) and rule (correct vs. alternative) as within-subjects factors and age (children, adolescents, adults) as the between-subjects factor for each ROI (see **Figure 2B**).

Left DLPFC

The (age group × valence × rule) ANOVA for left DLPFC resulted in an interaction between valence and rule, $F(2, 64) = 6.32, p < 0.01$, showing that left DLPFC was more active for both negative and positive feedback after choosing the alternative rule compared to the correct rule, but this difference was larger for positive than negative feedback. In addition, there was an interaction between rule (AC vs BD) and age group, $F(2, 64) = 3.87, p = 0.02$, and a three-way interaction between rule, valence, and age group, $F(2, 64) = 6.77, p < 0.01$.

As can be seen in **Figure 2B**, children and adolescents showed more activity for positive feedback after choosing the alternative rule compared to the correct rule ($t(17) = 2.64$, $p < 0.01$ and $t(26) = 3.18$, $p < 0.004$, respectively), whereas this difference was not present in adults. In addition, adults and adolescents showed more activity for negative feedback after choosing the alternative rule compared to the correct rule, ($t(21) = –2.49$, $p = 0.02$ and $t(23) = –2.81$, $p < 0.01$ respectively), but this difference was not present in children.

Left parietal cortex

The (age group × valence × rule) ANOVA for the left parietal cortex revealed a similar three-way interaction which approached significance, $F(2, 64) = 3.16$, $p = 0.05$ (see **Figure 2B**). Although the pattern of activation for the different conditions in the left parietal cortex appears similar to the pattern for left DLPFC, it did not survive Bonferroni correction and none of the *post hoc* comparisons resulted in significant effects.

dACC

The (age group × valence × rule) ANOVA for the dACC resulted in a rule × valence interaction, $F(2, 64) = 14.14$, $p < 0.001$, an age × valence interaction, $F(2, 64) = 4.11$, $p < 0.01$, and an age × rule interaction, $F(2, 64) = 4.81$, $p = 0.03$, but the three-way interaction failed to reach significance $F(2, 64) = 0.28$, $p = 0.75$.

As can be seen in **Figure 2B**, adults showed more activation in dACC after negative feedback than after positive feedback, $F(1, 21) = 8.25$, $p < 0.01$, but this was not found for the younger age groups. Children and adolescence, in contrast, showed more dACC activation after positive feedback for the alternative rule relative to the correct rule ($t(17) = 2.51$, $p < 0.01$ and $t(26) = 3.44$, $p < 0.01$ respectively). In addition, adults and adolescents showed more activity for negative feedback after choosing the alternative rule compared to the correct rule, ($t(21) = -2.89$, $p < 0.01$ and $t(26) = -3.32$, $p < 0.003$ respectively), but this difference was not present in children.

Left and right caudate

Finally, we performed an (age group × valence × rule) ANOVA for the left caudate nucleus. This analyses did not reveal any age effects, but a main effect for feedback, $F(1, 64) = 33.17$, $p < 0.001$, and a feedback × rule interaction $F(2, 64) = 17.21$, $p < 0.01$. All age groups showed more activity for the alternative (low probability) compared to the correct rule (high probability) positive feedback (all p's < 0.001), but there were no additional main or interaction effects (**Figure 2B**). Similar analyses for right caudate yielded the same results; a main effect of feedback, $F(1, 64) = 28.16$, $p < 0.005$, and a feedback × rule interaction $F(2, 64) = 19.33$, $p < 0.01$.

WIN STAY – LOSE SHIFT STRATEGIES: BEHAVIOR AND BRAIN ANALYSES

Finally, to further investigate differences in feedback processing we explored developmental changes in decision-making strategies on the behavioral and neural level. In order to investigate the strategy used on the task we examined how often participants chose either the same stimulus after positive feedback (win-stay) or the other stimulus after negative feedback (lose-shift). For this set of analyses we further broke down the trials based on the subsequent choice when presented with the same stimulus pair; win-stay, win-shift, lose-stay and lose-shift. The factor 'win-stay' was computed by calculating the proportion of choice repetitions following positive feedback as a function of the total number of positive feedback events. Likewise, the factor 'lose-shift' was computed by calculating the proportion of choice shifts following negative feedback as a function of the total number of negative feedback events. Because previous analyses revealed that positive and negative feedback were processed differently dependent on rule type we analyzed the sequential effects for the correct and alternative rule separately.

Behavior

For correct rules, the univariate ANOVAs with age group as the between-subjects factor revealed a significant age difference in lose-shift strategies, $F(2, 64) = 4.04$, $p < 0.02$ as well as in win-stay strategies, $F(2, 64) = 4.51$, $p < 0.02$ (see **Figure 3A**). These results illustrate that adults showed more optimizing behavior than adolescents and children; they stayed more often with the correct rule after positive feedback and shifted less often after negative feedback.

For the alternative rules, the univariate ANOVAs revealed no age differences for win-stay strategies, $F(2, 64) = 0.85$, $p = 0.43$, but there was a significant age difference in lose-shift strategies, $F(2, 64) = 3.91$, $p < 0.03$. In the latter case, children showed less optimal behavior compared to the adolescents and adults; surprisingly, they stayed more often with the alternative (incorrect) rule after negative feedback.

ROI analyses

In order to explore the relation between brain activity and behavior on the subsequent trial, we compared brain activity after positive and negative feedback that resulted in staying or shifting for the two rule types separately. We explored the same ROIs as reported above. These analyses revealed significant shift and age effects only in the dACC and left DLPFC, but not in the caudate or the parietal cortex. In general, the ANOVAs showed that in adults, dACC and DLPFC were more active when participants shifted on the next trial. There were some differences in significance levels, but overall this effect seemed generally independent of feedback valence or rule. The analyses are described in more detail below.

The dACC showed the strongest relation between brain activity and subsequent behavioral change. When applying the correct rule, the shift × age group ANOVA for positive feedback revealed a main effect of shifting, $F(1, 65) = 6.27$, $p < 0.01$ but no interaction with age, $F(2, 64) = 2.29$, $p = 0.11$ (see **Figure 3B**). There was more dACC activity when shifting after positive feedback. The same ANOVA for negative feedback revealed an age × shift interaction, $F(2, 64) = 3.62$, $p = 0.03$. *Post hoc* comparisons revealed that there was more dACC activity when shifting compared to staying after negative feedback for adults ($t(21) = -2.76$, $p < 0.01$) but not for the adolescents and children (both p's > 0.1).

When applying the alternative rule, the shift × age group ANOVA for positive feedback revealed no significant effects of age or shifting. However, the same ANOVA for negative feedback revealed an age × shift interaction ($F(2, 63) = 5.31$, $p < 0.01$). *Post hoc* comparisons revealed that there was more dACC activity when shifting after negative feedback for adults ($t(21) = -3.01$, $p < 0.01$) but not for adolescents and children (both p's > 0.2).

Finally, the pattern of activation in the left DLPFC appeared similar to that of the dACC (**Figure S2** in Supplementary Material). The shift × age ANOVAs for the correct rule resulted in significant shift × age interactions for both positive and negative feedback ($F(2, 63) = 4.46$, $p = 0.03$ and $F(2, 64) = 4.91$, $p = 0.02$, respectively). *Post hoc* test revealed that there was more left DLPFC activity when shifting on the next trial after positive and negative feedback, but this was only significant for the adults ($t(21) = -2.54$, $p < 0.01$ and $t(21) = -2.32$, $p = 0.03$, respectively). There were no significant effects for the alternative rule (all p's > 0.2).

DISCUSSION

The goal of this study was to examine the neural developmental changes when processing positive and negative feedback signals in a probabilistic decision-making task. As predicted, all participants learned to choose the correct rules (high probability stimuli A and C) more often than the alternative rules (low probability

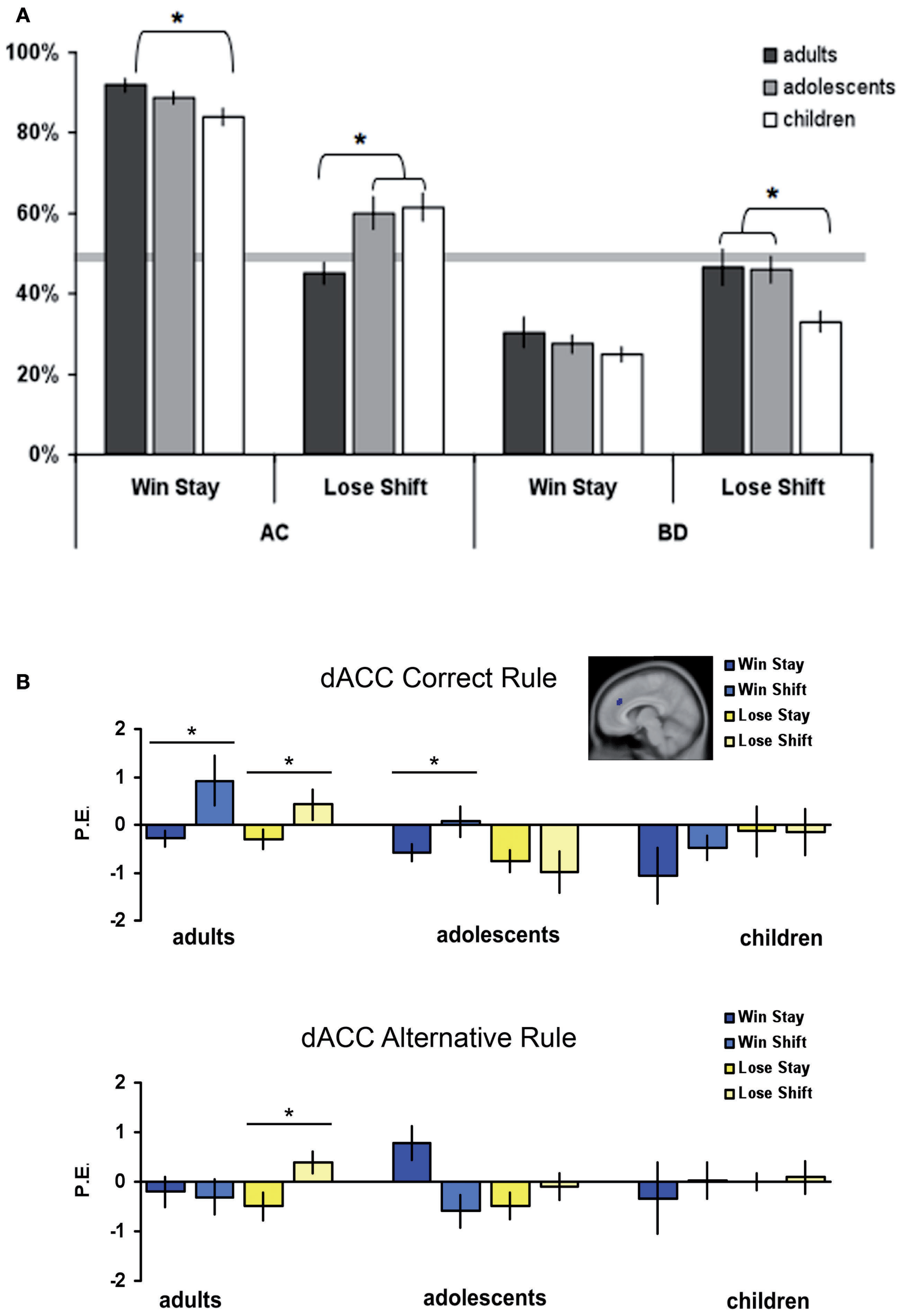

FIGURE 3 | (A) Percentages of win-stay and lose-shift choices per age group and rule type, error bars represent standard error. **(B)** Parameter estimates and standard errors for positive and negative feedback that followed by either staying or shifting, displayed for each age group and rule type separately. Significant differences between brain activity in two conditions are indicated with an asterisk (*Bonferroni corrected).

stimuli B and D) (Frank et al., 2004; Klein et al., 2007). After approximately 40 trials, participants adapted a performance pattern consistent with 'probability matching behavior', and this behavioral phase was the focus of our further analyses.

Behavioral analyses showed two important patterns: (1) probability matching behavior occurred in all age groups, but there were no age differences in overall learning rate, and (2) task adaptive win-stay, lose-shift strategies were observed, but age differences in adaptive behavior indicated more task-adaptive optimizing behavior in adults. These task and age differences in decision-making strategy were paralleled by changes in functional brain activity; (1) neural responses in DLPFC, dACC, and caudate were sensitive to rule × feedback interactions and an age-related difference was observed in DLPFC and dACC, and (2) activity in DLPFC and dACC predicted behavioral change on subsequent trials more strongly in adults than in adolescents and children. These behavioral data and their neural correlates provide important new insights in feedback processing in general and across development. The discussion will be organized according to these themes.

FEEDBACK PROCESSING IN ADULTS

Our analysis of positive and negative feedback processing in a probabilistic environment demonstrated that feedback-related activity in the DLPFC, dACC and caudate was dependent on valence and information value. We started out with a general whole-brain comparison for positive versus negative feedback and used ROI analyses to explore the areas identified in this contrast. This analysis revealed that especially left DLPFC, dACC and bilateral caudate were sensitive to feedback × rule context interactions. Before interpreting age differences in these activation patterns, we start out with the interpretation of feedback sensitivity observed in adults, which will set the stage for interpreting the developmental effects.

When exploring the data for adults separately, the results showed increased recruitment of DLPFC after receiving negative feedback following the alternative compared to the correct rule. Given that negative feedback after choosing the alternative, but not the correct, rule indicates the need for a switch in behavior, the adult findings are consistent with previous studies demonstrating negative feedback-related sensitivity in DLPFC for feedback that is important for subsequent behavioral adjustment (Kerns, 2006; van Duijvenvoorde et al., 2008; Zanolie et al., 2008) and not for negative feedback *per se*.

Besides DLPFC, the parietal cortex has previously been implicated in feedback processing (Crone et al., 2008, van Duijvenvoorde et al., 2008) and implementing cognitive control as part of the fronto-parietal network (Brass et al., 2005; Bunge et al., 2002; Dosenbach et al., 2008). In support of this hypothesis our whole-brain analyses revealed that the left superior parietal cortex was involved in feedback processing. However, in contrast with previous studies (van Duijvenvoorde et al., 2008), our subsequent *post hoc* analyses could not confirm a strong contribution of the superior parietal cortex. Possibly, the parietal cortex was more engaged in prior studies because these involved trial-to-trial learning, whereas in the current study we investigated feedback processing when rules were already learned. Future research is necessary to elucidate the role of the superior parietal cortex in feedback processing in relation to learning.

The analyses of dACC revealed a very similar activation pattern as DLPFC, however the dACC activation pattern in adults was more supportive of a general increase in activity after negative feedback regardless of rule type. Possibly, this finding indicates that, at least in adults, the dACC has a more general role in processing negative feedback; both in terms of detecting general conflict (Brown and Braver, 2005) and signaling the need for behavior change (Holroyd and Coles, 2008; Rushworth, 2008).

Finally, the caudate nucleus also showed sensitivity to feedback and rule type, but this region was more active after positive compared to negative feedback when participants chose the alternative rule. Given that this effect was specific for positive feedback, and that the probability for positive feedback for the alternative rule was low, the signal in the caudate could reflect a positive prediction error; i.e., signaling that the outcome is better than predicted (for review see Schultz, 2007).

Together, analysis of the adult activation pattern confirms prior findings showing that DLPFC and dACC are sensitive to negative feedback and the caudate is sensitive to positive feedback, but the findings further elucidate that these neural responses are dependent on the extent to which these feedback signals provide a learning signal of future performance. That is, DLPFC and caudate responses were more pronounced after selecting the incorrect rule which had a low probability of resulting in positive feedback, but which may have been important to explore. In contrast, when applying over-learned high probability rules, DLPFC and caudate were less involved, possibly because the informative value was smaller.

FEEDBACK PROCESSING: DEVELOPMENTAL COMPARISONS

The neural activation patterns described above were differentially sensitive to age modulations. The first notable finding is that of differential activation patterns in the DLPFC. All participants, regardless of age, showed increased recruitment of DLPFC when choosing the alternative rule compared to the correct rule. However, children, but not adults, showed more activation in DLPFC after positive feedback when choosing the alternative rule. In contrast, adults, but not children, showed more activation in DLPFC after negative feedback when choosing the alternative rule. Adolescents seemed to be in a transition phase, because their neural response to positive feedback was similar to that observed in children, but their neural response to negative feedback was similar to that observed in adults. Thus, consistent with prior studies, these developmental differences indicate a shift from focus on positive to a focus on negative feedback with age (Somsen, 2007; Crone et al., 2008; van Duijvenvoorde et al., 2008), which appears to continue across adolescence. In addition, the current results extend previous findings by showing that developmental differences in neural responses to feedback are not related to valence *per se*, but suggest an age-related change in processing learning signals with different informative value.

In contrast, for all age groups the caudate nucleus was more active for positive compared to negative feedback, in particular when participants chose the alternative rule. This finding indicates that part of the feedback processing network, which is implicated in processing statistical regularities of reward (Schultz, 2007) matures already at an early age, whereas the part of the network that is involved in processing negative feedback and the subsequent control of behavior has a more protracted developmental time course. These findings are consistent with prior reports using cognitive tasks, as these studies have also reported early maturation of subcortical regions and protracted development of cortical brain areas (Casey et al., 2004; van Duijvenvoorde et al., 2008; Velanova et al., 2008). It should be noted that other developmental studies have reported increased sensitivity of the striatum in early adolescence, however, these studies have employed paradigms with a more affective content, such as gambling tasks with real monetary rewards or emotion recognition (Ernst et al., 2005; Galvan et al., 2006; McClure-Tone et al., 2008; van Leijenhorst et al., 2009). In future studies, it will be of interest to examine whether the caudate activation can be modulated by the use of affective task modulations when learning rules or processing performance feedback.

ADAPTIVE BEHAVIOR AND BRAIN ACTIVATION ACROSS DEVELOPMENT

One of the challenging questions for future studies is how the neural activation is associated with trial-to-trial learning. For example, we did not observe age differences in general learning performance, despite differences in neural activation. This was unexpected, and again demonstrates that differences in neural activation can be present without differences in observable behavior (Ladouceur et al., 2004). However, consistent with prior studies, the sequential analyses revealed that with age, participants became better at using the negative feedback signals to adjust their behavior on subsequent trials (Crone and van der Molen, 2004). As expected, when receiving positive feedback after having applied the correct rule, participants were more likely to stay and select the same stimulus on the subsequent trial. Likewise, when receiving negative feedback after having applied the incorrect alternative rule, participants were more likely to shift and select the correct stimulus on the subsequent trial. Overall, adults appeared better at optimizing than adolescents, and adolescents performed better than children. Based on these findings, in combination with the developmental differences in neural activation, the data are supportive of a linear increase across adolescence. Although these findings differ from earlier reports which have showed larger differences in early adolescence than in later adolescence (e.g. Ladouceur et al. 2004) the findings are consistent with prior fMRI results showing late changes in brain activation and behavior (e.g. Scherf et al., 2006; van Duijvenvoorde et al., 2008).

Intriguingly, even though children were more likely than adults to shift after receiving negative feedback when applying the correct rule, they were also more likely to stay after receiving negative feedback when applying the incorrect alternative rule. The reason for this behavioral pattern is still unclear, but it is possible that children waited with shifting when applying the incorrect alternative rule until they received positive feedback (20%). Future research should use task manipulations that allow for further investigation of this hypothesis.

We performed exploratory analyses to investigate the relation between brain activity and win-stay, lose-shift behavior, although it should be noted that these analyses are preliminary as our study design was not optimized to test for these differences. The analyses on the ROIs identified in the main analyses revealed that, consistent with prior research, dACC and left DLPFC activity predicted behavioral adjustment on the subsequent trial in adults (Kerns et al., 2004; Jocham et al., 2009). However, this pattern was observed for both rule types and appeared independent of feedback valence. Possibly, the dACC and left DLPFC were important for trial-by-trial adjustment (Kerns et al., 2004). We found a similar pattern in adolescents, but only when applying the correct rule. We failed to find similar relations in children, which may indicate that the neural mechanisms that facilitate future behavioral adjustment are still immature or that they employed different strategies to perform the task. These interpretations are consistent with an ERP study showing increased error related negativity across adolescence (Ladouceur et al., 2007). Furthermore, the same study showed that only in adults the ERN amplitude was related to task performance.

The current study is limited by the relatively small number of trials for some of the contrasts examining the neural correlates of shifting behavior. Future studies should make use of tasks that are optimized for studying these developmental differences in more detail.

In addition, a challenging direction for future research will be to investigate the developmental differences in the learning phase. The combined use of computational reinforcement learning models (Klein et al., 2007) with imaging techniques could be a promising endeavor to parse out the developmental changes in different phases of learning (e.g. learning rate) and their neural correlates. These methods could be combined with trial-to-trial data categorization to understand how the observed developmental change in sensitivity from positive to negative feedback hinders or facilitates learning locally versus oriented towards future goals.

CONCLUSION

Taken together, the current findings confirm that DLPFC, dACC and caudate are important for probabilistic feedback processing, and show that they have dissociable roles as reflected in differential sensitivity to feedback valence and rule types. The DLPFC and dACC were sensitive to information value in response to negative feedback, but the caudate was sensitive to information value in response to positive feedback. These findings are consistent with previously suggested computational models of feedback learning (Cohen, 2008; Frank and Kong, 2008).

The results of this study replicate the previously reported developmental shift in sensitivity from positive to negative feedback as reflected in neural activation in the DLPFC, with a transition phase in adolescence. Using probabilistic feedback stimuli, we could dissociate between two competing hypotheses with respect to this developmental change. The results confirm the hypothesis that this shift is associated with different attention focus on learning signals and disconfirm the hypothesis that this shift reflects a simple valence effect. Further understanding of the age related changes in strategy differences, and how to influence decision-making strategies by guiding attention regulation, promise to be useful sources to improve learning behavior of children and adolescents.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/humanneuroscience/paper/10.3389/neuro.09/052.2009/

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Decoding developmental differences and individual variability in response inhibition through predictive analyses across individuals

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Response inhibition is thought to improve throughout childhood and into adulthood. Despite the relationship between age and the ability to stop ongoing behavior, questions remain regarding whether these age-related changes reflect improvements in response inhibition or in other factors that contribute to response performance variability. Functional neuroimaging data shows age-related changes in neural activity during response inhibition. While traditional methods of exploring neuroimaging data are limited to determining correlational relationships, newer methods can determine predictability and can begin to answer these questions. Therefore, the goal of the current study was to determine which aspects of neural function predict individual differences in age, inhibitory function, response speed, and response time variability. We administered a stop-signal task requiring rapid inhibition of ongoing motor responses to healthy participants aged 9–30. We conducted a standard analysis using GLM and a predictive analysis using high-dimensional regression methods. During successful response inhibition we found regions typically involved in motor control, such as the ACC and striatum, that were correlated with either age, response inhibition (as indexed by stop-signal reaction time; SSRT), response speed, or response time variability. However, when examining which variables neural data could predict, we found that age and SSRT, but not speed or variability of response execution, were predicted by neural activity during successful response inhibition. This predictive relationship provides novel evidence that developmental differences and individual differences in response inhibition are related specifically to inhibitory processes. More generally, this study demonstrates a new approach to identifying the neurocognitive bases of individual differences.

Keywords: development, predictive analysis, fMRI, response inhibition, stop-signal

INTRODUCTION

The ability to rapidly cancel planned or ongoing behaviors, referred to as response inhibition, is crucial to adaptive behavior and is known to improve throughout childhood and into adulthood (Casey et al., 1997; Williams et al., 1999; Durston et al., 2002). A network of cortical and basal ganglia regions that are not fully developed until adulthood (Giedd et al., 1999; Sowell et al., 1999) has been identified as being critical for this form of executive control, including the right inferior frontal gyrus (IFG), pre-supplementary motor area (preSMA), and subthalamic nucleus (STN). The necessity of these regions for response inhibition has been established by lesion (Aron et al., 2003; Floden and Stuss, 2006; Picton et al., 2007) and transcranial magnetic stimulation (Chambers et al., 2007, 2006; Chen et al., 2009) studies, which demonstrate that disruption of right IFG and preSMA functioning specifically impairs response inhibition, as compared to response execution, while disrupting other prefrontal areas has no effect. Additionally, deep brain stimulation of the STN in Parkinson's disease has been shown to enhance response inhibition (van den Wildenberg et al., 2006). Moreover, there is evidence for direct white matter connectivity between the STN, right IFG, and preSMA (Aron et al., 2007), which further supports the existence of this network. Lastly, both age and behavior have been found to correlate with brain activity in these regions, indicating that individual differences in the engagement of this network may relate to variability in behavior. For example, adults have greater activity than children during inhibition in the right IFG (Bunge et al., 2002; Durston et al., 2002; Rubia et al., 2007). It has also been found that activity in the right IFG and STN rises with increasingly successful response inhibition in adults (Aron and Poldrack, 2006).

The stop-signal paradigm (Logan, 1994) is commonly used to measure the rapid engagement of response inhibition. In this task, participants are presented with a simple choice response task (e.g., pressing a left or right button in response to an arrow pointing left or right), but a small proportion of trials are presented with a "stop-signal" (often an auditory tone), which signals to participants to withhold their response. A unique feature of this task is the ability to estimate a continuous measure of response inhibition, known as the "stop-signal reaction time" (SSRT), using a model known as the independent race model (Logan and Cowan, 1984;

Band et al., 2003). As a measure of response inhibition ability, SSRT is both highly reliable and has substantial external validity, exhibiting relations with self-reported impulsivity in the population (Logan et al., 1997). In addition, SSRT is significantly lengthened in a number of impulse control disorders, including attention deficit/hyperactivity disorder (ADHD; Logan et al., 2000; Lijffijt et al., 2005), substance use (Fillmore and Rush, 2002; Monterosso et al., 2005), and obsessive-compulsive disorder (OCD; Chamberlain et al., 2006).

Despite the strong observed relations between age, SSRT, and neural activity during successful response inhibition, there remain questions regarding the degree to which these relationships truly reflect underlying variability in inhibitory control functions. In particular, it has been noted that although individuals with ADHD show impairments in SSRT, they show even greater impairments in the variability of response times on "Go" trials (Lijffijt et al., 2005). Because increased variability in response times can result in spurious increases in SSRT, it is possible that the previous findings of slower SSRT in children as compared to adults could instead reflect variability in behavior rather than reduced inhibitory control in children. Unfortunately, with analyses being limited to how individual difference variables are correlated with neural activity and each other, it has not been possible to determine if there is a predictive relationship between neural activity and these variables during response inhibition or if they are simply correlated.

Therefore, the goal of the present study was to determine which aspects of neural function enable prediction of individual differences in age, response inhibition, response execution, and response time variability. More precisely, we wished to clarify whether neural function during successful inhibitory control is specifically predictive of age and SSRT. A large body of work has shown that individual difference variables often exhibit substantial correlations with functional neuroimaging data (Miller et al., 2002). These findings are often couched in terms of "prediction". However, the correlation within a particular sample generally overestimates the ability to predict to new samples because the predictions will include a contribution from the noise in the data in addition to the true signal. This distinction between training versus generalization error is a fundamental concept in the field of statistical learning, which focuses on the development of methods that enable optimal predictions about new observations based on existing data (Hastie et al., 2001). These methods have been used extensively within the fMRI literature to decode mental states based on fMRI data (Haynes and Rees, 2006; O'Toole et al., 2007); they have largely been used to predict within participants (i.e., predict mental states on one set of scans based on another set of scans from the same individual), but recent work has shown that these methods can also predict across individuals (Poldrack et al., 2009). Further, previous work has focused on classification into discrete categories (e.g., different stimulus classes), rather than prediction of quantitative differences (i.e., regression). We applied high-dimensional regression methods to fMRI data from a healthy developmental sample of participants performing the stop-signal task in order to determine which aspects of the task can be used to decode quantitative differences in age and behavior. We hypothesized that if age and SSRT could be decoded from neural activity during successful inhibitory control then we would provide stronger evidence that individual and developmental differences in these variables are directly related to inhibitory function, as opposed to reflecting some aspect of response execution. We found that while neural activity during successful response inhibition was correlated with age, response inhibition performance, speed of response execution, and variability of response execution, only age and response inhibition performance were successfully decoded from activation during successful inhibition. This specific predictive relation provides novel evidence that developmental differences as well as individual differences in SSRT are specifically related to inhibitory processes.

MATERIALS AND METHODS

PARTICIPANTS

Sixty seven healthy typically-developing right-handed participants between the ages of 9 and 30 were recruited from the community as control participants for a study of childhood onset schizophrenia. They were recruited with online advertisements and by randomly calling families found through a commercially available list of households within a 25-mile radius of UCLA (Survey Sampling Inc., Fairfield, CT, USA). Potential participants (or their parents, for minors) were interviewed to determine whether they met inclusion/exclusion criteria for the study. Participants were eligible if they had no history of CNS disease, DSM-IV disorders, or learning disabilities and no treatment with anti-psychotic drugs or substance use in the past 2 years. Additionally, they could not have any metal in their bodies other than dental fillings, and could not be pregnant. Of these 67 potential participants, 61 were eligible to participate in the MRI study. Of these, 25 were excluded because of: technical issues (1; field of view did not cover the entire brain), excessive motion (3; more than one translational displacement of 3 mm or greater), or poor performance (21; poor performance defined a priori as a response rate on the Go task of less than 90%, more than 10% incorrect trials on the Go task, percent inhibition on the Stop task less than 25% or greater than 75%, or an SSRT of less than 80 ms). This proportion of participants excluded for behavior is comparable to that in other fMRI studies of the stop-signal task using a developmental population (Leibenluft et al., 2007). Of the remaining 36 participants included in this analysis, there were 27 youth aged 9–19 (mean age 13.7, 16 females) and 9 adults aged 25–30 (mean age 26.6, 4 females). All participants (and their parents if they were under 18) provided written informed consent or assent (for minors) according to the procedures of the UCLA Institutional Review Board.

EXPERIMENTAL DESIGN AND PROCEDURE

Participants performed four runs of the stop-signal task (Logan, 1994; **Figure 1A**). Two training runs were conducted before the functional scans, one approximately a week before the scanning session and one just before functional scanning. These two training runs were administered to ensure that participants understood the task; feedback was given after each run. The last two runs were during fMRI acquisition. The primary task was a simple two-choice reaction time task with spatially compatible stimulus-response mappings. On Go trials, participants pressed the right button with their right middle finger if an arrow pointed rightwards and the left

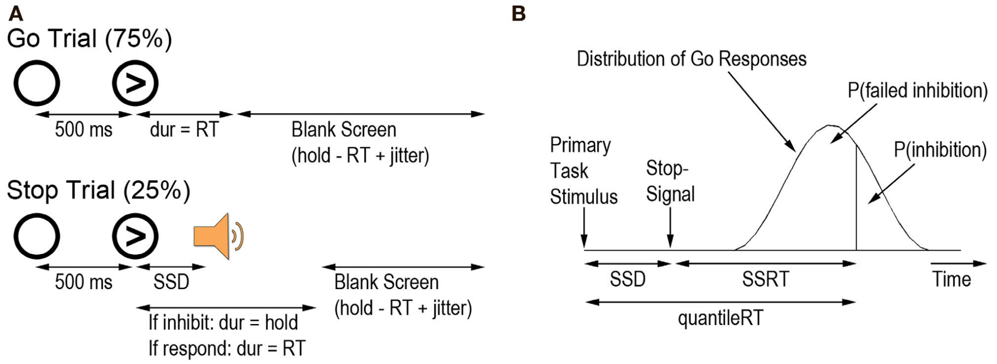

FIGURE 1 | (A) Schematic of go trials and stop trials. **(B)** The race model of stopping (Logan and Cowan, 1984). All correct RTs were arranged in ascending order in an assumption-free distribution to calculate the RT at each participant's proportion of failed inhibition (quantileRT). SSRT could then be calculated as quantileRT – SSD. Figure adapted from Aron et al. (2006).

button with their right index finger if the arrow pointed leftwards. On each Go trial, a warning signal (open circle) appeared on the computer monitor for 500 ms (visual angle subtended 4.9° × 4.9°). An arrow then appeared in the center of the circle until either the participant responded or 1000 ms elapsed. The duration of the blank screen between the trials was jittered between 500 and 4000 ms (mean 1000 ms, sampled from an exponential distribution) so as to optimize the contrast of successful Stop trials with successful Go trials. For the two behavioral runs, instead of a jittered inter-trial interval, each delay lasted 500 ms.

On 25% of the trials (Stop trials), a tone was sounded and participants tried to inhibit their already initiated response to the arrow. The tone was presented at varying delays (the stop-signal delay; SSD) after the onset of the primary stimulus. If participants responded on a Stop trial, the trial proceeded as if it were a Go trial. If participants inhibited their response, the arrow remained on the screen for 1000 ms, followed by the jittered interval between trials. An adaptive tracking procedure (1-up, 1-down staircase) was used to adjust the SSD to produce approximately 50% successful inhibition. Two independent staircase functions with a step size of 50 ms were used, with starting delays on the first run of the stop-signal task of 200 ms and 320 ms respectively. For runs 2–4, the average SSD of each staircase on the previous run was used as each staircase's starting value.

Each run had 128 trials: 96 Go trials and 32 Stop trials. For each eight trials, there were four left arrows and four right arrows. There were also two Stop trials (one for each ladder). Order of arrow direction, Stop trial ladder, and on which trials the stop-signal occurred were randomized.

The MATLAB (The MathWorks, Inc., Natick, MA, USA) Psychophysics Toolbox (Brainard, 1997) version 7.4 was used to present the stimuli to participants and to record their responses. Participants viewed the task through LCD goggles and responded using an MR-compatible button box.

FMRI DATA ACQUISITION

Imaging data were collected with a 3T head-only Siemens Allegra scanner at the UCLA Ahmanson-Lovelace Brain Mapping Center. For each functional run we collected 182 T2*-weighted echo-planar images (33 slices, slice thickness 4 mm, TR = 2000 ms, TE = 30 ms, flip angle = 90°, matrix 64 × 64, field of view 200 mm). A T2-weighted matched-bandwidth high-resolution anatomical scan with the same slice prescription as the functional images was also acquired. Lastly, a magnetization-prepared rapid-acquisition gradient echo (MPRage; 160 sagittal slices, slice thickness 1 mm, TR = 2000 ms, TE = 2.1 ms, matrix 192 × 192, field of view 256) was collected.

BEHAVIORAL DATA ANALYSIS

Go task response time (GoRT) and accuracy (GoAcc), standard deviation of GoRT (SDRT), percent successful inhibition, average SSD, and SSRT were calculated for each participant. Only correct Go trials were included in the GoRT analyses. SSRT was calculated according to the race model of stopping (Logan and Cowan, 1984). The race model assumes that the Go and Stop processes occur in parallel and are stochastically independent (although when using the tracking method this assumption is not critical; see Band et al., 2003). To calculate SSRT, first all correct RTs were arranged in an assumption-free distribution in ascending order. Then the proportion of failed inhibition (i.e., the proportion of Stop trials on which the participant responded) was determined. The RT corresponding to that proportion was computed: the quantileRT (i.e., if failed inhibition was 0.55, the RT corresponding to 55% of the area under the RT distribution curve was the quantileRT). SSRT was calculated as the difference between the quantileRT and the average SSD (**Figure 1B**). Correlations with behavior were conducted using robust linear regression with iteratively reweighted least squares to deweight outliers.

FMRI DATA ANALYSIS

Imaging data were processed and analyzed using FSL (FMRIB's Software Library[1]). For preprocessing we used FSL 3.3, including BET to extract the brain from the skull and MCFLIRT for motion correction. Following motion correction, the data were submitted to independent components analysis using MELODIC ICA, and the results from this analysis were used to identify and remove

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[1] www.fmrib.ox.ac.uk/fsl

potentially artifactual components in the data. After manually identifying artifactual components from one run for each of 11 randomly selected participants, we trained a classifier (Tohka et al., 2008) to automatically identify these components, and then used MELODIC to remove them from the data.

Statistical analysis was conducted in FSL 4.1 using FEAT 5.98. The statistical model included events for successful go responses, successful stop responses, and unsuccessful stop responses. Incorrect and missed Go trials were included in a nuisance regressor. All events began at stimulus onset and lasted the duration of the fixation plus the stimulus (1.5 s).

For the first-level analysis, images were spatially smoothed using a Gaussian kernel of FWHM 5 mm. Time-series statistical analysis was carried out using FILM (FMRIBs Improved Linear Model) with local autocorrelation correction after highpass temporal filtering (Gaussian-weighted least squares straight line fitting, with sigma = 33.0 s). Regressors of interest were created by convolving a delta function representing each event of interest with a canonical (double-gamma) hemodynamic response function (Woolrich et al., 2001). Parametric regressors were created by modulating the amplitude of a delta function using a demeaned version of the parameter of interest. In addition to regressors of interest, estimated motion parameters and their temporal derivatives (i.e., displacement) were included as nuisance regressors. Linear contrasts were performed for comparisons of interest.

A 3-step registration process was applied using FSL's FLIRT module for linear registration. EPI functional images were first registered to an inplane T2-weighted structural image (matched bandwidth; 7 DOF). The inplane structural image was registered to the high-resolution structural image (MPRage; 7 DOF), and the high-resolution image was registered to standard MNI152 space using FLIRT linear registration with 12 degrees of freedom. These transformation matrices were combined to provide the transform from EPI to MNI space, and this transform was applied to the results from the first-level analysis.

Data were combined across runs using a fixed effects model, and then modeled using mixed effects at the group level with FSL's FLAME model (Stage 1 only). The model included a regressor modeling mean activity and demeaned regressors for age, gender, SSRT, GoRT, and SDRT so we could examine neural effects of age, gender, and relevant behavioral performance during going and stopping. Contrasts included positive and negative relationships with each of the regressors. Outlier deweighting was performed using a mixture modeling approach (Woolrich, 2008). Results were thresholded at a whole-brain level using cluster-based Gaussian random field theory, with a cluster-forming threshold of $z > 2.3$ and a whole-brain corrected cluster significance level of $p < 0.05$. Cortical surface renderings were performed using CARET software[2]. Group statistical maps were mapped to the Population Average Landmark and Surface–based (PALS) atlas using the multi-fiducial mapping technique described by Van Essen (2005). For the purposes of presentation, data are overlaid on the average atlas surface.

PREDICTIVE ANALYSIS

Multivariate analyses were performed using the PyMVPA toolbox (Hanke et al., 2009). Prediction of individual differences from whole-brain fMRI data was performed using Gaussian process regression (Rasmussen and Williams, 2006) as implemented in PyMVPA, and support vector machines (Scholkopf and Smola, 2000) as implemented in libsvm (Chang and Lin, 2001). Individual differences for four different variables were included: age, SSRT, GoRT, and SDRT. These four variables are referred to as "labels." Contrast values from each voxel included in the whole-brain analysis mask were included as features in the analysis; because these are centered around zero, no scaling was performed. Four-fold balanced cross-validation was performed for each label (**Figure 2A**). On each run, participants were randomly assigned to one of the four folds, with the constraint that the mean label value did not differ across folds according to a one-way ANOVA ($p > 0.98$). This approach was used because it prevents overfitting that can occur when leave-one-out cross-validation is used with small sample sizes,

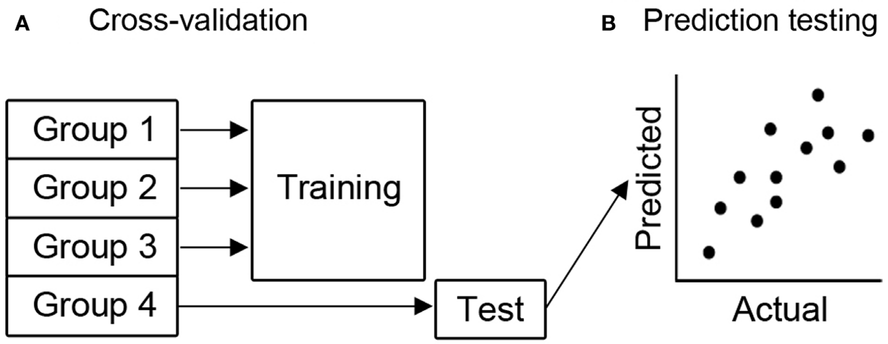

FIGURE 2 | Depiction of the predictive methods used. (A) All subjects were randomly assigned to 1 of 4 groups in such a way as to balance the distribution of the target variable across groups. Cross-validation was used, meaning that 3 groups were used to train the classifier (in this case 1, 2 and 3, although all iterations were used) and the classifier predicted the label value for the 4th group. **(B)** To quantify the accuracy of the classifier, the correlation between the actual label value and the label value predicted by the classifier was computed. Higher correlations imply that the actual and predicted values were similar, and thus the classifier was successfully predictive.

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[2] http://brainmap.wustl.edu

resulting in systematically incorrect predictions (Kohavi, 1995). Several different approaches for high-dimensional regression were employed:

- Gaussian process regression using a linear kernel and noise SD = 40.
- Gaussian process regression using a squared exponential (i.e., radial basis function) kernel with noise SD = 40, length scale = 12,000 and sigma $f = 10{,}000$.
- Support vector regression (epsilon-regression) with a linear kernel and cost function = 0.001.

In each case, parameters were determined using a set of increasingly fine grid searches over a broad parameter space. Predicted values were obtained for the left-out observations on each run and compared to the true label values using Pearson correlation (**Figure 2B**). This was performed 100 times for each analysis in order to obtain an estimate of prediction accuracy that was robust to the random group assignments. Statistical significance of prediction accuracy was determined by running each analysis 1000 times using randomly permuted labels; the correlation coefficients for predicted versus "true" labels in this analysis were used as an empirical null distribution against which the observed value was tested. Correlations were considered significant if the probability of such a correlation occurring was < 0.05.

Sensitivity maps were generated for the linear Gaussian process regression by averaging the linear regression weights for each voxel obtained for each training fold (it is not possible to create such maps for the non-linear case).

RESULTS

BEHAVIORAL RESULTS

Behavioral results are summarized in **Table 1**. There was no relationship between age and Go task performance ($r = 0.04$, robust $p = 0.92$). Older participants were, however, better at the Stop task than younger participants, as noted by decreasing SSRTs with increasing age ($r = -0.40$, robust $p = 0.03$). Additionally, there was no relationship between Stop task performance and Go task performance ($r = -0.008$, robust $p = 0.78$ for median GoRT and $r = -0.07$, robust $p = 0.66$ for SDRT).

STANDARD GLM ANALYSIS

We explored neural activity during successful Go task performance (successful go – baseline) and successful Stop task performance (successful stop – successful go and successful stop – unsuccessful stop). We included two contrasts exploring different aspects of successful stopping because both are commonly used in the literature. We also looked at correlations between neural activity during each of the contrasts and the four labels tested during the predictive analysis (age, SSRT, GoRT, and SDRT). All fMRI results were corrected for multiple comparisons using cluster-based Gaussian random field theory (cluster-forming threshold of $z > 2.3$, whole-brain FWE-corrected $p < 0.05$).

In order to determine which brain regions were associated with completing a planned response to a visual stimulus, we contrasted successful going with baseline. We found that the left motor cortex (corresponding to a right-handed button press), the left postcentral gyrus and the right occipital cortex were the only regions significantly active (**Figure 3A**; **Table 2A**). Similar to the behavioral data, there were no significant correlations with age or stopping ability (SSRT) and brain activity during the going process. There were, however, significant negative correlations between GoRT and SDRT and brain activity during the Go task. Regions correlated with GoRT included the right posterior middle frontal gyrus into the precentral gyrus, left angular gyrus, left superior lateral occipital cortex and left precuneus. All these regions displayed less activity with longer response times (**Figure 4A**; **Table 3A**). In a region in the left postcentral gyrus and supramarginal gyrus there was a significant negative correlation between SDRT and brain activity during the Go task, such that when participants' response speeds were more variable, there was less brain activity (**Figure 4B**; **Table 3B**).

Next, we explored regions associated with successful response inhibition by comparing activity during successful stopping to activity during successful going. We saw significant activity in a bilateral but predominantly right-lateralized network including the frontal pole, IFG, anterior insula, orbitofrontal gyrus, anterior cingulate gyrus, paracingulate gyrus, middle frontal gyrus, superior frontal gyrus, preSMA, posterior cingulate gyrus, central opercular cortex, striatum, thalamus, STN, brainstem, superior temporal gyrus, middle temporal gyrus, occipital fusiform cortex, precuneus cortex, intracalcarine cortex, supracalcarine cortex, lateral inferior occipital cortex, occipital pole, and cerebellum (**Figure 3B**; **Table 2B**). Many of these regions are typically active during successful stopping performance in adults (Aron and Poldrack, 2006). When examining correlations between age and neural activity during successful response inhibition we found one region, in the left medial prefrontal cortex, including part of the rostral anterior cingulate gyrus, that was significantly related to age. Activity in this region was decreased in older participants as compared to younger participants (**Figure 5A**; **Table 4A**). We then examined whether neural activity was related to behavior. To this end, we looked at brain regions correlated with SSRT, GoRT, and SDRT. During response inhibition, we found signifi-

Table 1 | Accuracy and response times of youth (ages 9–19) and adults (ages 25–30) on the stop-signal task.

Behavioral Data	GoAcc (SD)	GoRT (SD)	SDRT (SD)	PctInhib (SD)	SSRT (SD)
Youth	98.4% (1.9)	498.1 ms (73.4)	123.4 ms (25.3)	48.3% (4.8)	221.8 ms (56.1)
Adults	99.5% (0.5)	514.4 ms (83.9)	116.0 ms (23.8)	52.3% (5.0)	169.5 ms (43.1)

GoAcc, accuracy on the Go task; GoRT, response times on the Go task; SDRT, standard deviation of GoRT; PctInihib, percent inhibition on Stop trials; SSRT, stop-signal reaction time; SD, standard deviation.

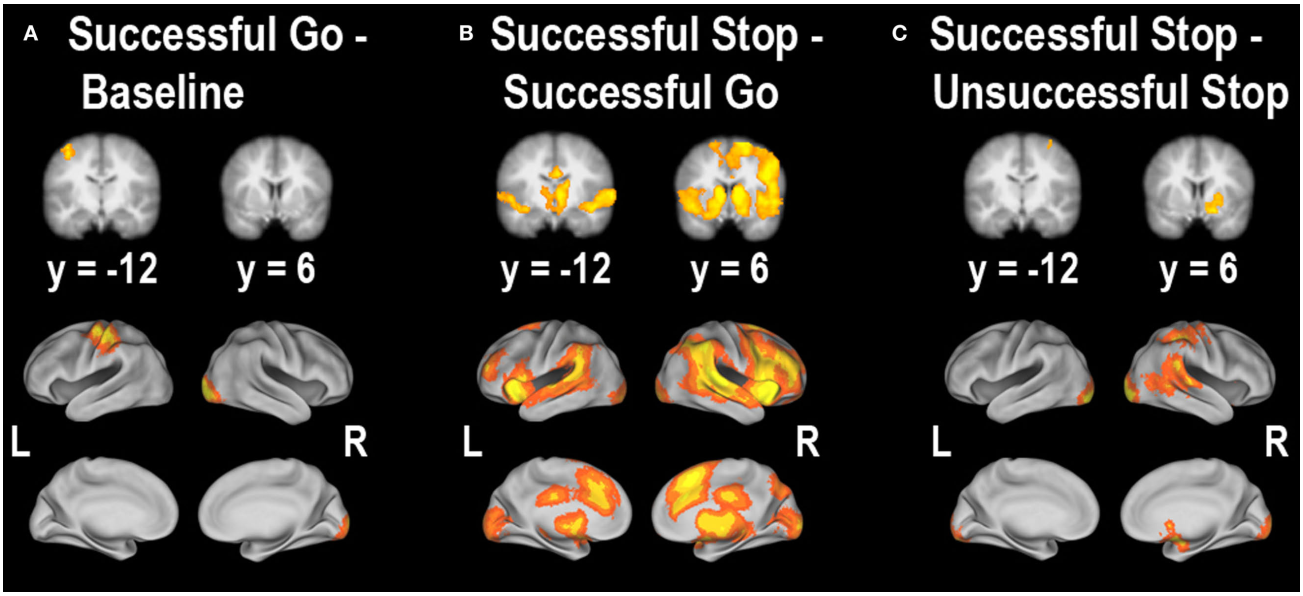

FIGURE 3 | Whole-brain main effects of (A) successful going – baseline, **(B)** successful stopping – successful going, and **(C)** successful stopping – unsuccessful stopping. All clusters survived whole-brain correction at $z > 2.3$, $p < 0.05$. For a list of clusters of activity, see **Table 2.**

Table 2 | Clusters associated with (A) successful going – baseline, **(B)** successful stopping – successful going, and **(C)** successful stopping – unsuccessful stopping.

Region	Coordinates (*x,y,z* in mm)	Max *z*	Extent (voxels)
A. SUCCESSFUL GO – BASELINE			
R inferior lateral occipital cortex, R occipital pole	24, –98, 8	5.87	1870
L precentral gyrus, L postcentral gyrus	–58, –26, 50	5.68	1589
B. SUCCESSFUL STOP – SUCCESSFUL GO			
R frontal pole, B inferior frontal gyrus, B anterior insula, B orbitofrontal gyrus, B anterior cingulate cortex, B paracingulate gyrus, R middle frontal gyrus, B superior frontal gyrus, B pre-supplementary motor area, B frontal opercular cortex, B central opercular cortex, B posterior cingulate gyrus, B striatum, B thalamus, B subthalamic nucleus, B brainstem, R superior parietal lobule, B supramarginal gyrus, B angular gyrus, B temporal pole, B superior temporal gyrus, R middle temporal gyrus, R precuneus cortex, B superior lateral occipital cortex	46, 20, –6	7.24	45928
B occipital fusiform gyrus, B intracalcarine cortex, B supracalcarine cortex, B lateral inferior occipital cortex, B occipital pole, L cerebellum	16, –100, 0	5.37	6077
R lateral frontal pole, R middle frontal gyrus	–38, 54, 22	4.62	1052
C. SUCCESSFUL STOP – UNSUCCESSFUL STOP			
R occipital fusiform gyrus, R lateral occipital cortex, R occipital pole	24, –90, –8	4.38	1419
L occipital fusiform gyrus, L lateral occipital cortex, L occipital pole	–16, –96, –12	4.57	1373
R posterior supramarginal gyrus, R angular gyrus, R superior temporal cortex, R middle temporal cortex	60, –42, 24	4.07	1180
R precentral gyrus, R postcentral gyrus, R superior parietal lobule, R anterior supramarginal gyrus	46, –30, 44	3.96	790
R putamen, R amygdala	20, 6, –16	3.82	696

All clusters survived whole-brain correction at $z > 2.3$, $p < 0.05$ and are reported in MNI space (mm). B, bilateral; L, left; R, right.

cant negative correlations between SSRT and successful response inhibition in regions relevant to the stopping process, such as the right medial prefrontal cortex, bilateral rostral anterior cingulate gyrus and paracingulate gyrus, right superior frontal gyrus, bilateral striatum, right subcallosal cortex, bilateral thalamus, right STN, right superior parietal lobule, right supramarginal gyrus,

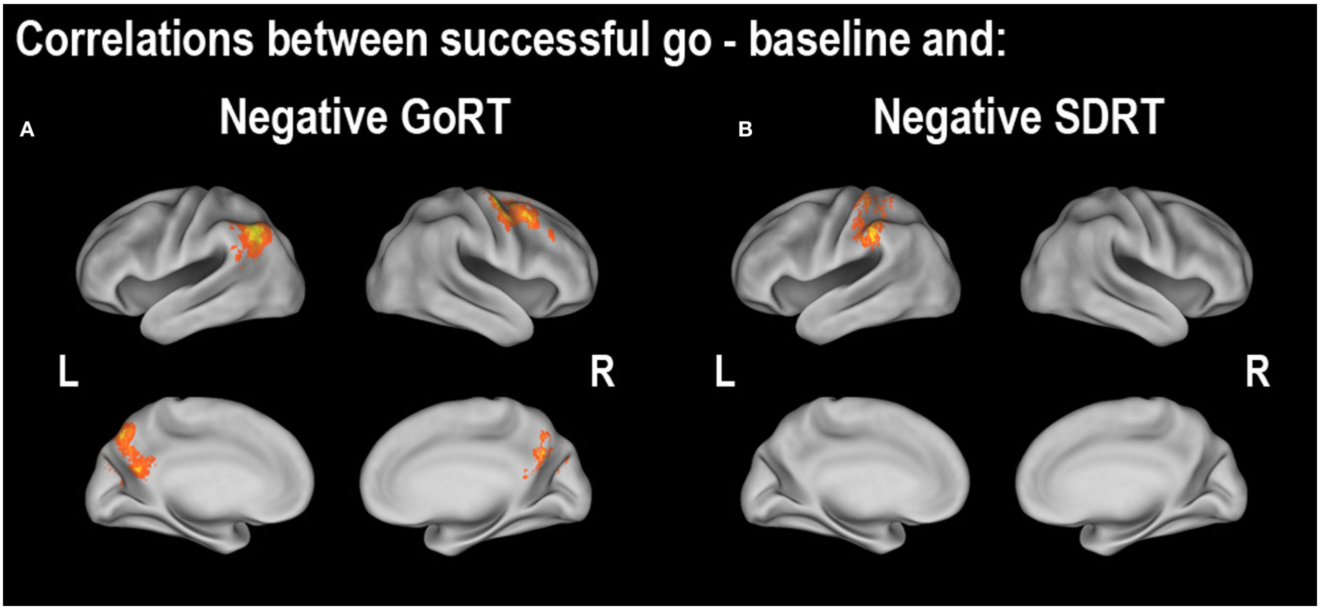

FIGURE 4 | Regions showing correlations between successful going vs. baseline and (A) median Go response time (GoRT) and **(B)** the standard deviation of Go response time (SDRT). All correlations were corrected at the whole-brain level at $z > 2.3$, $p < 0.05$. For cluster details, see **Table 3.**

Table 3 | Clusters of activity associated with correlations between successful going vs. baseline and (A) median Go response time (GoRT) and **(B)** the standard deviation of Go response time (SDRT).

Region	Coordinates (x,y,z in mm)	Max z	Extent (voxels)
A. NEGATIVE GORT			
L angular gyrus, L superior lateral occipital cortex	–42, –56, 46	3.38	614
R middle frontal gyrus, R precentral gyrus	42, –2, 56	3.74	395
B precuneus cortex	–2, –74, 50	3.47	291
B. NEGATIVE SDRT			
L postcentral gyrus, L supramarginal gyrus	–38, –32, 66	3.93	268

There were no significant correlations for this contrast with age or SSRT. All clusters survived whole-brain correction at $z > 2.3$, $p < 0.05$ and are reported in MNI space (mm). B, bilateral; L, left; R, right.

right parietal and central opercular cortex, right temporal pole, right superior temporal gyrus, right middle temporal gyrus, and right cerebellum. People with better inhibitory performance had more brain activity in these regions (**Figure 5B**; **Table 4B**). We also found a significant positive correlation between GoRT and left-lateralized activity in the insula, superior temporal gyrus and transverse temporal gyrus (including the auditory cortex), and middle temporal gyrus, suggesting that when the auditory cortex was more engaged, GoRTs on the Go task were slower (**Figure 5C**; **Table 4C**). Lastly, we found regions that were significantly negatively correlated with SDRT, including mostly left-lateralized frontal pole, middle frontal gyrus, superior frontal gyrus, preSMA, SMA, anterior cingulate cortex and paracingulate cortex, pre- and postcentral gyri, insula and frontal opercular cortex, striatum, pallidum, thalamus, supramarginal gyrus, superior parietal lobule, temporal pole, right inferior temporal gyrus, and bilateral occipital cortex (**Figure 5D**; **Table 4D**). It has been proposed that variability in stopping ability may be due to Go response time variability (Bellgrove et al., 2004), but the present results suggest that variability and inhibitory function are largely related to activity in different regions.

Another manner by which to explore successful stopping is to compare successful stopping with unsuccessful stopping (Rubia et al., 2003, 2007; Li et al., 2006). We examined this contrast as well and found that a right-lateralized network including the ventral striatum, amygdala, supramarginal gyrus, superior temporal gyrus, and bilateral occipital cortex was significantly active (**Figure 3C**; **Table 2C**). The limited significant neural activity with this contrast supports our previous findings in which the differences between successful and unsuccessful stopping conditions are weak compared to the differences between Stop and Go conditions (Aron and Poldrack, 2006). There were no significant correlations with either age or behavior when comparing successful to unsuccessful stopping.

PREDICTIVE ANALYSIS

Each of the contrasts outlined above (successful go – baseline, successful stop – successful go, and successful stop – unsuccessful stop) was entered into the predictive analysis to determine how well each label value could be predicted from fMRI data. We found that none of the label values (age, SSRT, GoRT, and SDRT) could be significantly predicted from successful go – baseline or successful stop – unsuccessful stop (**Figures 6A,C**). Instead, predictive accuracy was specific to successful response inhibition (as defined by the contrast successful stop – successful go)

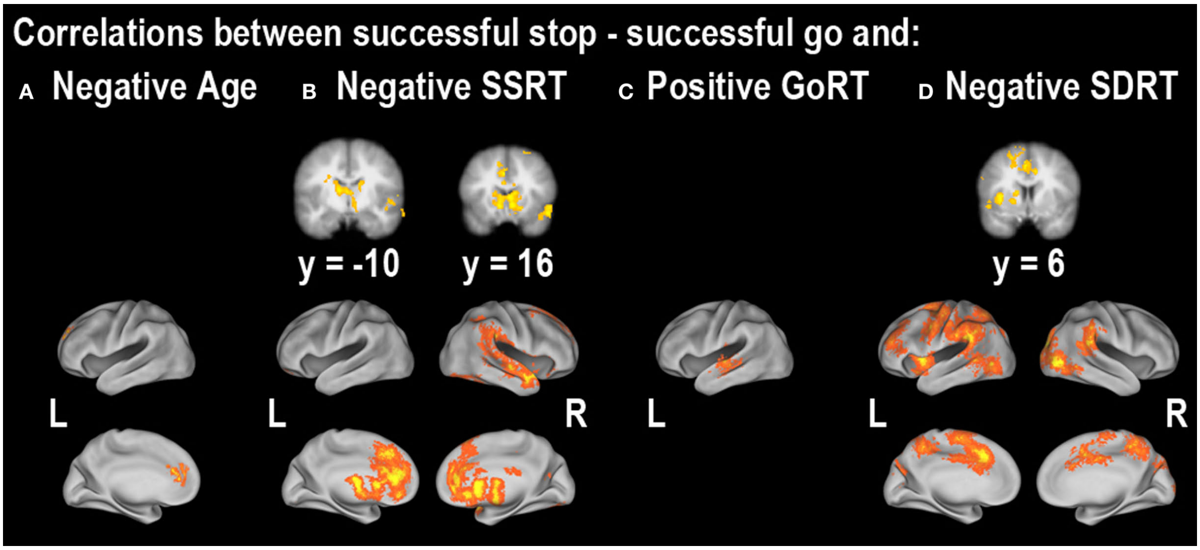

FIGURE 5 | Regions showing correlations between successful stopping vs. successful going and (A) age, **(B)** SSRT, **(C)** median Go response time (GoRT) and **(D)** the standard deviation of Go response time (SDRT). All correlations were corrected at the whole-brain level at $z > 2.3$, $p < 0.05$. For cluster details, see **Table 4**.

Table 4 | Clusters of activity associated with correlations between successful stopping vs. successful going and (A) age, **(B)** SSRT, **(C)** median Go response time (GoRT) and **(D)** the standard deviation of Go response time (SDRT).

Region	Coordinates (*x,y,z* in mm)	Max *z*	Extent (voxels)
A. NEGATIVE AGE			
L medial prefrontal cortex, L rostral anterior cingulate cortex	–12, 28, 14	4.00	310
B. NEGATIVE SSRT			
R medial prefrontal cortex, B rostral anterior cingulate cortex, B paracingulate gyrus, R superior frontal gyrus, B striatum, R subcallosal cortex, B thalamus, R subthalamic nucleus	–4, 16, 36	4.51	4959
R supramarginal gyrus, R superior parietal lobule, R parietal opercular cortex, R central opercular cortex, R temporal pole, R superior temporal gyrus, R posterior middle temporal gyrus	64, –18, 4	4.91	2025
R cerebellum	40, –80, –20	4.19	555
C. POSITIVE GORT			
L insula, L superior temporal gyrus, L middle temporal gyrus, L transverse temporal gyrus	–42, –26, 8	3.92	427
D. NEGATIVE SDRT			
L superior frontal gyrus, B anterior cingulate gyrus, B paracingulate gyrus, L pre-supplementary motor area, B supplementary motor area, B precentral gyrus, L postcentral gyrus, L posterior cingulate cortex, L supramarginal gyrus, L posterior middle temporal gyrus, L inferior lateral occipital cortex	–66, –54, 6	5.59	4022
R posterior inferior temporal gyrus, R cuneal cortex, R lateral occipital cortex, R occipital pole	20, –82, 24	3.69	1358
L superior parietal lobule, L cuneal cortex, L superior lateral occipital cortex	–28, –58, 36	3.53	705
L insula, L frontal opercular cortex, L caudate, L putamen, L pallidum, L thalamus, L temporal pole	–32, 8, 6	4.24	669
B precuneus cortex	12, –46, 20	3.33	558
R parietal opercular cortex, R supramarginal gyrus	52, –46, 20	4.85	511
L frontal pole, L middle frontal gyrus	–50, 44, 14	3.57	493

All clusters survived whole-brain correction at $z > 2.3$, $p < 0.05$ and are reported in MNI space (mm). B, bilateral; L, left; R, right.

and the labels that have been shown in previous literature to be related to response inhibition, namely age and SSRT (**Figure 6B**). Significant above-chance prediction of age from fMRI data was obtained using Gaussian process regression with linear [mean $r(\text{predicted, actual}) = 0.51$, $p = 0.003$] and squared exponential (mean $r = 0.38$, $p = 0.016$) kernels, as well as with a linear

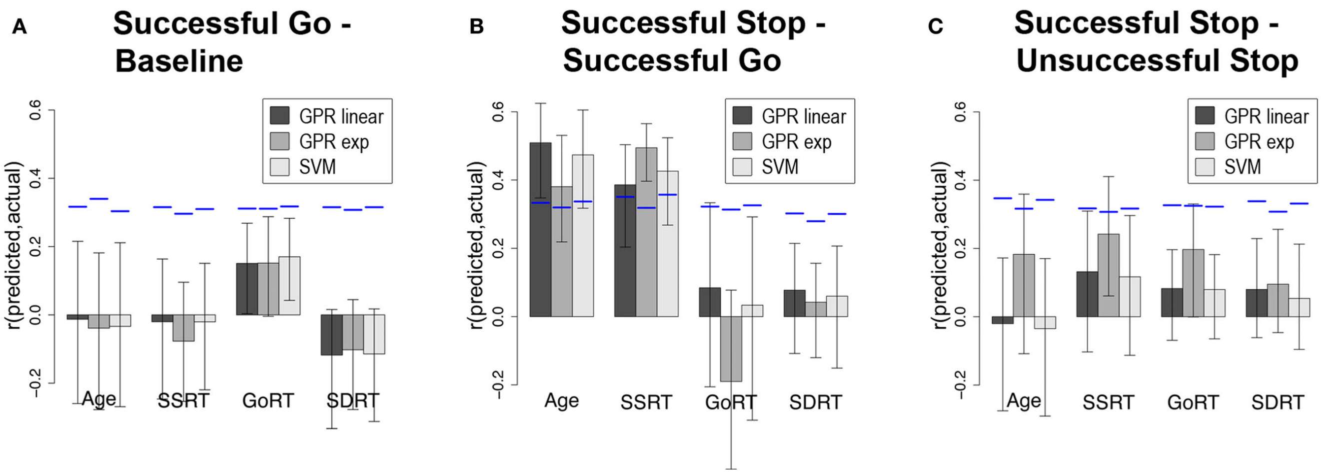

FIGURE 6 | Correlations between actual label values for age, SSRT, GoRT, and SDRT and predicted label values for (A) successful go – baseline, **(B)** successful stop – successful go and **(C)** successful stop – unsuccessful stop. Correlations shown for all three methods of prediction: Gaussian process regression with a linear kernel (GPR linear), Gaussian process regression with a squared exponential kernel (GPR exp), and linear support vector machine (SVM) regression. Blue lines depict the 95th percentile of an empirical null distribution, whereas the error bars depict the 95% confidence interval of correlation values across cross-validation samples. Thus, the blue depicts the threshold for statistical significance against the null hypothesis of zero predictability, whereas the error bars depict the stability of the prediction estimates across samples. Note that only predicted age and SSRT label values during successful stop – successful go are significantly related to actual age and SSRT.

support vector machine (mean $r = 0.47$, $p = 0.010$). Significant above-chance prediction of SSRT from fMRI data was obtained using Gaussian process regression with linear (mean r(predicted, actual) $= 0.39$, $p = 0.028$) and squared exponential (mean $r = 0.49$, $p = 0.002$) kernels, as well as with a linear support vector machine (mean $r = 0.43$, $p = 0.017$). The mean prediction accuracy for the permutation runs for both age and SSRT was very close to zero (ranging between −0.011 and −0.036 across all classifiers and labels), suggesting that there was no systematic bias due to the prediction method.

Sensitivity maps displaying which neural regions are most predictive of both age and SSRT were obtained for the linear Gaussian process regression (it is not possible to create such maps for the non-linear case; **Figures 7A,B**).

DISCUSSION

According to the race model of stop-signal response inhibition (Logan and Cowan, 1984), SSRT is a measure of the time needed to engage an inhibitory process. Individual differences in this quantity have long been linked to development (Williams et al., 1999) and impulsivity (Logan et al., 1997); they have also shown significant impairments in impulse control disorders such as ADHD (Logan et al., 2000; Lijffijt et al., 2005), substance use (Fillmore and Rush, 2002; Monterosso et al., 2005), and OCD (Chamberlain et al., 2006). However, some of these groups also appear to show increased variability in their performance on the primary (go) task as well (Williams et al., 1999; Lijffijt et al., 2005), which could inflate the estimated SSRT value. We strove to demonstrate that SSRT, not SDRT, is critically related to successful response inhibition. We found that both SSRT and SDRT were correlated with neural activity during successful inhibition, but only SSRT was predictable from that neural activity. This supports the claim that SSRT is the only behavioral variable critically related to the neural mechanisms of response inhibition. Moreover, we sought to extend previous findings that age and SSRT are related to the neural correlates of successful response inhibition by demonstrating that neural activity is predictive of these variables. Our results supported this claim as well, given that age and SSRT were the only two variables that could be predicted by neural activity, and that they were specifically predicted by neural activity during successful response inhibition, but not successful response execution.

Using a standard GLM analysis, we found much of the variability in neural activity in regions typically involved in successful response inhibition performance, such as the anterior cingulate gyrus, superior frontal gyrus, striatum and STN, was uniquely correlated with SSRT, while a widespread network of regions not specific to response inhibition were correlated with SDRT, including much of the left prefrontal cortex and primary sensory and motor areas. One region, a portion of the rostral anterior cingulate gyrus, showed activity related to age. Additionally, only one region, which included the superior, middle, and transverse temporal gyri, showed activity related to GoRT. During successful Go task performance, only response time behavior was related to neural activity (both GoRT and SDRT).

While the GLM analysis was useful in determining where neural activity was related to age or performance, we could not use those results as an indication of how neural activity may predict those variables. Thus, we implemented three methods of predictive analysis (Gaussian process regression with a linear kernel, Gaussian process regression with a squared exponential kernel, and support vector regression) to determine which aspects of the stop-signal task caused neural activity that was predictive of age or behavioral performance across individuals.

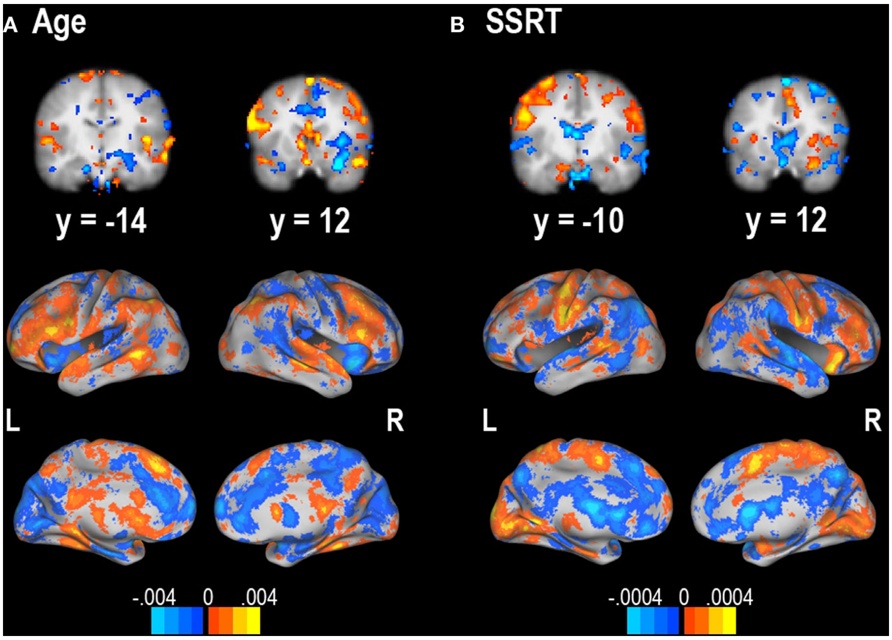

FIGURE 7 | Sensitivity maps for predictability of (A) age and **(B)** SSRT from successful stop – successful go contrast. Units are regression weights from the linear Gaussian process regression classifier. Orange areas are those that positively predict the label value; blue areas are those that negatively predict the label value. The color bars indicate the scale for each contrast. It is important to note that these regions may reflect the sensitivity of a particular classifier and could potentially change with different classifiers.

We found that neural activity during successful response inhibition as defined by contrasting successful stopping vs. successful going was predictive of age and SSRT, but not GoRT or SDRT. This was the case even though there were significant correlations between SDRT and activation during successful inhibition in our standard GLM analysis. This discrepancy between significant correlations and unsuccessful prediction may reflect the fact that the correlations were driven by a small number of observations. It also highlights the need to be careful regarding the conflation of correlation and prediction, as is common in the neuroimaging literature.

Additionally, we found that neural activity during successful Go task performance was not predictive of age or any behavioral variables. Taken together, these results imply that individual differences in SSRT are specifically related to response inhibition processes as opposed to response execution processes. Further evidence for a linkage between developmental changes in SSRT and changes in inhibitory processing was seen in that similar neural regions were predictive of both age and SSRT, including regions known to be utilized during successful motor inhibition, such as the right IFG, the striatum and the right STN. Lastly, individual differences in SSRT were related to inhibitory processes and not variability of Go task performance.

It is important to note that any conclusions from the present results must be qualified by the fact that only a small portion of the possible space of statistical learning methods was chosen. In particular, though we examined a range of statistical machines (linear and non-linear support vector machines and linear Gaussian processes), it could be the case that other methods would be more sensitive to specific effects. In addition, we did not employ any feature selection but rather included whole-brain data in the analyses. Finally, additional parameter optimization could have potentially improved predictive performance. However, the presence of significant results across multiple methods suggests that the significant prediction observed here is at least somewhat robust to methodological choices.

The current findings support and extend much previous developmental and response inhibition research. It has been demonstrated that cortical and subcortical neural regions involved in response inhibition are not fully developed structurally (Giedd et al., 1999; Sowell et al., 1999) or functionally (Casey et al., 2000) until adulthood. More specific to response inhibition, previous studies have demonstrated that adults have greater activity in the right IFG, a brain region known to be critical for successful response inhibition (Bunge et al., 2002; Durston et al., 2002; Rubia et al., 2007), and that response inhibition ability increases with increasing age (Casey et al., 1997; Williams et al., 1999; Durston et al., 2002). Even in fully developed, healthy adults, right IFG activity has been shown to be related to response inhibition ability (Aron and Poldrack, 2006). Therefore, the current study extends these correlational findings by demonstrating that neural activity during successful response inhibition in the right IFG, the striatum and the right STN is predictive of age and SSRT. It is important, however, to qualify that even though we extended the results of previous research from correlational to predictive, without a direct manipulation of neural functioning (i.e., a lesion or TMS), we cannot make strong claims about the causal relationship between regional brain function and behavior.

A debate in developmental neuroscience centers around how researchers can tease apart age-related neural differences from performance-related differences, especially when performance improves with age, as occurs in the stop-signal task (Durston and Casey, 2006). The results from our predictive analysis indicate that patterns of neural activity during successful response inhibition predicted both age and SSRT equally well in our participants. This implies that in a task where age and performance are inextricably linked, a combination of age effects and performance effects underlies neural activity. Future research could further enhance our knowledge of the relationship between age and SSRT by more specifically exploring whether age influences SSRT directly or if the two variables are related via a separate, mediating factor. A combination of predictive analyses and formal modeling of the causal relationships between these and other variables may help to elucidate this relationship.

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BDNF genotype modulates resting functional connectivity in children

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A specific polymorphism of the brain-derived neurotrophic factor (BDNF) gene is associated with alterations in brain anatomy and memory; its relevance to the functional connectivity of brain networks, however, is unclear. Given that altered hippocampal function and structure has been found in adults who carry the methionine (met) allele of the BDNF gene and the molecular studies elucidating the role of BDNF in neurogenesis and synapse formation, we examined the association between BDNF gene variants and neural resting connectivity in children and adolescents. We observed a reduction in hippocampal and parahippocampal to cortical connectivity in met-allele carriers within both default-mode and executive networks. In contrast, we observed increased connectivity to amygdala, insula and striatal regions in met-carriers, within the paralimbic network. Because of the known association between the BDNF gene and neuropsychiatric disorder, this latter finding of greater connectivity in circuits important for emotion processing may indicate a new neural mechanism through which these gene-related psychiatric differences are manifest. Here we show that the BDNF gene, known to regulate synaptic plasticity and connectivity in the brain, affects functional connectivity at the neural systems level. In addition, we demonstrate that the spatial topography of multiple high-level resting state networks in healthy children and adolescents is similar to that observed in adults.

Keywords: fMRI, children, adolescents, resting-state, functional connectivity, BDNF, gene

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) plays an essential role in the early growth of neural networks in the human brain (McAllister et al., 1999; Huang and Reichardt, 2001). BDNF is a polypeptide growth factor in a family of signaling molecules known as *neurotrophins*. These proteins regulate axonal and dendritic growth (Huang and Reichardt, 2001), synaptic structure and plasticity (McAllister et al., 1999; Lu and Gottschalk, 2000), neurotransmitter release, and long-term potentiation (LTP)-associated-learning (Lu and Gottschalk, 2000; Chao, 2003). Considerable progress has been made in understanding the ways in which neurotrophins exert their effects on neuronal health and synaptic plasticity (e.g., cellular signaling and ligand-receptor binding) (Lewin and Barde, 1996; Stoop and Poo, 1996); how neurotrophin-related differences in neuronal health and synaptic plasticity may lead to selective impairments in nervous system function, however, is not well understood.

BDNF is a unique neurotrophin because activation of its cognate receptor, tropomyosin-related kinase B (TrkB), results in a wider set of functional consequences than is the case with other Trk receptors (Bath and Lee, 2006). BDNF is expressed predominantly in the central nervous system, including cortical regions, the hippocampus, limbic structures, the cerebellum, and the olfactory bulb (Huang and Reichardt, 2001). A common single-nucleotide polymorphism (SNP) in the BDNF gene produces an amino acid substitution (valine to methionine) at codon 66 (val66met) that alters secretion and intracellular trafficking of the mature peptide (Egan et al., 2003; Chen et al., 2004). This SNP has been useful in linking BDNF to human cognitive processes (Egan et al., 2003; Gabrieli and Preston, 2003; Hariri et al., 2003; Dempster et al., 2005; Ho et al., 2006) and, as a result, interest in BDNF has come from researchers spanning fields from developmental neurobiology to neurodegenerative and psychiatric disorders (Chao, 2003).

Compared with val-allele homozygotes, individuals with the altered methionine (met) allele of the BDNF gene have been found to exhibit reduced hippocampal volume (Pezawas et al., 2004; Szeszko et al., 2005; Bueller et al., 2006; Frodl et al., 2007; Chepenik et al., 2009), memory impairment (Egan et al., 2003; Hariri et al., 2003; Dempster et al., 2005; Ho et al., 2006), increased susceptibility to schizophrenia and other psychotic disorders (Gratacos et al., 2007), and abnormal hippocampal activity during memory processing (Egan et al., 2003; Hariri et al., 2003). Given the established role of BDNF in mediating processes related to neural excitability, learning, and memory (Korte et al., 1995; Patterson et al., 1996; Desai et al., 1999), this gene is likely implicated in the formation and maintenance of major functional circuits in the human brain. To date, however, the effects of this BDNF polymorphism on the functioning of large-scale neural networks have not been examined.

Currently, one of the most informative methods for interrogating the integrity of neural networks is to measure activation across distinct brain regions by conducting functional connectivity (FC) analysis on data obtained through whole-brain functional magnetic resonance imaging (fMRI). FC can be operationally defined to refer to temporal correlations across cortical regions representing an index of function (Friston et al., 1993; Horwitz, 2003) and anatomical connectivity

(Koch et al., 2002; Quigley et al., 2003; Hagmann et al., 2008; Greicius et al., 2009). In the present study we used fMRI to probe resting FC as a function of BDNF gene variants across the three most frequently examined high-order cognitive- and emotion-processing networks of the brain: the default-mode network (DMN), the executive network (EN), and the salience network (SN). Because neurotrophins profoundly affect the development of the nervous system (Lewin and Barde, 1996), we examined BDNF and FC in a sample of children and adolescents, in whom neural development is actively progressing and for whom BDNF may therefore be expected to exert significant effects (for detailed discussion, see Casey et al., 2009).

We hypothesize that because BDNF secretion is decreased in met-allele carriers compared to val-allele homozygotes (Chen et al., 2004), participants who carry a BDNF met allele will be broadly characterized by reduced FC, although this may differ across the three resting-state networks. Moreover, because BDNF is widely expressed in the hippocampus (Conner et al., 1997) and because, compared to val-allele homozygotes, met-allele carriers have been found to have poorer memory (Egan et al., 2003; Dempster et al., 2005), diminished hippocampal function (Egan et al., 2003) and smaller hippocampal volume (Pezawas et al., 2004; Szeszko et al., 2005; Bueller et al., 2006; Frodl et al., 2007; Chepenik et al., 2009), we hypothesize that differences between genetic groups in resting FC will be particularly pronounced between the hippocampal formation and regions that support memory processing (e.g., parietal association cortices, prefrontal regions, cingulate).

MATERIALS AND METHODS

PARTICIPANTS

Participants were 38 children and adolescents (25 females) between the ages of 9 and 16 years ($M = 12.2$, SD = 2.1). They were recruited through their mothers via Craigslist and other online advertisements and parent networks, and each mother-child pair was compensated $25/hour. All participants had no reported history of brain injury, no behavioral indications of possible mental impairment, no past or present Axis I disorder, were right-handed, fluent in English, and had no learning disorder. Parents and children gave informed consent and assent, respectively, as approved by the Stanford Institutional Review Board.

PROCEDURE

Participants were assessed in two sessions. In the first session, participants were administered the Schedule for Affective Disorders and Schizophrenia for School-Aged Children-Present and Lifetime version (K-SADS) (Geller et al., 1996, 2001) to assess current and lifetime psychopathology in order to ensure the absence of any current or past diagnosable DSM-IV psychiatric disorder. During this session, children and parents also provided saliva samples for genetic testing and viewed a video to prepare them for the MRI scan session. In the second session, brain-imaging data were acquired using a whole-brain MRI scanner.

GENETIC DATA

DNA was extracted from saliva using the Oragene DNA saliva kit. The target 300 bp BDNF gene fragment was amplified using the G196A primer 5′-ATC CGA GGA CAA GGT GGC-3′ (forward) and 5′-CCT CAT GGA CAT GTT TGC AG-3′ (reverse). The PCR amplification was carried out in a final volume of 20 µl consisting of 50 ng of genomic DNA, 200 nM each of sense and antisense primers, 200 µM of dNTP mix, 2 units of Expand High Fidelity PCR System (Roche, Cat# 11-795-078-001). Annealing was carried out at 60 C for 45 s, extension at 72 C for 1 min, and denaturation at 95 C for 30 s for a total of 35 cycles. 5 µl of the PCR products were digested by 10 units of Pml I (New England Biolabs) in a 15 µl reaction at 37 C for 3 h. The digestion mixture was electrophoresed through 7% Polyacrylamide gel (Acrylamide/bis-Acrylamide ratio 19:1) at 150 V for 40 min. 100 bp and 10 bp marker was used to measure the digestion and PCR product size. Allele A (methionine) was not digested showing one 300 bp band, but the G allele (valine) was digested showing two bands, at 180 and 120 bp. As we describe below, this genotyping yielded two groups of children: val-allele homozygotes ($n = 23$) and met-allele carriers ($n = 15$).

fMRI DATA ACQUISITION

Magnetic resonance imaging was performed on a 3.0-T GE whole-body scanner. Participants were positioned in a purpose-built single channel T/R head coil and stabilized by clamps and a bite bar formed with dental impression wax (made of Impression Compound Type I, Kerr Corporation, Romulus, MI) to reduce motion-related artifacts during scanning. During the resting-state experiment, participants completed a 6-min scan during which they were instructed to lay still with eyes closed. For this study, 29 axial slices were taken with 4 mm slice thickness. High-resolution T2-weighted fast spin echo structural images (TR = 3000 ms, TE = 68 ms, ETL = 12) were acquired for anatomical reference. A T2*-sensitive gradient echo spiral in/out pulse sequence (Glover and Law, 2001) was used for all functional imaging (TR = 2000 ms, TE = 30 ms, flip angle = 77°, FOV = 22 cm, 64 × 64). An automated high-order shimming procedure, based on spiral acquisitions, was used to reduce B0 heterogeneity (Kim et al., 2002). Spiral in/out methods have been shown to increase signal-to-noise ratio and BOLD contrast-to-noise ratio in uniform brain regions, as well as to reduce signal loss in regions compromised by susceptibility-induced field gradients generated near air-tissue interfaces such as PFC (Glover and Law, 2001). Compared to traditional spiral imaging techniques, spiral in/out methods result in less signal dropout and greater task-related activation in PFC regions (Preston et al., 2004). A high-resolution volume scan (140 slices, 1 mm slice thickness) was collected for every participant using a spoiled grass gradient recalled (SPGR) sequence for T1 contrast (TR = 3000 ms, TE = 68 ms, TI = 500 ms, flip angle = 11°, FOV = 25 cm, 256 × 256). During the resting-state scan, children's heart-rate and respiration waveform were recorded.

fMRI PREPROCESSING

fMRI data were then preprocessed using AFNI[1] (Cox, 1996). Preprocessing included slice-timing correction, volume registration, smoothing (4 mm), bandpass filtering ($0.008 < f < 0.15$), and co-registration of functional and anatomical images.

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[1] http://afni.nimh.nih.gov/afni

ICA VERSUS ROI-BASED CONNECTIVITY ANALYSIS

Previous resting state connectivity analyses have used FSL's Independent Component Analysis (ICA) software (MELODIC) and an automated MATLAB analysis routine (Greicius et al., 2004, 2007). This processing pathway was not suitable for the present study because in a small number of cases (<20%) the MELODIC ICA process separated the executive control network for the participant into two lateralized maps, one right and one left. For this reason, and because in our previous work we demonstrated that seed-based FC and ICA-based network analyses yield comparable DMNs in children (Thomason et al., 2008), we used ROI-based connectivity analysis to evaluate multiple resting networks in the present study.

FUNCTIONAL CONNECTIVITY (FC) ANALYSIS

We conducted ROI-based connectivity analysis on the functional data. Selected seed regions were based on those reported in published studies (Default: 10, −50, 30; Executive: 44, 36, 20; Salience: 38, 26, −10) (Krasnow et al., 2003; Seeley et al., 2007; Thomason et al., 2008), as they have been shown to yield robust connectivity across the major resting-state networks. We began our analysis with image reconstruction using a correction that removes respiratory variations and HR signal extraction. In brief, this correction removes the effect of low-frequency respiratory variations (i.e., the "envelope" of the respiratory belt waveform) and heart rate (average rate in a 6-s sliding window) by first convolving those signals with appropriate filters and then regressing them out of the time series for each voxel (described in Birn et al., 2008; Chang et al., 2009).

Following signal extraction, the trace from each participant's seed region (3D sphere with a radius of 3, centered on the previously presented coordinates) was detrended for three translational and three rotational motion regressors (AFNI 3dDetrend) and was used to calculate the correlation between the seed region and time-course data in all of the other voxels in the brain. Correlation estimates were controlled for estimated translational and rotational motion and a white matter nuisance time-course (3d sphere with radius of 3 mm, centered at (27, −8, 27). In contrast to some of the previous resting-state protocols conducted with adults (e.g., Fox et al., 2005), in the present analysis rather than removing variance from motion, white matter, cerebral spinal fluid and from global signal, we removed only motion and a timecourse sampled from a white matter region of interest. We made this choice based on recent work indicating that the use of global signal correction may force the presence of anticorrelated networks (Chang et al., 2009; Murphy et al., 2009; Weissenbacher et al., 2009); indeed, this methodological and interpretive topic is currently being actively debated for resting-state studies (Fox et al., 2009). After correlation coefficients were calculated for each voxel in the brain, we normalized the distribution of these values using Fishers *r*-to-*z* transformation. We submitted the resulting correlation maps to main-effects analysis, conducting two-tailed *t*-tests to identify regions in which whole-brain *z*-converted regression maps differed between the two genotype groups. We conducted group-level analyses separately for each resting network and report results at $p < 0.01$.

We analyzed motion and found groups differed at $p < 0.05$. Therefore, before signal was extracted from the seed regions, we detrended the signal for motion. Then, the six motion parameters (three translational, three rotational) were modeled as regressors of non-interest at the individual subject level to adjust the error term appropriately.

REGION OF INTEREST (ROI) ANALYSIS

ROI analysis was conducted for hippocampal ROIs created using the AFNI software package and subroutines for creating smoothed (3 mm), resampled (3.75 mm^3), binary masks for the left and right hippocampi[2]. Results within the masks were held to a liberal threshold ($p < 0.05$) for completeness in reporting.

HIPPOCAMPAL VOLUME ANALYSIS

To determine whether the connectivity analysis required correction for hippocampal volume, we conducted manual tracing of hippocampal volumes within SPGR images using Insight Toolkit's SNAP program (Yushkevich et al., 2006). Tracings were performed in reoriented native space, and hippocampal volumes were divided by total brain volume to control for the potentially confounding factor of head size. The anatomical features used to guide manual tracing of the hippocampus have been described elsewhere (Pruessner et al., 2000). Briefly, the hippocampal head-body boundary was delineated by the clear appearance of the uncal recess, while the body-tail boundary was delineated by the opening of the crus of the fornix. Final volumes were output using SNAP. Data were analyzed in SPSS16 using a multivariate general linear model framework and including participant age as a covariate. Because there was not a significant gene-group effect in hippocampal volume, $F(2, 32) = 0.36$, $p = 0.69$, correction of functional connectivity data for volume differences was not required.

RESULTS

PARTICIPANTS

Participants were 26 Caucasians (68%), 3 Asian Americans (8%), 2 Hispanic Americans (5%), and 7 participants of multi- or biracial descent (18%). BDNF genotyping yielded two groups of children: val-homozygotes ($n = 23$) and met-allele carriers ($n = 15$). These allelic frequencies were in Hardy-Weinberg equilibrium, $\chi^2 = 0.65$, $p = 0.42$. Demographic data for the two genotype groups are presented in **Table 1**. The two groups did not differ significantly with respect to age, $F(1,36) = 0.96$,

Table 1 | Participant demographics and summary statistics.

	val/val	val/met	Statistic
N	23	15	$\chi^2_{(1)} = 0.65$, $p = 0.42$
Gender (F:M)	14:9	11:4	$\chi^2_{(1)} = 0.63$, $p = 0.43$
Mean age (s.d.)	11.9 (2.0)	12.6 (2.2)	$F_{1,36} = 0.96$, $p = 0.33$

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[2] We should note that AFNI ROIs are based on an adult-derived brain template. Validity of using the same stereotactic space for adults and children in this age range (>9 years old) has been demonstrated empirically (Burgund et al., 2002).

$p = 0.33$, or gender, $\chi^2 = 0.63$, $p = 0.43$. BDNF alleles were distributed similarly in Caucasian and non-Caucasian participants, $\chi^2 = 0.035$, $p = 0.85$.

NETWORK MAPS

Complete network maps generated using three separate one-sample *t*-tests (**Figure 1**), each collapsing across the full sample, yielded maps similar to those produced in previous studies of adults (Fox et al., 2005; Fransson, 2005; Seeley et al., 2007; Taylor et al., 2008; Habas et al., 2009) and children (Thomason et al., 2008; Kelly et al., 2009), and regarding reproducibility, see (Meindl et al., 2009). These maps demonstrate reliable generation of the high-order cognitive and/or affective resting network maps in a sample of children and adolescents that have previously been reported in samples of adults.

DEFAULT-MODE NETWORK (DMN) COMPARISON

Figure 2 shows val-allele homozygotes have greater and wider extent of suprathreshold functional connectivity within and beyond the DMN than do met-allele carriers. The between-groups whole-brain comparison of DMN statistical maps (**Figure 3**) showed significantly ($p < 0.01$) increased hippocampal, fusiform, insula, caudate, cingulate, frontal and cerebellar contribution to the DMN in children homozygous for the BDNF val allele. The reverse contrast for areas with significantly greater contribution in met-allele carriers produced fewer significant clusters, and included regions of the parietal and temporal lobes; see **Table 2**.

A total of 10 voxels in the between-groups, within-hippocampal, ROI comparison of DMN statistical maps were significant at $p < 0.05$. The largest significant cluster was $k = 5$ voxels in the left hippocampus (−34,−19,−7) where resting functional connectivity to the DMN seed region was greater in children homozygous for the BDNF val allele ($z = 4.10$); there was no significant correlation in children who carried a met allele ($z = 0.34$). There were no areas in the hippocampal ROI analysis in which the contribution of met-allele carriers to the DMN network were significantly greater than val-allele homozygotes; see **Figure 4**.

EXECUTIVE NETWORK (EN) COMPARISON

As seen in **Figure 2**, val-allele homozygotes have a greater and wider extent of suprathreshold functional connectivity within and beyond the EN than do met-allele carriers. Whole-brain between-groups analysis of the EN (**Figure 3**) showed that children homozygous for the BDNF val allele showed significantly increased ($p < 0.01$) contributions from a number of clusters in temporal and parietal cortices, including parahippocampal and neighboring temporal lobe regions and a large area in the precuneus. In addition, there were frontal areas, insula, anterior cingulate, and striatal regions that showed

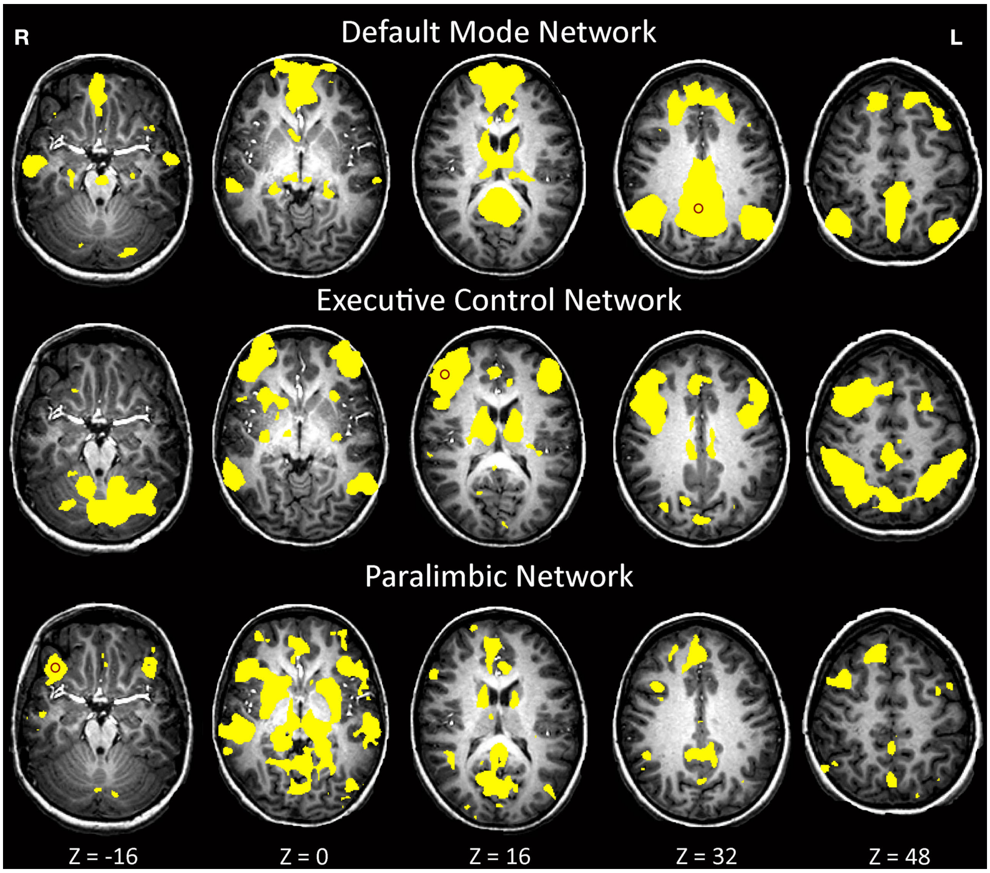

FIGURE 1 | Map of neural connectivity for the three major resting-state networks across all subjects ($n = 38$). $p < 0.0001$. Red circles denote approximate locations of seed-point ROIs.

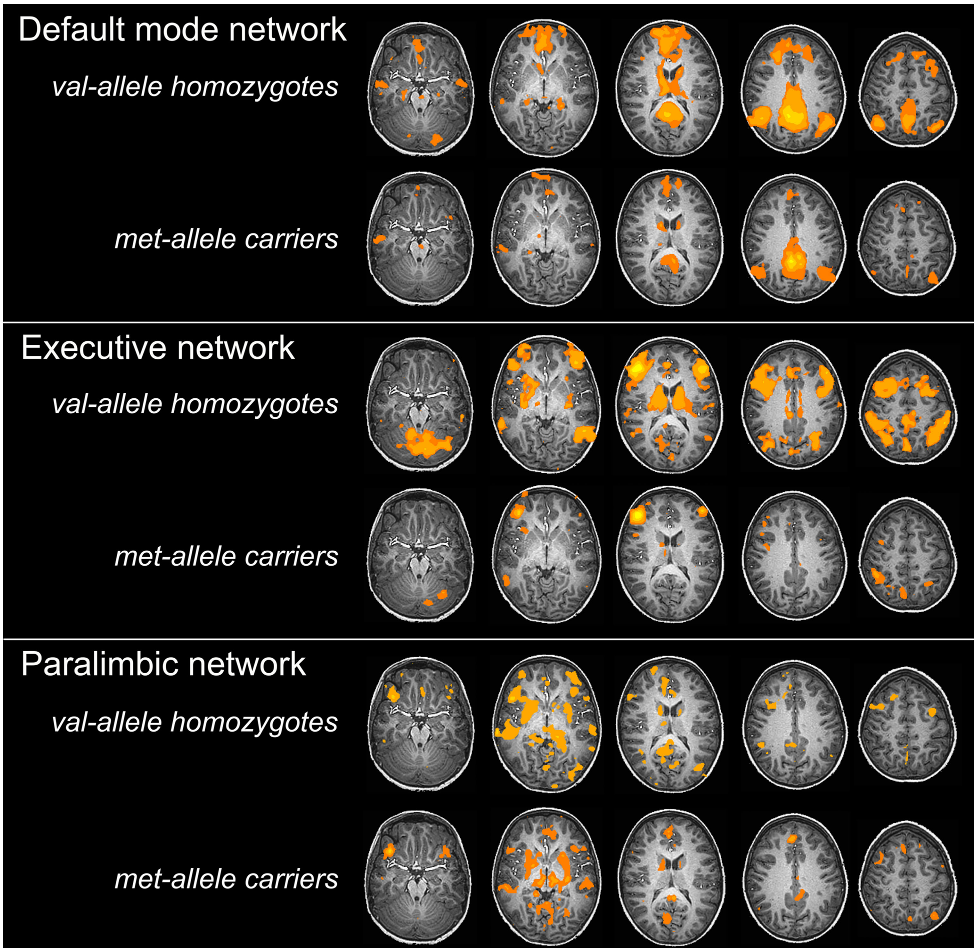

FIGURE 2 | One-sample *t*-tests within each genetic group depicting group effects for three major resting-state networks at $p < 0.0001$.

greater FC in the val-allele homozygotes than in the met-allele carriers within this network. In the reverse contrast, met-allele carriers again had fewer significant clusters, and these were located in inferior frontal and temporal regions, insula, and cingulate cortex.

The between-groups, within-hippocampal ROI comparison of EN resulted in 17 voxels that showed significantly increased contribution to the EN by children homozygous for the BDNF val allele compared to met-allele carriers. Of these, 11 fell within the left hippocampal ROI and 6 fell within the right hippocampus ROI. The largest, most significant cluster was $k = 4$ voxels in the left hippocampus (−26, −26, −7) where the average correlation to the executive seed was $z = 3.87$ in val-allele homozygotes compared to $z = 1.15$ in met-allele carriers. There were no areas in the hippocamal ROI analysis in which met-allele carrier contributions to the EN network were significantly greater than was the case for val-allele homozygotes; see **Figure 4**.

PARALIMBIC NETWORK (PN) COMPARISON

The whole-brain between-groups analysis produced a different pattern of results in the PN. Whereas the DMN and EN comparisons were dominated by regions in which val-allele homozygotes had significantly higher FC than did met-allele carriers, the reverse was true for the PN (see **Table 2**); many more regions showed significantly increased connectivity in the PN in met-allele carriers. In a number of cortical areas, amygdala, insula, and caudate, met-allele carriers showed significantly greater FC than did val-allele homozygotes. In contrast, val-allele homozygotes showed significantly greater reliance than did met-allele carriers only in regions of the posterior cingulate and a region of the parahippocampal gyrus.

The hippocampal ROI analysis also yielded different results in the PN. Only three voxels showed significant differences between the gene groups, all of which were located in the left hippocampus. Here, in contrast to what was observed for the other networks, significantly ($p < 0.05$) greater FC was observed in met-allele carriers than in val-allele homozygotes. In the peak of this difference (−30, −11, −22), which occurred in a region of the hippocampus that was more anterior than were peaks of group differences observed for the other networks, the average correlation to the executive seed was $z = 1.95$ in val-allele homozygotes and $z = 3.84$ in met-allele carriers.

DISCUSSION

Studies of the BDNF gene polymorphism have reliably documented episodic memory deficits associated with the met allele (Egan et al., 2003; Hariri et al., 2003). Contemporary models of declarative

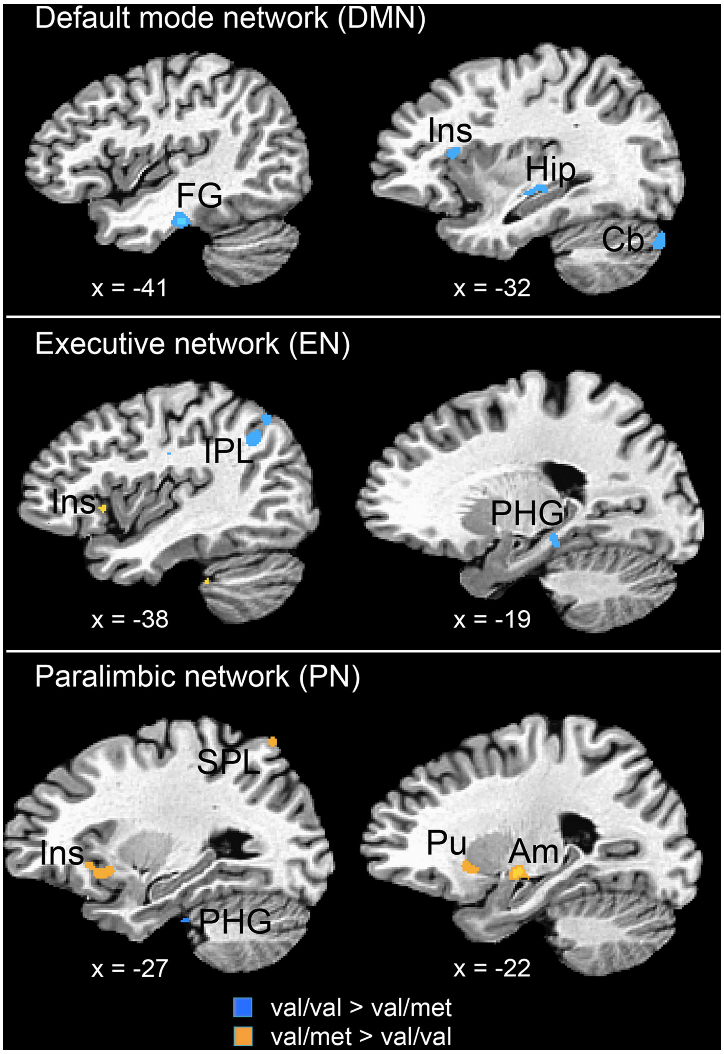

FIGURE 3 | Two-sample *t*-tests for three major resting-state networks. BDNF gene group differences (val/val > val/met: blue; val/met > val/val: orange) across three resting networks. FG = fusiform gyrus, Ins = insula, Hip = hippocampus, IPL = inferior parietal lobule, PHG = parahippocampal gyrus, SPL = superiorparietal lobe, Pu = putamen, Am = amygdala; $p < 0.01$.

memory function suggest that strong neural connectivity between neocortical association areas and medial temporal lobe (MTL) regions underlies successful memory encoding, long-term maintenance, and retrieval (Ranganath et al., 2005). In this study we did not restrict differences in FC to only within-network regions; consequently, we observed reliable effects across networks. More specifically, we observed a reduction in hippocampal and parahippocampal to cortical connectivity at rest in carriers of the met allele within each of the three resting networks we examined: the default-mode, executive, and paralimbic networks (see **Table 2**). This work suggests that differences are present between genetic groups both within networks and in the connectivity of these networks to other parts of the brain. Analyses of all three networks provided evidence of higher basal connectivity between MTL structures and neocortical association areas in val-allele homozygotes than in met-allele carriers. These differences in resting FC may result from differences in anatomical connectivity, in which met-allele carriers have less robust cortical-to-MTL projections, particularly in the network important for executive control and in the default-mode network.

Association studies have linked the BDNF gene to substance-related disorders, eating disorders, and schizophrenia; the association of the BDNF gene with major depression disorders and bipolar disorders, however, has been inconclusive (see, for example, meta-analysis by Gratacos et al., 2007). Contemporary theory suggests that the allele (met vs. val) that confers risk may change across development, differing in trajectory for various disorders (Casey et al., 2009). Comprised primarily of insular and cingulate cortices that are connected with subcortical limbic structures, the paralimbic network may be the neural network most broadly relevant to psychiatric disorders for the role it is expected to exert in the detection, integration, and filtering of interoceptive, autonomic, and emotional information (Seeley et al., 2007; Habas et al., 2009). The insula plays a critical role in the experience of emotion, interoceptive awareness,

Table 2 | Regions of significant ($p < 0.01$, uncorrected) BDNF gene-group differences functional connectivity in across three resting-state networks.

	BA	*x*	*y*	*z*	Volume (mm^3)	*Z* score
DEFAULT MODE NETWORK						
val/val > val/met						
Frontal						
Inferior	R10	49	45	–3	105	2.714
Inferior	R46	45	38	4	158	2.762
Temporal						
Parahippocampal gyrus	L34	8	–8	–14	158	2.95
Parahippocampal gyrus	L34	–15	–4	–14	264	3.004
Hippocampus	L	–34	–26	–3	316	3.161
Fusiform	L20	–41	–23	–22	475	3.521
Uncus	L28	–11	–4	–26	211	3.331
Parietal						
Inferior	R40	34	–38	31	158	2.72
Limbic						
Cingulate	L24	–15	11	23	158	2.632
Cingulate	L24	–19	4	34	105	2.794
Insula	L13	–34	19	12	158	3.068
Subcortex						
Caudate	R	15	19	16	105	2.764
Caudate	L	–23	8	19	422	3.049
Cerebellum						
Tuber	L	–34	–86	–29	633	3.428
val/met > val/val						
Temporal						
Superior	L38	–45	15	–14	158	3.071
Middle	R21	68	0	–7	105	3.454
Middle	R21	49	–11	–11	527	3.071
Parietal						
Inferior/Precuneus	R39	45	–68	42	264	3.014
Angular	R39/40	49	–68	31	422	3.219
EXECUTIVE NETWORK						
val/val > val/met						
Frontal						
Medial	R10	11	53	8	158	2.786
Medial	R9	11	49	34	158	3.22
Precentral	L4	–26	–15	46	158	2.783
Temporal						
Superior	R38	41	11	–18	264	3.315
Inferior	L20/37	–60	–56	–11	105	3.441
Parahippocampal gyrus	R28	19	–15	–18	105	2.816
Parahippocampal gyrus	L28	–23	–26	–7	264	2.693
Fusiform/Inferior	R20	53	–11	–22	158	3.051
Uncus	L28	–11	–4	–26	105	3.458
Parietal						
Superior	R7	19	–60	61	105	2.714
Superior	R7	8	–60	68	105	2.987
Inferior	L39	–41	–64	42	264	2.776
Angular gyrus	L39	–34	–60	34	264	2.892
Angular gyrus	L39	–41	–56	34	211	2.944
Supramarginal gyrus	R	41	–41	23	105	2.923
Supramarginal gyrus	R	34	–49	27	475	2.837
Precuneus	L31	–11	–56	31	1266	3.37
Limbic						
Anterior Cingulate	R25	4	15	–7	105	3.238
Insula	L13	–30	–19	27	369	3.143
Subcortex						
Putamen/Lentiform Nucleus	R	26	–4	19	105	2.77
val/met > val/val						
Frontal						
Inferior	R44	60	15	4	105	2.924
Temporal						
Inferior	R20	45	–8	–33	105	2.988
Limbic						
Insula	L13	–38	15	1	105	2.694
Cingulate	L24/32	0	15	38	369	2.915
PARALIMBIC NETWORK						
val/val > val/met						
Temporal						
Parahippocampal gyrus	L19	–38	–41	1	158	2.928
Limbic						
Posterior cingulate	L31	–19	–41	27	158	3.025
val/met > val/val						
Frontal						
Middle	R6	53	4	38	264	2.911
Inferior	L47	–30	19	–18	211	2.916
Medial	L10	–11	56	–7	316	3.152
Medial	R10/11	11	64	–11	105	2.968
Precentral	L4	–49	–4	46	475	2.993
Temporal						
Superior	L22	–60	–23	4	316	3.077
Superior	L38	–45	11	–18	105	3.148
Superior	L38	–34	11	–33	211	2.967
Middle	L21	–45	4	–29	211	2.931
Middle	R39	49	–68	23	105	3.071
Middle	L19	–41	–79	23	105	2.776
Inferior	R20	45	–8	–33	158	3.144
Inferior	L20	–45	–4	–29	158	2.824
Parietal						
Postcentral	L5	–41	–41	61	211	2.81
Occipital						
Inferior	R19	41	–79	–3	105	2.936
Limbic						
Amygdala/Lentiform Nucleus	L	–19	–8	–7	949	5.067
Insula	L13	–26	15	–7	475	3.106
Subcortex						
Caudate	L	–8	8	19	105	3.244

Coordinates are given in Talairach and Tournoux convention. BA = Brodmann's area.

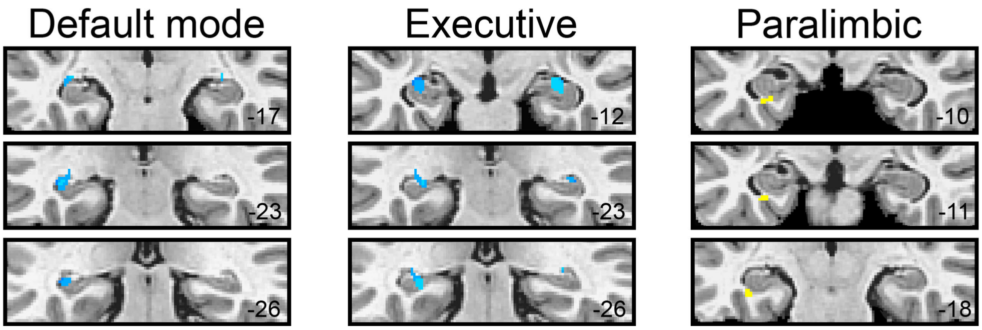

FIGURE 4 | BDNF gene group differences (val/val > val/met: blue; val/met > val/val: yellow) for each of the three resting networks within hippocampal ROIs.

and bodily homeostasis (Critchley et al., 2004; Pollatos et al., 2007). In a complementary fashion, the cingulate cortex is implicated in resolution of emotional conflict, in part through mediation of MTL structures, in particular, the amygdala (Etkin et al., 2006). Overall, the system is posited to play an important role in response selection and orientation toward action by integrating its numerous inputs. Given the unique role of the paralimbic neural network in integrating sensory stimuli and in generating responses to those stimuli, it is noteworthy that the two genotype groups differ in resting connectivity in this circuit. In the present study we demonstrate that connectivity between this network and neocortical association areas and the amygdala is enhanced in met-allele carriers. It is possible, therefore, that greater connectivity between the cortico-limbic structures underlies both the risks associated with this allele (e.g., disorders, schizophrenia) and the protective effects of this allele (e.g., reduced substance abuse, lower levels of neuroticism).

We found lower resting connectivity in widespread brain regions in met-allele carriers than in val-allele homozygotes in both the default-mode and executive networks. We emphasized the differences observed in parahippocampal and hippocampal regions because of the major role these structures play in memory formation and the known association between the met allele and poorer episodic memory (Egan et al., 2003).

In this paper, we interpret the obtained genotype differences as reflecting greater coherence between the MTL and our seed regions in individuals who are homozygous for the polymorphism of BDNF that confers higher levels of that growth factor in the MTL. Based on our analysis, the relations between our seeds and the MTL peaks range by group from $z = 0.3$ to 4.1. The finding that val-allele homozygotes have greater FC between hippocampal and both executive and salience networks is surprising given previous reports suggesting that these networks are negatively correlated with the MTL (Fox et al., 2005). It is possible that by not performing global normalization, our results are more reliable in assessing the nature of the relation between these seeds and MTL, and this may account for the observed difference (Chang et al., 2009; Murphy et al., 2009; Weissenbacher et al., 2009).

As we point out above, effective memory processing relies on distributed cortical and subcortical brain regions. In fact, the resting-state approach has been particularly fruitful in demarcating major memory networks by examining healthy participants and showing that regions of the precuneus, posterior cingulate, and parietal lobule that show greater activity to successfully remembered items are the same regions that are connected at rest to the hippocampal formation (Vincent et al., 2006), and by drawing conceptual links between memory impairments in Alzheimer patients and reduced resting-state connectivity in the DMN in the hippocampus (Greicius et al., 2004). Collectively, studies examining resting-state networks have found that levels of connectivity and integrity in these networks are related to the anomalies in the psychological and cognitive operations served by those networks (for reviews of DMN and neuropsychiatric disorders, see Greicius, 2008; Broyd et al., 2009). In the present study we found reduced connectivity in default-mode and executive networks in a number of regions known to be implicated in memory processing (hippocampus, precuneus, BA 31, inferior parietal lobule). We suggest, therefore, that reductions in FC in met-allele carriers observed for the DMN and EN may represent anomalous development of these cognitive networks in children and adolescents, a process that may give rise to the kinds of memory impairments previously reported in adult BDNF met-allele carriers (Egan et al., 2003; Hariri et al., 2003; Dempster et al., 2005; Ho et al., 2006).

There are a number of caveats concerning this work that warrant mention. First, genotype interacts with several factors, including age, pubertal status, and gender, to influence the intermediate phenotype of brain biology. As is typical of most neuroimaging studies, our sample size limited the number of analyses that we could reliably conduct. Moreover, our sample of children is relatively heterogeneous, and other factors will certainly contribute variance to the observed findings. Second, the data are not reported using multiple comparisons correction. Without this correction we have accepted more chance for Type 1 error. Our future work will attempt to replicate these findings in an independent sample. The final caveat concerns the ROI-based approach and the selection of seed regions. We have attempted to differentiate connectivity between each network and the medial temporal lobe, but have done so using seed regions that are not completely independent of one another. Indeed, investigators have documented some degree of overlap among these networks, and between these networks and sensorimotor networks (Seeley et al., 2007; Habas et al., 2009). Consequently, resting state networks should not be interpreted as independent networks of the brain, but instead, as having some degree of interplay.

In summary, the reduced mnemonic function, altered MTL function, reduced hippocampal and cortical volume, and disposition to affective illness attributed to the BDNF met allele, combined with the functional connectivity analysis in our study, suggest that reduced BDNF has implications for the formation of large-scale neural networks. Our results extend previous fMRI findings by identifying altered neural system-level functional cooperativity in carriers of the met-allele. It remains for future research to elucidate the nature of the associations among these measures in affecting specific behavioral phenotypes. The role of exogenously applied neurotrophins, for example, is an active area of investigation, and advances in this area will inform our understanding of how these large-scale networks are formed and maintained. In addition, this work will help to generate novel hypotheses about the neural underpinnings of phenotypes associated with specific BDNF alleles. For instance, increased FC in paralimbic networks may also characterize individuals with an eating disorder, given the association of the met allele with that phenotype. The present results highlight the importance of integrating neural and genetic data in elucidating phenotypic behaviors.

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Task control signals in pediatric Tourette syndrome show evidence of immature and anomalous functional activity

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Tourette Syndrome (TS) is a pediatric movement disorder that may affect control signaling in the brain. Previous work has proposed a dual-networks architecture of control processing involving a task-maintenance network and an adaptive control network (Dosenbach et al., 2008). A prior resting-state functional connectivity MRI (rs-fcMRI) analysis in TS has revealed functional immaturity in both putative control networks, with "anomalous" correlations (i.e., correlations outside the typical developmental range) limited to the adaptive control network (Church et al., 2009). The present study used functional MRI (fMRI) to study brain activity related to adaptive control (by studying start-cues signals), and to task-maintenance (by studying signals sustained across a task set). Two hypotheses from the previous rs-fcMRI results were tested. First, adaptive control (i.e., start-cue) activity will be altered in TS, including activity inconsistent with typical development ("anomalous"). Second, group differences found in task-maintenance (i.e., sustained) activity will be consistent with functional immaturity in TS. We examined regions found through a direct comparison of adolescents with and without TS, as well as regions derived from a previous investigation that showed differences between unaffected children and adults. The TS group showed decreased start-cue signal magnitude in regions where start-cue activity is unchanged over typical development, consistent with anomalous adaptive control. The TS group also had higher magnitude sustained signals in frontal cortex regions that overlapped with regions showing differences over typical development, consistent with immature task-maintenance in TS. The results demonstrate task-related fMRI signal differences anticipated by the atypical functional connectivity found previously in adolescents with TS, strengthening the evidence for functional immaturity and anomalous signaling in control networks in adolescents with TS.

Keywords: Tourette syndrome, fMRI, control, development, adolescence

INTRODUCTION

TASK CONTROL SIGNALS

Control over one's behaviors, both overt and covert, is needed on different timescales to best achieve one's goals. In particular, the focus here will be on two sets of control processes: the short-term choosing and initiation of desired information processing operations for task performance, and the longer-term maintenance of those operations. Over typical human development, capacity increases for control at both of these timescales (Crone et al., 2004; Luna and Sweeney, 2004; Davidson et al., 2006).

In a meta-analysis of healthy young adults, we have described signals putatively related to these sets of control processes (Dosenbach et al., 2006). The activity that was analyzed included signals related to task onset (start-cue), and signals related to task-maintenance (sustained). For start-cue activity, a consistent set of regions was found including regions in frontal cortex, parietal cortex, mid-cingulate, precuneus, anterior cingulate, and bilateral anterior insula regions. The regions consistently active for positive sustained activity included anterior cingulate, bilateral anterior insula, and anterior prefrontal cortex. Studies of resting-state functional connectivity MRI (rs-fcMRI), confirmed the segregation of the regions into two networks (Fair et al., 2007; Dosenbach et al., 2008).

The combined results from the functional MRI (fMRI) meta-analysis and the subsequent rs-fcMRI analyses (Fair et al., 2007, 2009; Dosenbach et al., 2008; Church et al., 2009) suggest that there are brain regions consistently involved in separable aspects of top-down control over a variety of tasks. One network of regions has positive sustained activity across the duration of a task, suggesting its involvement as a "task-maintenance" network. The other network contains regions showing primarily task initiation signal, suggesting that it may act as a faster, adaptive control network. In principle, should abnormal processing occur at either of these timescales, the result could be unintentional and/or undesired behaviors.

TOURETTE SYNDROME

Tourette Syndrome (TS) is a developmental disorder characterized by unwanted, irresistible behaviors, which are manifested as movements and vocalizations called tics (American Psychiatric

Association, 2000). Though the precise etiology of the disorder is unknown, it may in part stem from abnormal processing in the two control networks. Tics often first become apparent around ages 6–7 years, or school age. The symptoms of vocal and motor tics often increase in severity to peak intensity around the ages 10–12 years (Leckman et al., 1998). Once thought to be rare, the prevalence of tics in the pediatric population is now estimated to be around 6–12%, with TS affecting 1–10 in 1,000 children (Kurlan et al., 2001; CDC, 2009). TS is not typically associated with general cognitive impairment, except when present with co-morbid disorders (Como, 2001). Attention-deficit hyperactivity disorder (ADHD) and obsessive-compulsive disorder (OCD) are strongly co-morbid with TS (50–62% with TS also have ADHD, while 20–60% have OCD) (Freeman et al., 2000; CDC, 2009). Generalized anxiety (i.e., other than OCD) and affective disorders also have increased prevalence in TS (Singer, 2005). Indeed, TS in isolation, without any co-morbid diagnosis, is relatively uncommon, constituting only 10–15% of TS patients (Freeman et al. 2000).

TS is thought to affect frontal cortex and/or its interaction with subcortical structures including the thalamus and basal ganglia (Singer, 2005; Albin and Mink, 2006). Many anatomical studies of TS have suggested alterations of frontal and/or parietal cortex (e.g., Peterson et al., 2001; Fredericksen et al., 2002). Additionally, a recent study of children with TS has suggested that there is significant cortical thinning in bilateral ventral frontal and bilateral parieto-occipital cortex (Sowell et al., 2008). There is evidence for altered neuronal distribution within subcortical structures, indicative of an abnormality in cortico-subcortical circuitry (e.g., Kalanithi et al., 2005). Recent fMRI studies of TS in children have found regions of left prefrontal cortex that are significantly more active in affected than in unaffected children during a Stroop interference task (Marsh et al., 2007; Baym et al., 2008). While each study identified different regions, the overall findings are suggestive of compensatory prefrontal activity for difficulties in top-down control.

While the interest in the neural underpinnings of TS is growing, research in co-morbid disorders in pediatric groups is also increasing [e.g. ADHD: (Durston et al., 2007; Vance et al., 2007; Rubia et al., 2009b), and OCD: (Lazaro et al., 2008; Gilbert et al., 2009; Rubia et al., 2009a)]. As these disorders are hypothesized to have related neural substrates, research on any of them can be mutually informative to all of them. Results may help to identify any consistent differences between TS and its co-morbidities, which could allow for the creation of biomarkers for TS, thus assisting diagnosis in the future.

A previous report from our group, investigating putative control networks using rs-fcMRI signals in a group of adolescents with TS, found two intriguing results (Church et al., 2009). First, the TS group's functional connections showed significant and widespread functional immaturity (correlation strengths more similar to younger children) compared to age-matched controls involving both putative control networks. Second, for a number of connections, the TS average correlation coefficient was not similar to the correlation strengths observed at any age between 7 and 31 years. These functional connections were termed "functionally anomalous", and predominantly involved regions in the fronto-parietal, adaptive control network (Dosenbach et al., 2008). The combined results suggest that both task initiation and sustained, maintenance control signals may be affected in TS, but that regions related to the two signal types would be impacted differently. These suggestions can be captured in two related hypotheses.

First, because the most significant functional connectivity deviations in the TS group were in regions thought to be involved in adaptive control, the TS group should show anomalous start-cue signals in frontal and parietal regions associated with the adaptive control network. Further, because the adaptive control network anomalous connections did not fall along a measurable developmental trajectory, the group differences in start-cue signals should not conform to developmental differences found in typical control subjects.

Second, outside of the adaptive control network, the TS group should exhibit "immature" start-cue or sustained control signals. This immaturity would manifest itself as differences between TS and control adolescent signals more like those found in younger children than adolescent or adult subjects. The presence of child and adult data on similar tasks allowed for assessment of this possibility.

MATERIALS AND METHODS

SUBJECTS

All subjects were recruited to participate in two separate visits to the Washington University Neuroimaging Laboratories. The first visit, which lasted about 1.5 h, included the consenting process, an introduction to the scanning environment via a mock scanner, and a battery of neuropsychological tests (see below). The second visit, which lasted about 2 h, was an fMRI scanning session during which anatomical and functional scans were obtained. The Institutional Review Board at Washington University School of Medicine approved all aspects of the study.

Subjects: direct comparison

Forty-five adolescent subjects (ages 9–15 years) with TS were recruited for this study through the Washington University School of Medicine Movement Disorder Center (via database search for pediatric patients with TS, who were interested in research) and the local chapter of the Tourette Syndrome Association (TSA) (presentations at meetings and mailed advertisement). All subjects had a clinical diagnosis of TS from a licensed clinician in the community, with many diagnosed by an author of this study (BLS, who is a pediatric neurologist). Forty-one subjects completed the study, but because of excessive movement or behavioral non-compliance, only 27 had sufficient data (at least five out of eight total runs, median = eight runs) for analysis. Twenty-five of the 27 subjects overlapped with the 33 TS subjects used by Church et al. (2009). Two 9-year olds were not used in the previous study due to age matching of those groups.

Forty-six unaffected adolescent subjects (ages 10–15 years) were recruited in an attempt to create a set of subjects matched to the TS group by age, sex, IQ, and behavioral measures for the two tasks[1]. Subjects were recruited from our database of past subjects, flyers in the local community, and through word of mouth by previous subjects. From this dataset, a group of 27 unaffected subjects was matched to the TS group (**Table 1**). Twenty-six of the 27 overlapped

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[1] "Button press" reaction times were recorded for 17 of the TS subjects. Reaction time matching for the two tasks is based on comparing reaction times for these TS participants with the unaffected group. Accuracy measures include all 27 TS participants.

with the 42 control subjects reported in Church et al. (2009). Fewer subjects overall were used in the current study in order to control for task performance between the control and TS groups.

Subjects: region application

Because our hypotheses depend on the ability to assess whether certain regional activity in the TS group is "younger" than expected, we also utilized a "developmental" dataset of typically-developing younger children and unaffected adults. A healthy group of 47 7- to 9-year old children (22 female, mean 8.7 years) was compared to a healthy group of 35 21- to 29- year-old adults (19 female, mean 24.3 years) (**Table 2**). These data were used in two different ways. First, regions resulting from the comparison of children and adults were applied to the adolescent groups. Second, regions showing significantly different activity between the adolescents with and without TS were applied to the children and adults to see if the regions showed activity changes over typical development.

NEUROPSYCHOLOGICAL MEASURES

Each adolescent participant underwent a battery of neuropsychological tests and rating scales during the first visit to assess individual characteristics of interest. All tests were administered by author JAC. The Yale Global Tic Severity Scale (YGTSS) (Leckman et al., 1989), children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS) (Scahill et al., 1997), and DSM-IV based ADHD rating scale were administered. An estimated IQ from the vocabulary and matrix reasoning sections of the WISC-IV (Wechsler, 2003) was obtained, as well as measures of working memory (digit span forwards and backwards from WISC-IV), verbal fluency [controlled oral word association COWA-FAS (Benton and Hamsher, 1989)], and other aspects of executive control [Stroop color-word interference (Golden and Golden, 2002), and Trails A and B (Reitan, 1971)]. An estimated IQ from the vocabulary and matrix reasoning sections of the WAIS-III (for adults) and WISC-IV (for children) was also obtained for the unaffected adult and child groups.

TASK

A semantic judgment task presented in either a visual or auditory modality was employed.

For the visual modality version of the task, participants saw single black-on-white, line-drawn pictures on the projection screen and pressed one button for "living" items, and the other button for "non-living" items. In between stimulus presentations, a black fixation cross was projected onto the screen. The 152 picture stimuli were chosen from the Snodgrass and Vanderwart standardized set (Snodgrass and Vanderwart, 1980; Berman et al., 1989). Eight picture lists of 19 stimuli each were balanced for living/non-living status, and for Battig and Montague category (e.g., four-footed animal, insect, tool, furniture), as described by Snodgrass and Vanderwart (1980). As viewed by the participants, stimuli subtended between 1.6 and 5.7 visual degrees horizontally and between 1.1 and 5.2 visual degrees vertically. Relative size is balanced within category in the Snodgrass and Vanderwart set (e.g., a cat is smaller than an elephant; a cherry is smaller than a peach).

For the auditory modality version of the task, participants heard single syllable words (spoken by an adult female native English speaker) through their headphones, and pressed one button for "living" words, and the other button for "non-living" words. Throughout the auditory runs, a black fixation cross was projected onto the screen, and participants were instructed to watch the fixation cross. The 152 auditory stimuli used were derived from the English Lexicon database (Balota et al., 2007), and early reading word lists. The referents partially overlapped with the picture stimuli (22% of words were names of presented picture objects). Each of eight word lists had 19 stimuli, and was balanced for both living/non-living status and for Battig and Montague category similar to the pictures.

Button assignments were counterbalanced for living/non-living status across subjects.

fMRI PARADIGM

A method for extracting and separating start-cue, sustained, and trial-related signals from fMRI blood oxygenation level-dependent (BOLD) data has been demonstrated in multiple papers studying adults (Chawla et al., 1999; Donaldson et al., 2001; Visscher et al., 2003; Wenger et al., 2004), and children (Wenger et al., 2004; Burgund et al., 2006). These studies used the "mixed blocked/event-related design" (henceforth "mixed design") that allows the separation of signals tied to (1) the start of a specific task, and (2) activity sustained at a constant level across a task period, and (3) individual trials of a task, including error-related activity.

In brief, the mixed design uses aspects of both blocked designs and event-related designs for fMRI. Trials are jittered within a given task block, and task blocks are interspersed with blocks of fixation. Cues indicate the start and end of task blocks. Visscher et al. (2003) demonstrated that the mixed design could successfully separate sustained activity from transient activity and noise. For this study, the mixed design was used to study the cue-related and sustained signals in a group of TS adolescents and typical controls.

Each participant performed four experimental runs of the living/non-living visual modality of the task, and four runs of the auditory modality of the task. Each run consisted of 124 2.5 second frames of fMRI BOLD data (about 5 min 10 s duration). The modalities were not intermingled within a run. Each run consisted of three fixation-only periods and two task blocks. The black fixation cross changed to red for 1250 ms at the start and end of each task block to cue the participant to start or stop the task. The task blocks were 39 frames long (including start-cues), and within each task block, 19 picture or auditory stimuli were presented in a pseudorandomly jittered manner (the 19 trials within a single task block were jittered with a flat distribution of 0, 1, or 2 frames between stimulus onsets). Stimulus duration was 1300 ms for the pictures, and the average duration of the spoken words for the auditory modality was 696 ms (range 512–996 ms). Run type and list position (i.e., which lists appear together in a run) were varied pseudorandomly across subjects, approximating a counterbalanced design. Button-press responses were recorded, and accuracy and either button press or button release reaction times were noted.

MOVEMENT

Participant motion was corrected and quantified using an analysis of head position based on rigid body translation and rotation. The data derived from adjustments needed to realign head movement on a frame-by-frame basis were calculated as root mean square (rms) variance values using in-house software. Movement was assessed in six directions for each frame of every run in each individual: amount in millimeters of x, y, and z translation, and degrees of rotation around x, y, and z axes. Runs with movement greater than 2.0 mm rms variance were excluded from analysis.

SCANNING PROCEDURES

Participants were encouraged to minimize movements throughout the scan, but TS participants were instructed not to inhibit their tics. In order to enhance participants' abilities to maintain head position, a thermoplastic mask that molds to an individual's face and attaches to the head coil was applied. Headphones dampened scanner noise and enabled communication with participants. A Power Macintosh computer (Apple, Cupertino, CA, USA) and PsyScope software (Cohen et al., 1993) were used for display of visual stimuli. An LCD projector (Sharp model PG-C20XU) was used to project stimuli onto a MRI-compatible rear-projection screen (CinePlex) at the head of the bore, which the participants viewed through a mirror attached to the coil.

MRI data acquisition

MRI data were acquired on a Siemens 1.5-T Vision system (Erlangen, Germany). Structural images were acquired using a sagittal MP-RAGE three-dimensional T1-weighted sequence (repetition time = 9.7 ms, echo time = 4 ms, flip angle = 12°, inversion time = 300 ms, voxel size = 1.25 mm × 1 mm × 1 mm). Functional images were acquired using an asymmetric spin-echo echo-planar sequence sensitive to BOLD contrast during visual stimulation (Kwong et al., 1992; Ogawa et al., 1992) (repetition time = 2.5 s, T2* evolution time = 50 ms, flip angle = 90°, voxel size = 3.75 mm × 3.75 mm in-plane resolution) (Conturo et al., 1996). One volume, or one MR frame, was acquired every 2.5 s. During each functional run, 124 frames of 16 contiguous interleaved 8 mm thick axial slices were acquired parallel to the plane transecting the anterior and posterior commissures (i.e., the AC-PC plane). This plane was defined with an automated program utilizing a low-resolution MP-RAGE image. The first 4 frames in each run were discarded to allow stabilization of longitudinal magnetization.

fMRI processing and data analysis

Functional images were preprocessed to remove artifacts, including intensity correction, motion correction, and temporal realignment using sinc interpolation (Lancaster et al., 1995; Snyder, 1996).

BOLD activity related to the cues, trials, and task period were modeled using the general linear model (GLM) as previously described for the mixed blocked/event-related design (Donaldson et al., 2001; Visscher et al., 2003; Wenger et al., 2004). Sustained maintenance-related activity during task performance was modeled with a square-wave, starting seven MR frames after the beginning of each task block and terminating at the end of each task block. In addition, four types of event-related activity (activity related to start-cues, stop-cues, correct trials, and error trials) were modeled without assuming a shape for seven frames. This approach is equivalent to estimating the finite impulse response evoked by each event and avoids assumptions about the shape of the hemodynamic response function (Worsley et al., 1995; Josephs et al., 1997; Zarahn et al., 1997; Miezin et al., 2000; Ollinger et al., 2001). Additionally, baseline and trend effect terms for each BOLD run were included in the GLM. Individual subject data were transformed into a standardized stereotactic atlas space (Talairach and Tournoux, 1988; Lancaster et al., 1995; Snyder, 1996) and interpolated to isotropic voxels 2 mm on a side. The atlas-representative target image was produced by mutual co-registration of independent groups of 12 healthy adults and 12 healthy children. This target image strategy minimizes systematic age differences in the atlas location of cortical structures (see Burgund et al., 2002). The atlas-transformed image for each participant was checked against a reference average to ensure appropriate fit.

Methods for assessing start-cue signals in the TS and unaffected groups

A voxel-by-voxel analysis of the start-cue signal was conducted through a 2 × 2 × 7 ANOVA which modeled a within factor of modality (auditory vs. visual; two levels) and a between factor level of group (TS vs. unaffected; two levels) in addition to the effect of time (seven levels). Regions of interest (ROIs) for the start-cue analysis of group differences were derived from the resulting ANOVA images including: the main effect of time ("time"), which showed regions where there was a significant timecourse across groups; the interaction of time by group, ("time-by-group"), which identified regions where timecourses differed by group membership; and any interactions of time by modality by group, ("time-by-modality-by-group"). Regions were created from those images using in-house peak-finding software (developed by Dr. Abraham Snyder), using a hard sphere preblur of 4 mm, and were limited to those regions with a main effect of time surpassing a monte-carlo corrected z-score of 3.5 ($p < 0.0002$) and a minimum size of 24 voxels. The ROIs were then interrogated for their expected effects (e.g. a time by group effect from a region derived from the time by group image from the ANOVA), as well as for any additional effects (e.g. evaluating that same region for time by modality-by-group interaction effects). Regions that showed a significant effect of time by group ($p < 0.006$, sphericity-corrected) are reported and discussed here. The resulting region set for the start-cue analysis was displayed on an averaged brain anatomical image created from the 54 subjects. Regions centered in white matter or located off of the brain were removed from the analysis. In cases of region overlap (e.g. a region in the main effect of time image and a region in the time-by-modality image closer than 10 mm in coordinate space), the larger of the two regions was preserved.

Methods for assessing sustained signals in the TS and unaffected groups

The analysis of the sustained signal was largely similar to that described for examination of start-cues, though because it was modeled as a boxcar, the sustained signal did not include time as a factor. A voxel-by-voxel analysis of the sustained signal was conducted with a t-test combining signals across the two groups (TS and unaffected adolescents) and two modalities (auditory and visual), creating one image of the sustained signals across these factors. Regions were created using the same in-house peak-finding software, and were limited to those with a main effect surpassing a monte-carlo corrected z-score of positive or negative 2.5 ($p < 0.006$) and a minimum size of 42 voxels. A lower threshold was used than for the start-cue signal due to the weaker sustained effects. These regions were interrogated for group effects. Regions that showed a significant effect of group ($p < 0.05$, sphericity-corrected) are reported and discussed here. Regions centered in white matter or located off of the brain were removed from the analysis.

OVERALL ANALYSIS APPROACH: TWO ANALYSIS STREAMS

Regions resulting from an analysis of each signal type (start-cue or sustained, as described above) were examined separately, but using an identical approach. Two different analysis streams were used to examine each of the signal types.

First, ROIs resulting from the direct comparison of the adolescents with and without TS were examined for group differences. The regions showing group differences were subsequently interrogated for developmental effects (i.e., did the regions show differences between typical children and adults?) by applying them to unaffected child and adult groups.

The second analysis stream started with identification of regions of difference between the typical children and adults. For each of these regions, the TS and typical adolescent groups were assessed for difference. All results greater than a sphericity-corrected *p*-value of 0.05 were considered significant (Box, 1954; McAvoy et al., 2001). Given this rather liberal threshold, no single region is emphasized in this report.

Three potential conclusions were possible from this approach. First, the TS group activity was considered "functionally appropriate" for an ROI from a given signal analysis if it was not significantly different from the unaffected adolescent group activity. Second, the TS group activity was considered "functionally anomalous" if there was either a mismatch between the direction of activity change over typical development compared to the TS group activity, or if there was a significant difference between the affected and unaffected adolescents, but no difference was observed over typical development. Finally, an ROI was interpreted to be "functionally immature" in the TS group if there was a significant group difference between the TS and unaffected groups in the same direction as a developmental difference (e.g. 7- to 9-year-old children having smaller start-cue activity in a region than adults, and the TS group having smaller start-cue activity than the unaffected group).

RESULTS

DEMOGRAPHICS AND BEHAVIOR

Tic severity in the TS group ranged from mild to moderate, and 12 participants (44%) had diagnosed co-morbidities (primarily ADHD and/or OCD). Nineteen participants (70%) in the TS group were on one or more medications for tic or co-morbid symptoms. These medications were centrally acting adrenergic agents (11 subjects), SSRI anti-depressants (6 subjects), stimulants (5 subjects), atypical neuroleptics (5 subjects), benzodiazepines (2 subjects), or other (3 subjects). Ten subjects were taking more than one medication.

Performance in the adolescent groups on the two modalities of the task was not significantly different for accuracy, or for reaction times (**Table 1**). For both groups, accuracy was greater than 96% on the picture version, and 88% on the auditory version. Both groups performed the auditory task significantly slower and less accurately than the picture task ($p < 0.001$ for each group by *t*-test). There were no significant group differences on any of the neuropsychological measures (IQ, COWA-FAS, Stroop Interference, Trails B, Digit Span; $p > 0.1$ for each measure by *t*-test). As expected, the TS group had a significantly higher tic rating (YGTSS), obsessive-compulsive rating (CY-BOCS), and attention-deficit rating (DSM-IV ADHD rating) than the unaffected group ($p < 0.001$ for each measure by *t*-test).

The unaffected child and adult groups used in our analysis stream are summarized in **Table 2**.

Table 1 | Group characteristics of adolescents with and without TS.

	TS group	Unaffected group
n	27	27
Age (range)	12.53 (9.2–15.8)	12.47 (10.4–15.8)
Sex	19M/8F	18M/9F
% Correct visual modality	96.4	97.6
% Correct auditory modality	88.4	88.1
RT visual modality (msec)	852.99	857.6
RT auditory modality (msec)	1328.56	1369.94
IQ	108.7	110.4
Digit span *z*-score	–0.15	–0.2
COWA-FAS *z*-score	–0.19	–0.11
Stroop interference *z*-score	0.11	0.25
Trails B *z*-score	0.31	0.67
Tics, YGTSS rating (range)	15.6 (4–28)*	0
ADHD, DSM-IV rating (range)	29.5 (4–72)*	10.1 (0–28)
OCD, CY-BOCS rating (range)	4.63 (0–18)*	0.1 (0–2)

In brief, the 27 subjects with TS were ages 9–15 years, and the 27 unaffected subjects ranged from 10–15 years. Two subjects with TS were left-handed, while all subjects of the unaffected group were right-handed. As expected, there was a preponderance of males in the TS group, so the unaffected group was created to have a very similar sex distribution. The averages for each factor for each group are displayed. Significant differences on these factors ($p < 0.01$; two-tailed t-test) are indicated with an asterisk (*). YGTSS score was derived from motor and vocal tic assessment subscores (maximum 50 points) and does not include the global impairment rating subscore. The maximum score on ADHD rating scale was 123 points, while the maximum score on CY-BOCS was 40 points.

Table 2 | Group characteristics of unaffected adults and children.

	Adult group	Child group
n	35	47
Age (range)	24.3 (21.2–29.8)	8.7 (7.2–9.9)
Sex	16M/19F	25M/22F
% Correct visual modality	98.9	92.5
RT picture modality (msec)	1031.24*	1291.59*
IQ	120.9	114.6

*The 35 unaffected adults and 47 unaffected children only performed the visual modality of the task. *Reaction times for these groups were recorded for button release instead of button press, resulting in the slower recorded reaction times compared to the adolescent groups.*

START-CUE ACTIVITY

The region set showing a significant main effect of time for start-cue activity in the TS and unaffected adolescents largely replicates what was seen in a start-cue meta-analysis of adults (Dosenbach et al., 2006). The set includes bilateral frontal, parietal, occipital, thalamus, anterior cingulate, and insula regions (**Figure 1A**). This finding suggests a level of similarity between the TS and unaffected adolescents.

However, in a quantitative direct comparison, a significant effect of group was found for 19 regions. In all cases, the TS subjects showed decreased activity relative to their unaffected controls (**Table 3**, **Figure 2**). Of these ROIs, 13 overlap with adult-derived start-cue ROIs (Dosenbach et al., 2006). The other 6 regions include right thalamus, right putamen, right precentral gyrus, a left occipital ROI, and bilateral temporal ROIs.

These group differences occurred in several regions previously shown to have anomalous resting-state functional connectivity in adolescents with TS (Church et al., 2009). The group differences in start-cue activity were found in four adaptive control network regions including bilateral frontal, mid-cingulate, and precuneus ROIs. Significantly less start-cue activity in the TS group was also found in bilateral temporo-parietal junction and medial occipital ROIs.

When all 19 of these regions were applied to the typical adult and child groups to determine whether these regions also showed developmental differences, only 2 ROIs, the midcingulate and left fusiform, had a significant group effect between children and adults, and thus 17 regions could be deemed functionally anomalous by our criteria.

Only 5 ROIs showed start-cue differences when typical adults and children were compared. When these five regions were applied to the TS and unaffected adolescent groups, three regions, all in occipital cortex, showed differences between those groups (see **Table 4**, **Figure S1** in Supplementary Material). The TS group activity appeared more child-like in all three cases. These regions would be deemed "functionally immature" by our criteria. A dorsal medial frontal region and precuneus region were not significantly different between the TS and unaffected adolescents.

The majority of regions showing start-cue group differences in the TS group were regions that have start-cue activity functioning at mature levels in healthy children by the age of seven. The significantly smaller start-cue activity in the TS group for many regions, particularly those in the putative adaptive control network, then, is supportive of a "functionally anomalous" designation for TS adolescents. The decreased start-cue activity in the TS group for these regions cannot be explained by the presence of co-morbidities and/or medication use (see **Figure S2** in Supplementary Material).

SUSTAINED ACTIVITY

As stated earlier, immature but not anomalous functional connections were characteristic of the task-maintenance cingulo-opercular network in our previous work. We hypothesized, then, that investigation of sustained signaling, a hallmark of the task-maintenance control network, would reveal immature, but not anomalous activity in the TS group.

Regions resulting from the *t*-test of the sustained signal across groups and modalities in the current study largely replicate what has been seen previously in mixed design studies [i.e., (Dosenbach et al., 2006) and see **Figure S1** in Supplementary Material] (**Figure 1B**). Significant negative sustained signal across groups was found in bilateral precuneus, occipital, and ventral frontal regions, while positive sustained signal was evident in bilateral temporal, anterior cingulate, parietal and frontal regions.

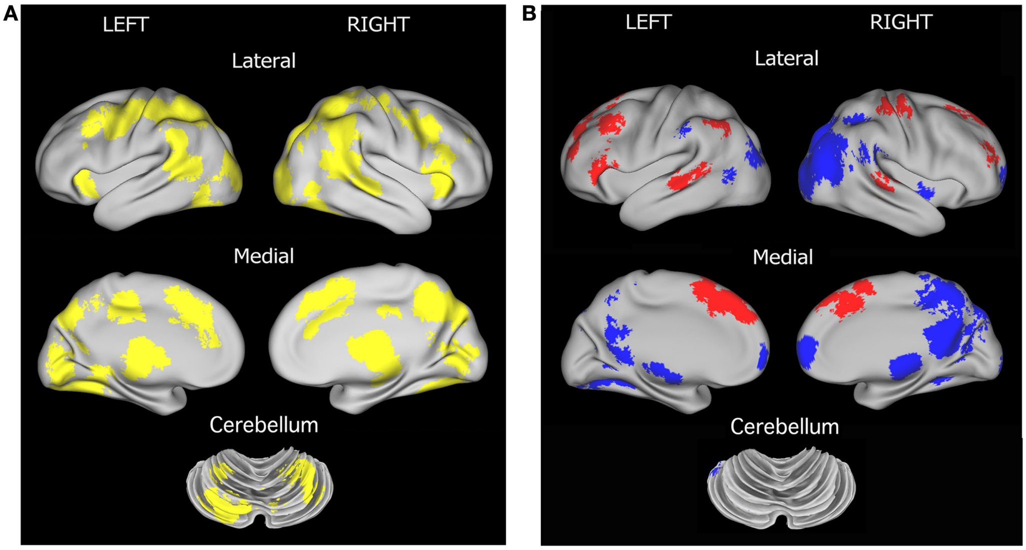

FIGURE 1 | Region sets for each signal type in the direct comparison of adolescent groups. (A) Regions with significant start-cue activity are shown in yellow (main effect of time *z*-score > 3.5); **(B)** Regions with significant sustained activity (*z*-score > 3); Regions with positive sustained signal activity are shown in red, and Regions with negative sustained signal activity are shown in blue. The lateral surfaces of the brain are presented in the top of each set of four, with the medial images on the bottom of each set. The left hemispheres are presented on the left side of each set. A dorsal view of the cerebellum is shown. All brain surfaces in this and subsequent figures were created using PALS and CARET software (Van Essen et al., 2001; Van Essen, 2002, 2005).

Table 3 | START-CUES: 19 brain regions showing a significant effect in the direct comparison (unaffected vs TS adolescent groups) of time × group; all have significantly smaller activity in the TS group (sphericity-corrected *p*-values < 0.01).

X	Y	Z	Size (# of voxels)	Location	Image source	Approximate Brodmann area	TS × unaffected adolescents	Unaffected child × adult
							Time × Group effect (*z*-score)	Time × Group effect (*z*-score)
LEFT								
–45	12	38	184	Middle frontal gyrus*	T × G	9	5.34	0.87
–56	–50	10	297	Superior temporal gyrus	Time	22	3.08	0.99
–46	–55	9	170	Middle temporal gyrus	Time, T × G	21	2.54	1.58
–44	–61	–9	128	Inferior temporal gyrus	T × G	37	5.42	1.16
–37	–74	2	43	Middle occipital gyrus	T × G, Time	19	4.40	1.84
–27	**–57**	**–9**	**325**	**Fusiform gyrus**	**Time**	**19**	**3.91**	**2.56**
–34	–64	–13	228	Fusiform gyrus	T × G, Time		5.92	1.73
INTERHEMISPHERE								
8	–70	37	512	Precuneus*	Time	7	2.84	1.52
–1	**–26**	**35**	**72**	**Cingulate gyrus***	**T × G, Time**	**31**	**4.67**	**3.46**
4	–64	11	61	Posterior cingulate	T × G, Time	31	4.85	1.32
10	–72	8	120	Cuneus	Time, T × G	31	3.47	1.80
8	–17	12	495	Thalamus*	Time		2.76	0.43
–9	–19	10	413	Thalamus*	Time		2.90	0.56
19	–9	14	127	Thalamus	T × G		4.93	1.27
RIGHT								
27	14	3	53	Putamen	T × M × G		2.72	0.38
37	–23	51	216	Precentral gyrus	Time	4	3.21	0.22
45	6	37	470	Middle frontal gyrus*	Time	9	2.96	1.01
46	–51	13	71	Superior temporal gyrus	T × G	22	4.80	1.33
55	–47	14	469	Superior temporal gyrus	Time	22	2.70	1.85

Bold indicates regions that also show a significant group effect between unaffected children and adults, sphericity-corrected p-values < 0.01. Asterisks indicate regions that overlap with regions from the two putative control networks. Abbreviations: T × G = time × group image; Time = main effect of time image; T × M × G = time × modality × group image.

Significant group differences, however, were found between the unaffected and TS groups for the sustained signal in 7 ROIs located in bilateral frontal cortex (**Table 5**, **Figure 3**). In all 7 ROIs, the TS group showed significant positive sustained signal across modalities, while the unaffected group did not.

When these regions were applied to the unaffected adult and child datasets, two regions showed a significant developmental difference in activity between healthy adults and children. An additional two regions were significantly positive in children but not in adults, but without a clear and significant group difference. In all four regions, the TS group was more similar to the child group, while the unaffected adolescents were more similar to the adult group.

Twenty-one regions were found to be significantly different between the typical child and adult groups for the sustained signal, and were located in frontal, fusiform, and temporal cortex, and the cerebellum (**Table 6**, **Figure S1** in Supplementary Material). Four of these regions, all located in frontal cortex, were significantly different between the TS and unaffected adolescents. All four regions were significantly positive in the unaffected child and TS groups, and not significantly active in the unaffected adolescent and adult groups. The findings for the TS group, as in the start-cue analysis above, cannot be explained by the presence of co-morbidities and/or medication use (**Figure S3** in Supplementary Material).

Overall, analysis of sustained signal indicates that, when different, the activation in adolescents with TS appears more similar to younger children than their age-matched control group, with this "functionally immature" sustained signaling being confined to frontal regions.

DISCUSSION

In the Introduction, we proposed two hypotheses concerning fMRI analysis of task control-related signaling in adolescents with TS. First, we hypothesized that the TS group will show anomalous start-cue activity in the adaptive control network. Second, we hypothesized that the TS group, when different from adolescent controls, will have "immature" start-cue and sustained activity in putative control regions outside of the adaptive control network.

TS GROUP HAS ANOMALOUS ADAPTIVE CONTROL ACTIVITY

The differences in start-cue signaling between the TS and unaffected adolescent groups were not similar to those observed over typical development. The results for start-cues in particular were suggestive

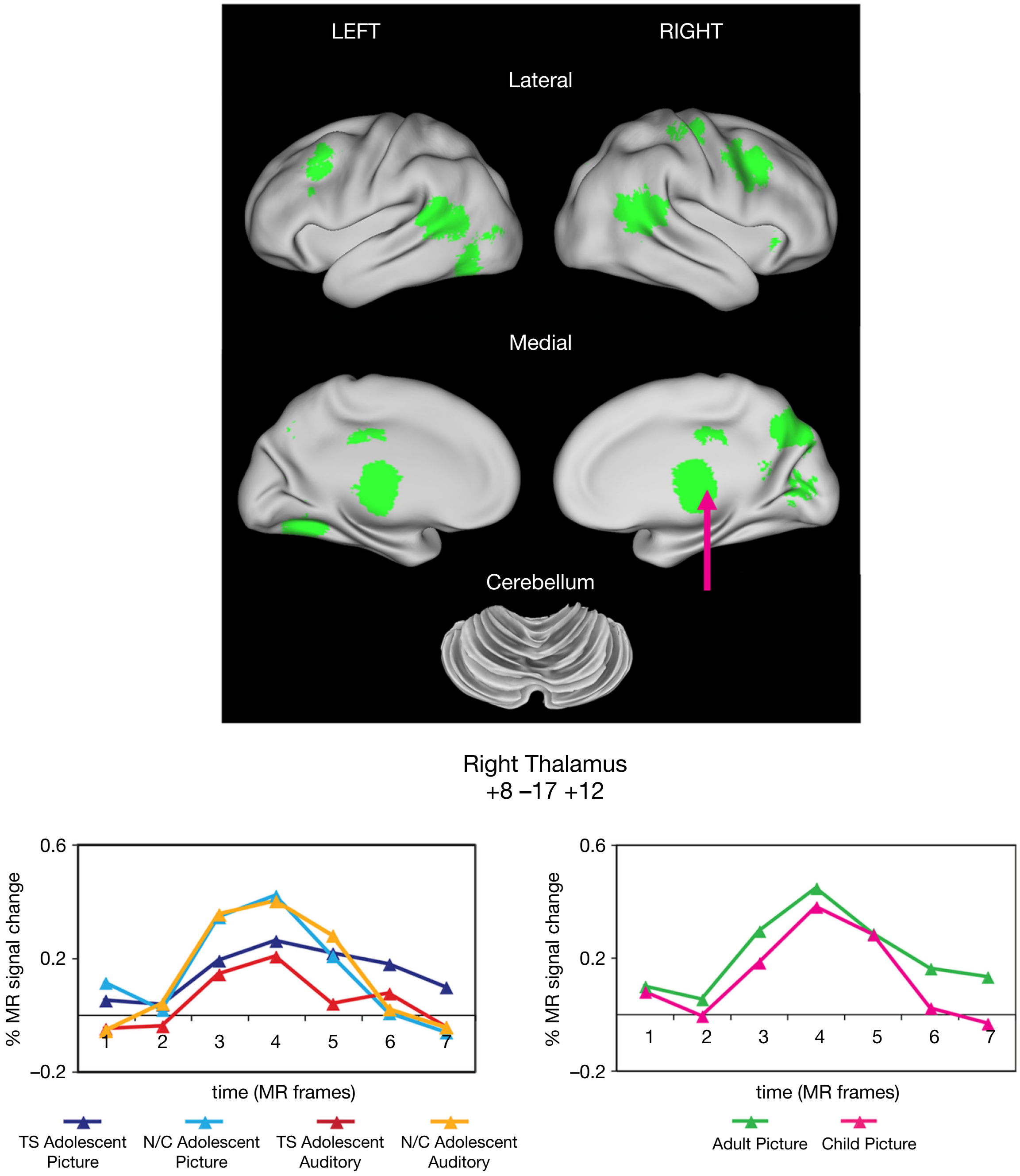

FIGURE 2 | Start-cue group effects. Time by Group effects between the adolescents with and without TS are displayed in green. In all cases, cue activity was greater for the unaffected adolescents (N/C) than those in the TS group. Example timecourses are shown for a region in medial thalamus (arrow). The timecourses for the adolescent groups for both task modalities are shown (left timecourses). There was no significant difference between unaffected child and adult groups for that region during the visual modality task (right timecourses).

of an abnormality that is not explained by functional immaturity in TS, at least within the typically-developing age range we collected (7–29 years).

Start-cue group differences were evident in regions that belong to the adaptive control fronto-parietal network, and in additional regions that consistently have start-cue activity at mature levels from the age of 7 years (i.e., no difference between typical children and adults). The transient nature of the start-cue activity makes it part of an adaptive as opposed to task-set maintenance control network (Dosenbach et al., 2006, 2008). Thus, anomalous start-cue activity in the TS group supports the previous rs-fcMRI finding of anomalous adaptive control (Church et al., 2009). However, in the present study, the extent of anomalous activity did not appear to be limited to the frontal and parietal regions found in the rs-fcMRI study. Rather, the

Table 4 | START-CUES: 5 brain regions showing a significant start-cue group effect between typical children and adults for the visual modality of the task (sphericity-corrected p-values < 0.05).

X	Y	Z	Size (# of voxels)	Location	Direction of effect	Approximate Brodmann area	Unaffected child × adult	TS × unaffected adolescents
							Time × Group effect (z-score)	Time × Group effect (z-score)
LEFT								
–37	**–77**	**–8**	**414**	**Middle occipital gyrus**	**Adult > Child**	**19**	**2.53**	**3.60**
–26	**–65**	**–10**	**206**	**Fusiform gyrus**	**Adult > Child**	**19**	**2.15**	**3.51**
INTERHEMISPHERE								
0	9	58	96	Medial frontal gyrus	Adult > Child	6	3.31	1.01
10	**–89**	**3**	**277**	**Cuneus**	**Adult > Child**	**17**	**2.37**	**3.65**
12	–66	36	430	Precuneus	Child > Adult	7	2.11	1.88

Regions were applied to the groups of adolescents with and without TS, for the picture task cue activity only; group effects where sphericity-corrected p-values < 0.05 are in bold; All significant statistics were instances of the TS group < unaffected group.

Table 5 | SUSTAINED: Seven brain regions showing a significant sustained signal group effect, such that TS adolescent group activity was greater than the unaffected adolescent group activity (sphericity-corrected p-values < 0.05).

X	Y	Z	Size (# of voxels)	Location	Approximate Brodmann area	Time × Group effect (z-score)	Direction of magnitude
LEFT							
–48	**13**	**39**	**92**	**Middle frontal gyrus**	**9**	**2.03**	+
–20	43	35	144	Superior frontal gyrus	9	2.59	+
–32	50	22	112	Superior frontal gyrus*	10	2.98	+
INTERHEMISPHERE							
–15	**29**	**37**	**123**	**Medial frontal gyrus**	**8**	**1.99**	+
–5	**40**	**36**	**160**	**Medial frontal gyrus**	**8**	**2.66**	+
12	**38**	**47**	**120**	**Superior frontal gyrus**	**8**	**2.10**	+
RIGHT							
39	–24	60	139	Precentral gyrus	4	2.16	+

Bold indicates regions that also showed either a significant sustained group effect between unaffected children and adults, such that child activity was greater than adult activity, or significant activity for children only (not adults) (sphericity-corrected p-values < 0.05). Asterisks indicate regions that overlap with regions from the two putative control networks.

anomalous activity seemed extensive throughout typical start-cue-related regions. A possible reconciliation between these results is that the additional start-cue-related regions demonstrating anomalous activity are mildly affected in TS, rendering them as evidently different in a particular task, but not in the rs-fcMRI correlations.

It is also possible that the observed differences in start-cue activity are related to extreme immaturity, such that the TS start-cue activity is most similar to an age group younger than that which we have scanned (younger than age 7 years). While the majority of start-cue-related regions appear to have adult-like levels of signaling by age seven, start-cue signals may be smaller (and thus more similar to the TS group) in younger children. Future work will need to include acquisition of data from younger subjects.

Anomalous activity in start-cue signaling suggests that TS might affect the ability to transition between task states, and that the signals related to initiation of desired task control might be competing with unwanted moment-to-moment activity (i.e., tics) that characterizes the disorder. Alternatively, some researchers have demonstrated that young individuals with TS actually show smaller task switching costs than age-matched unaffected individuals on an occulomotor switching task (Mueller et al., 2006; Jackson et al., 2007). They suggest this decreased switch cost is due to an enhanced control system resulting from years of tic suppression. Thus, the anomalous (decreased) adaptive control signaling that we observe in TS start-cue activity, and the increased sustained signals observed in frontal cortex, could be a reflection of a control system that has become more efficient for certain task demands. This possibility and the alternative (that patients with TS have an impaired adaptive control network) can be explored further in the future by attempting to tax the adaptive control system by manipulating task rules, frequency of switching, or task difficulty (e.g. Baym et al., 2008).

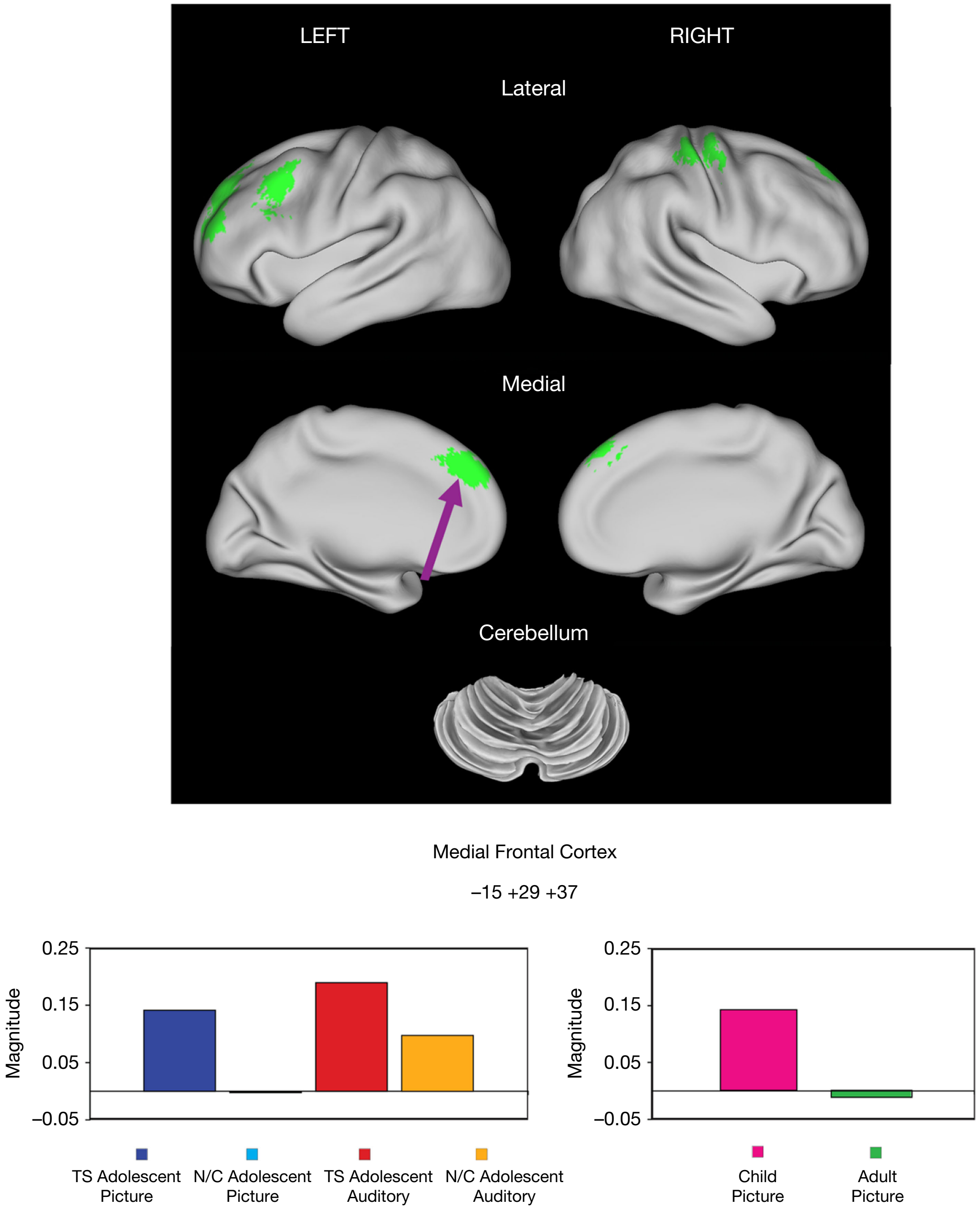

FIGURE 3 | Sustained activity group effects. Regions showing significant differences in sustained activity between TS and unaffected adolescents (N/C) are displayed in green. All significant differences were in frontal cortex. An example of the sustained magnitudes for each adolescent group for each task modality is shown for a medial frontal region (arrow) (left magnitude set). This same region also showed a significant developmental effect between healthy children and adults for the picture version of the task. Note the similarity between the TS group and the younger healthy children for the visual modality of the task, while activity for the age-matched unaffected adolescents is more similar to that observed in adults for the region for that task.

Previous research has pointed to abnormal cortical-striatal-thalamic-cortical signaling in TS (e.g., Singer, 2005; Albin and Mink, 2006). The significant under-activity observed in the thalamus for start-cue signals, and the increased activity in frontal regions for sustained signaling could be related to this type of signaling pathway. However, the lack of a common signal being significantly different in TS for these regions makes the explanation less straightforward. There could be sustained over-activity in frontal cortex that is related to transient under-activity in the subcortical structures. However, we also see this transient under-activity in other motor-related regions

Table 6 | SUSTAINED: Twenty-one brain regions showing a significant sustained group effect between typical children and adults for the picture living/non-living task (sphericity-corrected *p*-values < 0.05).

X	Y	Z	Size (# of voxels)	Location	Approximate Brodmann area	Direction of effect	Unaffected child × adult	TS × unaffected adolescents
							Time × Group effect (*z*-score)	Time × Group effect (*z*-score)
LEFT								
–40	26	33	344	Middle frontal gyrus	9	Adult n.s., child pos.	–2.72	2.31
–23	–9	54	63	Motor cortex	4	Adult pos., child neg.	3.09	0.50
–32	–86	1	473	Inferior occipital gyrus	18	Adult neg., child n.s.	–2.26	0.36
–22	–87	16	47	Middle occipital gyrus	19	Adult neg., child pos.	–3.22	–0.99
–35	–34	–16	187	Fusiform gyrus	20	Adult n.s., child pos.	–2.11	0.39
–51	–13	–28	66	Inferior temporal gyrus	20	Adult pos., child neg.	3.23	1.65
–48	5	–30	80	Middle temporal gyrus	21	Adult n.s., child pos.	–3.15	–0.34
INTERHEMISPHERE								
–17	11	50	30	Medial frontal gyrus	6	Adult pos., child n.s.	3.05	0.92
0	9	58	96	Medial frontal gyrus	6	Adult pos., child neg.	3.60	0.80
12	40	42	147	Medial frontal gyrus	8	Adult n.s., child pos.	–2.12	0.81
–7	30	39	256	Medial frontal gyrus	8	Adult n.s., child pos.	–2.13	1.98
9	**29**	**22**	**74**	**Cingulate gyrus**	**32**	**Adult n.s., child pos.**	**–2.26**	**2.00**
–17	–94	–1	147	Cuneus	17	Adult neg., child n.s.	–3.90	0.05
–2	–80	14	70	Cuneus	18	Adult neg., child pos.	–3.16	0.55
–1	–51	–12	78	Cerebellum		Adult n.s., child pos.	–2.11	1.41
RIGHT								
34	**54**	**0**	**52**	**Middle frontal gyrus**	**10**	**Adult neg., child pos.**	**–3.26**	**2.18**
57	1	33	23	Inferior frontal gyrus	44	Adult n.s., child neg.	3.49	0.33
34	–83	0	475	Inferior occipital gyrus	18	Adult pos., child n.s.	2.01	0.67
40	–64	47	228	Inferior parietal lobe	40	Adult pos.,<child pos.	–2.62	–0.54
46	–69	33	390	Angular gyrus	39	Adult n.s., child pos.	–2.11	0.05
43	–59	–32	74	Cerebellum		Adult pos.,<child pos.	–2.02	0.28

Direction of effect indicates the direction of magnitude for the child and adult groups: pos. = Positive; neg. = Negative; n.s. = non-significant. Regions were applied to the groups of adolescents with and without TS for the picture task only; group effects where sphericity-corrected p-values < 0.05 are in bold; All significant statistics were instances of the TS group > unaffected group.

including motor cortex and cerebellum, as well as left insula (trial-related activity; data not shown), suggesting that subcortical structures are not uniquely showing this activity pattern in TS.

TS GROUP APPEARS FUNCTIONALLY IMMATURE COMPARED TO AGE-MATCHED CONTROLS IN SUSTAINED SIGNALING

The sustained signal differences between the TS and unaffected adolescent groups overlapped substantially with the differences found between unaffected children and adults in frontal cortex (compare **Figure 3** and **Figure S1** in Supplementary Material). The observation that the TS group has positive sustained activity in medial and left dorsolateral frontal regions while the unaffected group does not, and that typical children have positive sustained activity in similar or overlapping frontal regions, while typical adults do not, suggests that sustained control is functionally more immature in the TS group than in the unaffected peers.

A key finding described in our previous rs-fcMRI research is that over development the adaptive control network and task-maintenance control network become more segregated, while in 7- to 9-year-old children they have a number of interconnections, particularly between frontal regions (Fair et al., 2007). Similarly, we report here that the sustained signal is elevated in typically-developing children in parts of the brain beyond the task-maintenance control network (**Figure S1** in Supplementary Material). We find, however, that sustained signals are apparently adult-like by typical adolescence. The presence of elevated sustained signal in frontal regions in adolescents with TS (similar to that observed in unaffected younger children) could be a reflection of a delay in segregation of the two control networks in TS.

A left dorsolateral region (Talairach coordinates −48, 13, 39) that has greater positive sustained signal in TS adolescents compared to age-matched unaffected adolescents is similar in location to the left frontal regions of difference found between children and adolescents with and without TS in Baym et al. (2008) (−48, 23, 24; 18 mm vector distance away) and Marsh et al. (2007) (−42, 14, 32; 9.3 mm vector distance away) where the TS group had greater activity than the unaffected group. Both of these studies have suggested that the greater frontal activity they observed in children with TS is related to engagement of compensatory processes to make up for impaired control signaling in other regions, including the striatum. These regions are also close to a region (Talairach coordinates −40, 26, 33; vector distance 16.4 mm away from TS region; see **Table 6**), which shows a difference between unaffected children and adults (and both adolescent groups). The similarity to a region of developmental change suggests that the hypothesized compensatory processes could instead be delayed maturity of this general region in TS, or an interaction of these concepts.

TYPICAL DEVELOPMENT IS AN IMPORTANT TOOL FOR UNDERSTANDING ATYPICAL DEVELOPMENT

These results, particularly in combination with the data from (Church et al., 2009), emphasize the importance of having an understanding of typical development in order to place results from atypical populations in context (see also Johnson et al., 2002). The set of regional differences we observe for each signal type (e.g. start-cue signals) between adolescents with and without TS is much enhanced by our understanding of whether those regions also change over development during the same semantic judgment task. An understanding of the developmental trajectories of regional activity during a given task is critical for interpreting results in the transitional environment of adolescence. In addition, it is important to note that we observe both elevated activity in the TS group (sustained signals) and decreased activity in the TS group (start-cue signals), suggesting that there may not be a simple shift in signaling level in the TS group. Thus, the developmental trajectories of these signals are all the more critical to providing a plausible interpretation of these results.

It is interesting to note that these functional activation differences were observed in the TS group despite a lack of behavioral and neuropsychological differences. These results suggest that despite a lack of performance differences on this relatively easy task, the brain activity in TS is functionally different, demonstrating the added value of neuroimaging tools to behavioral and clinical measures.

Investigating developmental trajectories across patients with TS is an important consideration for future study. The TS group size in the present study is not sufficiently large to address age-related changes across the span of 9–15 years, the age of the subjects from whom data has been acquired at this point. However, future studies could examine whether there are different signal results between younger and older TS participants, or could test whether TS participants experiencing a relief of symptoms with age also show a recovered maturity of functional signaling.

IS FUNCTIONAL IMMATURITY SPECIFIC TO TS?

While functional immaturity is not a feature that has been well characterized in TS, immaturity using neuroimaging measures has previously been reported in studies of other developmental disorders including autism (Courchesne and Pierce, 2005; Just et al., 2007; Turner et al., 2006; Langen et al., 2009) and ADHD (Tian et al., 2006; Durston et al., 2007; Kelly et al., 2007; Shaw et al., 2007; Sonuga-Barke and Castellanos, 2007; Vance et al., 2007; Rubia et al., 2009a). At present it remains unclear whether these functional immaturities are common across different developmental disorders and whether or not they involve the same or similar brain regions.

Furthermore, it is possible that there are confounding factors in the TS group that are contributing/accounting for the functional immaturity and/or anomalous signaling results. For example, ADHD is a common co-morbidity with TS, and thus it is also present in a subset of our TS participants. Similarly, medication could be playing a role in the observed sustained or start-cue activity differences observed in the TS group. While our study is underpowered to do a full analysis of the effects of co-morbidities and medications, when we examined subgroups on and off medication, or with and without co-morbidities, within our whole TS group, there was no consistent indication that the results reported here could be explained by medication use and/or co-morbidities (**Figures S2 and S3** in Supplementary Material). The use of medication and frequent diagnosis of co-morbidities in TS are challenging but important issues that need to be more fully addressed in future studies. Finally, movement during scanning, while strictly limited for all subjects in this analysis, and not correlated with any signal type (data not shown), could possibly account for some of our group differences.

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SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found online at http://www.frontiersin.org/humanneuroscience/paper/10.3389/neuro.09/038.2009/

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Neuroanatomical correlates of developmental dyscalculia: combined evidence from morphometry and tractography

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Poor mathematical abilities adversely affect academic and career opportunities. The neuroanatomical basis of developmental dyscalculia (DD), a specific learning deficit with prevalence rates exceeding 5%, is poorly understood. We used structural MRI and diffusion tensor imaging (DTI) to examine macro- and micro-structural impairments in 7- to 9-year-old children with DD, compared to a group of typically developing (TD) children matched on age, gender, intelligence, reading abilities and working memory capacity. Voxel-based morphometry (VBM) revealed reduced grey matter (GM) bilaterally in superior parietal lobule, intra-parietal sulcus, fusiform gyrus, parahippocampal gyrus and right anterior temporal cortex in children with DD. VBM analysis also showed reduced white matter (WM) volume in right temporal-parietal cortex. DTI revealed reduced fractional anisotropy (FA) in this WM region, pointing to significant right hemisphere micro-structural impairments. Furthermore, FA in this region was correlated with numerical operations but not verbal mathematical reasoning or word reading. Atlas-based tract mapping identified the inferior longitudinal fasciculus, inferior fronto-occipital fasciculus and caudal forceps major as key pathways impaired in DD. DTI tractography suggests that long-range WM projection fibers linking the right fusiform gyrus with temporal-parietal WM are a specific source of vulnerability in DD. Network and classification analysis suggest that DD in children may be characterized by multiple dysfunctional circuits arising from a core WM deficit. Our findings link GM and WM abnormalities in children with DD and they point to macro- and micro-structural abnormalities in right hemisphere temporal-parietal WM, and pathways associated with it, as key neuroanatomical correlates of DD.

Keywords: mathematical disability, white matter, grey matter, diffusion tensor imaging, voxel-based morphometry, development

INTRODUCTION

Mathematical skills are becoming increasingly critical for achieving academic and professional success. Dyscalculia is a developmental learning deficit estimated to have a prevalence of about 5–6% (Butterworth, 2005; Cohen Kadosh and Walsh, 2007; Rubinsten and Henik, 2009). Developmental dyscalculia (DD) can be defined as "a disorder of numerical competence and arithmetic skill which is manifest in children of normal intelligence who do not have acquired neurological injuries" (Temple, 2002). The prevalence of mathematical difficulties arising from non-specific attentional, working memory and reading disabilities has an even higher prevalence rate, estimated to range from 5% to 20% of young children depending on the precise criteria used (Berch and Mazzocco, 2007). Although their prevalence rates are at least as high as dyslexia and reading disabilities, dyscalculia and related mathematical disabilities have received much less attention from developmental neuroscientists.

Normative functional neuroimaging studies have implicated the intra-parietal sulcus (IPS) within the posterior parietal cortex (PPC) as a region specifically involved in the representation and manipulation of numerical quantity (Dehaene et al., 2003). With experience and learning, the IPS builds an increasingly amodal, language-independent semantic representation of numerical quantity (Bruandet et al., 2004; Ansari, 2008; Rosenberg-Lee et al., 2009). In addition to the IPS, depending on the nature and complexity of specific tasks, mathematical information processing also critically involves activation and deactivation in a more distributed network of regions within the dorsal visual stream encompassing the superior parietal lobule (SPL), the angular and supramarginal gyri in the PPC and the ventral visual stream encompassing the lingual and fusiform gyri in the inferior temporal cortex (Menon et al., 2000; Rickard et al., 2000; Zago et al., 2001; Delazer et al., 2003; Grabner et al., 2008; Wu et al., 2009). Functional neuroimaging studies examining the neural basis of dyscalculia have yielded mixed results in these regions. Price et al. (2007) reported hypoactivation of the IPS in dyscalculic children during number comparison tasks, while Simos et al. (2008) demonstrated increased neurophysiological activity in inferior and superior parietal regions in the right hemisphere in children with mathematical difficulties compared

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Abbreviations: DD, developmental dyscalculia; DTI, diffusion tensor imaging; FA, fractional anisotropy; GM, gray matter; IPS, intra-parietal sulcus; PPC, posterior parietal cortex; ROI, region of interest; SPL, superior parietal lobule; TD, typically developing; VBM, voxel-based morphometry; WM, white matter.

to controls. One potential reason for these discrepancies is that the profile of functional deficits in mathematical task processing varies with the level of task difficulty and type of operation performed. Thus, for example, Kesler et al. (2006) found that, compared with controls, children with Turner syndrome recruited additional neural resources in frontal and parietal regions during an easier, two-operand math task, whereas during a more difficult three-operand task, they showed significantly less activation in frontal, parietal and subcortical regions than controls. Moreover, mathematical abilities in children involve multiple cognitive processes, most notably visuospatial processing and working memory (Adams and Hitch, 1998; Swanson and Beebe-Frankenberger, 2004), and the relative contribution of these processes changes with instruction and development (Meyer et al., 2009). Little is currently known about functional abnormalities underlying the neural basis of these component processes in children with DD. Recent conceptual models of dyscalculia raise the possibility that heterogeneous deficits in DD can arise from common abnormalities (Rubinsten and Henik, 2009).

One approach to the systematic investigation of DD in children is to examine whether there are neuroanatomical and structural differences in the brains of these children when compared to well-matched TD controls. White matter (WM) and gray matter (GM) integrity are crucial for nearly all higher cognitive operations (Johansen-Berg and Behrens, 2006). However, only one study to date has used voxel-based morphometry (VBM) to examine GM and WM integrity in DD. Rotzer et al. (2008) reported decreased GM volume in the right IPS, anterior cingulate cortex, left inferior frontal gyrus, and bilateral middle frontal gyri in 9-year-old children, compared to IQ matched controls. They also reported WM volume reductions in the left frontal cortex and right parahippocampal gyrus in children with DD. It is, however, not clear whether the two groups were matched on reading and working memory abilities. Furthermore, morphometric studies leave unclear whether WM microstructural and tract deficits underlie DD. In TD children, van Eimeren et al. (2008) reported that FA in the left superior corona radiata and the inferior longitudinal fasciculus were correlated with mathematical abilities in TD children. The precise anatomical location, however, remains unknown because the analysis was constrained to large WM regions. Nevertheless, it is noteworthy that the laterality and location of the deficits differs considerably from those reported by Rotzer et al. (2008). Related studies in children with neurodevelopmental and neurogenetic disorders have suggested that mathematical difficulties may be related to GM abnormalities in the IPS and surrounding WM within the PPC. Isaacs et al. (2001) reported reduced left parietal GM in children with low birth weight. Molko et al. (2003) observed abnormal sulcal depth in bilateral IPS in adults with Turner syndrome (Bruandet et al., 2004). Barnea-Goraly et al. (2005) found that fractional anisotropy (FA) of WM tracts in left parietal WM matter adjacent to the IPS was significantly correlated with arithmetic ability in 7- to 19-year-old children and adolescents with 22q11.2 deletion (velocardiofacial) syndrome. However, mathematical difficulties in these neurodevelopmental disorders are typically accompanied by profound visuo-spatial and reading disabilities, thereby limiting their generalizability to pure DD.

Over the past several years diffusion tensor imaging (DTI) and VBM measures have been used to identify consistent white matter integrity and gray matter density deficits in children with reading disability (Ben-Shachar et al., 2007; Schlaggar and McCandliss, 2007; Kronbichler et al., 2008; Richards et al., 2008; Steinbrink et al., 2008). Parallel efforts in the domain of dyscalculia have been slow to develop, and the precise anatomical locations and identity of WM tracts that are impaired in children with DD is currently unknown.

The recruitment and assessment of a homogenous group of children with DD, as well as a well-matched TD control group, presents particular challenges. Except for the study by Rotzer et al. (2008), previous brain imaging studies of DD have been limited by relatively small sample sizes, wide age ranges, poor neuropsychological evaluation and assessments, and, most problematically, the presence of other comorbid cognitive disabilities. Here we report findings from a well-characterized cohort, comprising 23 children with DD and 24 TD children matched for IQ, age, gender, reading ability and working memory. We examined macro-structural and micro-structural integrity using measurements of GM and WM volume, and fractional anisotropy (FA) in DD. We also examined the relation between micro-structural deficits and key individual subscales of the Wechsler Individual Aptitude Test, 2nd edition (WIAT-II) (Wechsler, 2001). We used DTI tractography in order to delineate abnormal WM pathways and to link WM and GM abnormalities. Finally, we conducted a novel classification analysis to examine whether network-level connectivity differs between children with DD and TD children. Based on prior neuroimaging studies in children and adults, we hypothesized that children with DD would should deficits in dorsal PPC and inferior temporal cortex GM and WM.

MATERIALS AND METHODS

PARTICIPANTS

Forty-seven 7–9 year old children (25 females) in the 2nd and 3rd grades were selected from an ongoing longitudinal MRI study of brain structural and functional changes underlying the development of mathematical ability. A group of DD children ($N = 23$) was selected based on their scores on a standardized measure of mathematics ability, derived from two subscales of the WIAT-II. Children with either a Numerical Operations Score or Math Composite Score at or below 95 were considered DD. A group of gender-, IQ-, reading-ability- and working-memory matched children who did not qualify as DD formed a control group ($N = 24$). The participants were recruited from the San Francisco Bay Area using advertisements in school and local newspapers, community and electronic bulletin boards, community organizations, and other public locations. Written informed consent was obtained from the children as well as their legal guardians. All protocols were approved by the human participants Institutional Review Board at the Stanford University School of Medicine. All participants were volunteers and were treated in accordance with the APA "Ethical Principles of Psychologists and Code of Conduct."

STANDARDIZED NEUROPSYCHOLOGICAL ASSESSMENTS

The Child Behavior Check List (CBCL) was administered to all participants to exclude children with behaviors that are highly linked to later psychopathology (Achenbach, 1991). Participants were administered a demographic questionnaire, the Wechsler Abbreviated Scale of Intelligence (WASI; Weschler, 1999), Wechsler Individual Achievement Test, Second Edition (WIAT-II; Wechsler, 2001), and Working Memory Test Battery For Children (WMTBC;

Pickering and Gathercole, 2001). WASI provided a standardized measure of full-scale IQ, while WIAT-II yielded measures of reading ability (Word Reading & Reading Comprehension Scores), and math ability (Numerical Operations, Math Reasoning Scores and derived from them Math Composite Score). A shortened version of WMTBC was also administered to obtain several measures of working memory (Digit Recall, Block Recall, Counting Recall and Backward Digit Recall subscales). For each participant, a composite working memory score was calculated by averaging standardized scores across the obtained WMTBC measures. Neuropsychological tests were administered by trained research assistants, and individual scores were validated using multiple raters.

MRI DATA ACQUISITION

High-resolution structural images were acquired with T1-weighted spoiled grass gradient recalled (SPGR) inversion recovery 3D MRI sequence. The following parameters were used: TI = 300 ms, TR = 8.4 ms; TE = 1.8 ms; flip angle = 15°; 22 cm field of view; 132 slices in coronal plane; 256×192 matrix; 2 NEX, acquired resolution = $1.5 \times 0.9 \times 1.1$ mm.

The DTI data was collected using a diffusion-weighted single-shot spin-echo, echo planar imaging sequence (TE = 70.8 ms; TR = 8.6 s; field of view = 220 mm; matrix size = 128×128; bandwidth = ± 110 kHz; partial k-space acquisition; 4NEX). We acquired 63 axial, 2-mm thick slices (no skip) for $b = 0$ and $b = \sim 850$ s/mm^2 (the latter by applying gradients along 23 noncollinear diffusion directions). Structural MRI and DTI images were acquired in the same scan session.

VOXEL-BASED MORPHOMETRY ANALYSIS

Voxel-wise differences in brain anatomy were assessed using the optimized voxel-based morphometry (VBM) method described by Good et al. (2001). The analysis was performed using the VBM5 toolbox developed by Christian Gaser (University of Jena, Germany)[1] for Statistical Parametric Mapping Software (SPM5, Wellcome Department of Imaging Neuroscience, London, UK).

Prior to analyses, the structural images were resliced with tri-linear interpolation to isotropic $1 \times 1 \times 1$ voxels and aligned to conventional AC-PC space, using manually identified landmarks, including the anterior commissure (AC), the posterior commissure (PC) and the mid-sagittal plane.

As a part of the VBM5 pipeline, the images were spatially normalized to the Montreal Neurological Institute (MNI) common stereotactic space. Spatial transformation was nonlinear with warping regularization = 1; warp frequency cutoff = 25.

Spatially normalized images were then segmented into GM, WM, and cerebro-spinal fluid compartments, using a modified mixture model cluster analysis technique (Good et al., 2001) with the following parameters: bias regularization = 0.0001, bias FWHM cutoff = 70 mm, sampling distance = 3, HMRF weighting = 0.3. As recommended by Gaser for children or elderly populations, we used no tissue priors for segmentation. Voxel values were modulated by the Jacobian determinants derived from the spatial normalization: areas that were expanded during warping were proportionally reduced in intensity. We used modulation for nonlinear effects only (while the warping itself included both an affine and a nonlinear component). When using modulated images for performing subsequent group comparisons, the inference is made on measures of volume rather than tissue concentration (density). The use of modulation for nonlinear but not affine effects ensures that the further statistical comparisons are made on relative (that is, while controlling for overall brain size) rather than absolute measures of volume.

The segmented modulated images for WM and GM were smoothed with an isotropic Gaussian kernel (10 mm full width at half maximum). The size of the kernel for smoothing was chosen as recommended by Gaser for modulated images, since modulation introduces additional smoothing. Between-group comparisons for GM and WM volumes were performed in SPM5 using two-sample *t*-tests on smoothed images. A voxelwise significance threshold of $p < 0.001$ (uncorrected) was used in order to facilitate comparisons with previous studies (Isaacs et al., 2001; Rotzer et al., 2008). Additionally, in order to integrate VBM and DTI data, we thresholded statistical comparison maps adjusting for search volume with height threshold of $p < 0.01$, a nonstationary cluster extent (Hayasaka et al., 2004) threshold of $p < 0.05$ corrected for multiple comparisons with family-wise error (FWE) correction, and correction for non-isotropic smoothness, as implemented in VBM5 toolbox.

REGIONS OF INTEREST FOR THE ANALYSES OF WHITE MATTER MICROSTRUCTURE

We used the more liberally thresholded maps ($p < 0.01$, and a cluster extent threshold of $p < 0.05$ corrected for multiple comparisons) to define GM and WM regions of interest (ROIs), as noted above. Statistical maps corresponding to group differences (TD > DD) images were thresholded, binarized and used in further analyses. They are referred to as "WM ROI" and "GM ROI" for white and grey matter comparisons, correspondingly. Specifically, the voxels where the TD group had significantly greater relative volume measurements were included in the WM ROI mask.

To examine structures that contribute to group differences in WM integrity, the WM ROI was parcellated using the white matter tractography atlas provided by Laboratory of Brain Anatomical MRI, John Hopkins University, hereafter referred to as the JHU atlas (Hua et al., 2008).

FRACTIONAL ANISOTROPY ANALYSIS

The aim of this analysis was to examine group differences in WM integrity using complementary and independent measures derived from DTI. DTI data were processed using FMRIB Software Library (Beckmann and Smith, 2004). A diffusion tensor model was fitted on brain data after removing eddy current distortion effects and extracting brain voxels. For every voxel, FA was computed from voxel-wise tensor measures. Each subject's FA data were then warped into a common space using nonlinear registration with FSL Nonlinear Registration Tool to FMRIB58_FA standard space image available as a part of FSL tools. To examine FA in the regions where reduced WM intensity was observed in the DD children, the mean and standard deviation of the FA values across the voxels within the WM ROI was computed for each participant. The mean FA values were scaled by corresponding standard deviations in each individual, yielding standardized within-WM-ROI FA summary measures. These FA measures were compared between the groups using a two-sample *t*-test.

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[1] http://dbm.neuro.uni-jena.de/vbm

PROBABILISTIC TRACTOGRAPHY ANALYSIS

The aim of this analysis was to examine regional group differences in projection densities from the WM ROI. The primary question was whether the observed volumetric differences in GM and WM, identified by VBM analyses, can be linked via axonal pathways. Reduced projection densities in pathways linking the GM and the WM ROIs would provide evidence that the observed WM and GM structural differences between the groups reflect impairments of the same network. Probabilistic tractography was performed for each participant with FSL tools, using BEDPOST estimates of diffusion parameters, yielding connectivity distribution seeded from the WM ROI. Probtrackx algorithm draws samples from the probability distributions of fiber directions at each voxel, generating trajectories of fibers and computing the observed frequency of those that originate from the seed voxel (Behrens et al., 2003). The resulting image describes, for each voxel in the full brain volume, the likelihood of a connection with any voxel within the seed mask, based on 5000 samples. The obtained WM ROI connectivity maps were compared across participants using a two-sample *t*-test as implemented in SPM, inference limited to the GM ROI in order to test our original hypothesis on relationship between the regions of reduced volume in GM and WM. The resulting maps were thresholded at height threshold of $p < 0.05$ and non-stationary cluster extent threshold of $p < 0.05$ with family-wise error (FWE) correction for multiple comparisons and correction for non-isotropic smoothness.

DETERMINISTIC TRACTOGRAPHY

The aim of this analysis was to identify WM tracts traversing the WM ROI that showed deficits in children with DD. Fifty-eight candidate GM targets (**Table 1**) were selected from 116 regions defined in Anatomical Automated Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002). The ROIs were selected based on AAL regions that overlapped with voxels that showed VBM GM deficits in the DD group; thus, all 58 ROIs chosen were in the posterior part of the brain encompassing dorsal and ventral visual stream areas in the posterior parietal cortex, the inferior temporal cortex, and the occipital cortex, as well as the medial temporal lobe and the cerebellum. Importantly, we excluded regions such as the superior and middle temporal gyri which are typically not activated during mathematical tasks (Rivera et al., 2005; Wu et al., 2009). One reason for choosing such large AAL-based ROIs is to recover as many tracts linking distal brain regions as possible. DTI data were preprocessed, and tractography performed, using VISTALAB Software (Stanford University)[2]. Eddy current distortion effects were removed by determining a constrained non-rigid image registration using normalized mutual information (Bammer et al., 2002). The six elements of the diffusion tensor were determined by multivariate regression (Basser, 1995; Basser and Pierpaoli, 1996). The eigenvalue decomposition of the diffusion tensor was computed. For each subject, the T2-weighted ($b = 0$) images were coregistered to the corresponding T1-weighted 3D anatomical images. A rigid-body transform from the native T1-weighted image space to the conventional AC-PC aligned space was computed using several manually identified landmarks, including the anterior commissure (AC), the posterior commissure (PC) and the mid-sagittal plane. The DTI data were then resampled to this AC-PC aligned space with 2 mm isotropic voxels using a spline-based tensor interpolation algorithm (Pajevic et al., 2002), taking care to rotate the tensors to preserve their orientation with respect to the anatomy (Alexander et al., 2001).

Whole-brain fiber tracking was performed on AC-PC aligned tensor maps. The seeds for tractography were selected from a uniform 3D grid with 1 mm step, spanning the whole brain white matter mask (only voxels that had FA > 0.25 were chosen as seeds). Multiple fiber tracts were estimated by using a deterministic streamlines tracking algorithm (Conturo et al., 1999; Mori et al., 1999; Basser et al., 2000) with a fourth-order Runge–Kutta path integration method and 1-mm fixed-step size. A continuous tensor field was estimated by using trilinear interpolation of the tensor elements. Path tracing proceeded until the FA fell < 0.15 or until the angle between the current and previous path segments was >30°. Starting from the initial seed point, fibers were traced in both directions along the principal diffusion axis.

From the resulting whole-brain tracts we extracted the pathways traveling through the WM ROI. Since the tractography was performed in individual AC-PC aligned space, the WM ROI was nonlinearly warped into each individual space using SPM spatial normalization routines. Fiber pathways that did not travel through the WM ROI (minimum distance of 0.89 mm from any point of a fiber to any point within the ROI), as well as fiber tracks whose endpoints were not within the predefined 58 AAL atlas grey matter regions, were omitted from the further analysis. In the areas with high FA, the dense seeding schema often produces a significant number of duplicate pathways that are highly similar in their trajectory. Due to non-uniform spatial FA distribution, fiber pathway count has limited use as a quantitative metric comparable across brain regions and individuals. We removed redundant fibers, frequent in high FA regions, using an algorithm based on three metrics: the length of a pathway (minimum = 10.0 mm), the linear anisotropy along a pathway (minimum = 0.10), and the pairwise distance between pathways (minimum average point-to-curve distance = 2 mm, computed across portions of the pathways where these portions are at least 1 mm apart from each other). For the details on the culling algorithm, please see Zhang et al. (2003). We argue that this procedure provides a “resampling” of fiber space, approximately representing this space in terms of fascicles with a given unit diameter of 2 mm, subject to finite volume constraint. Pathway count in this regularized space becomes roughly proportional to the cross-sectional area of a fiber group and it can be used as a measure of structural connectivity.

NETWORK ANALYSIS

Based on the deterministic tractography described above, we conducted exploratory classification analysis to examine whether network-level connectivity differs in children with DD and TD children. Culled pathways (2 mm-diameter “fascicles”) in each individual were first classified to 58 AAL labels according to their projection target, each pathway contributing two targets. The two projection targets for each pathway were determined by the AAL labels of pathway origin and termination points. We then performed a classification analysis with a support vector machine (SVM) algorithm using sparse logistic regression with L1 and L2 norm regularization (Ryali et al., 2009). The number of pathways connecting each pair of ROIs was used as feature vectors and pairs of ROIs which contained

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[2] http://white.stanford.edu

Table 1 | Regions of interest for the network analysis.

AAL ID	AAL Label	Voxels in AAL mask	Average number of projections per voxel	AAL ID	AAL Label	Voxels in AAL mask	Average number of projections per voxel
78	Thalamus_R	8399	0.0084	66	Angular_R	14009	0.0009
50	Occipital_Sup_R	11149	0.0076	112	Vermis_6	2956	0.0008
60	Parietal_Sup_R	17554	0.0076	100	Cerebelum_6_R	14362	0.0007
68	Precuneus_R	26083	0.0065	93	Cerebelum_Crus2_L	15216	0.0007
96	Cerebelum_3_R	1600	0.0062	51	Occipital_Mid_L	25989	0.0007
52	Occipital_Mid_R	16512	0.0058	43	Calcarine_L	18157	0.0007
46	Cuneus_R	11323	0.0056	113	Vermis_7	1564	0.0007
38	Hippocampus_R	7606	0.0051	64	SupraMarginal_R	15770	0.0006
49	Occipital_Sup_L	10791	0.0036	104	Cerebelum_8_R	18345	0.0006
54	Occipital_Inf_R	7929	0.0029	97	Cerebelum_4_5_L	9034	0.0005
45	Cuneus_L	12133	0.0028	95	Cerebelum_3_L	1072	0.0004
67	Precuneus_L	28358	0.0028	99	Cerebelum_6_L	13672	0.0003
44	Calcarine_R	14885	0.0027	101	Cerebelum_7b_L	4639	0.0003
90	Temporal_Inf_R	28468	0.0021	47	Lingual_L	16932	0.0003
94	Cerebelum_Crus2_R	17038	0.0021	107	Cerebelum_10_L	1169	0.0003
40	ParaHippocampal_R	9028	0.0021	39	ParaHippocampal_L	7891	0.0002
48	Lingual_R	18450	0.0020	62	Parietal_Inf_R	10763	0.0002
59	Parietal_Sup_L	16519	0.0018	103	Cerebelum_8_L	15090	0.0002
92	Cerebelum_Crus1_R	21017	0.0015	109	Vermis_1_2	404	0.0002
77	Thalamus_L	8700	0.0013	114	Vermis_8	1940	0.0002
98	Cerebelum_4_5_R	6763	0.0011	53	Occipital_Inf_L	7536	0.0002
37	Hippocampus_L	7469	0.0010	116	Vermis_10	874	0.0002
108	Cerebelum_10_R	1280	0.0010	65	Angular_L	9313	0.0001
110	Vermis_3	1822	0.0010	105	Cerebelum_9_L	6924	0.0001
91	Cerebelum_Crus1_L	20667	0.0009	89	Temporal_Inf_L	25647	0.0001
56	Fusiform_R	20227	0.0009	61	Parietal_Inf_L	19447	0.0001
111	Vermis_4_5	5324	0.0009	55	Fusiform_L	18333	0.0001
106	Cerebelum_9_R	6462	0.0009	115	Vermis_9	1367	0.0000
102	Cerebelum_7b_R	4230	0.0009	63	SupraMarginal_L	9907	0.0000

The fifty-eight posterior cortical and subcortical regions of interest used in the network analysis. ROIs were based on the AAL atlas (Tzourio-Mazoyer et al., 2002) and rank ordered by the average normalized number of projections from WM regions that showed deficits in children with DD to each ROI.

no pathways in any participant were excluded. The pathway counts were transformed to *z*-scores to control for differences in sizes of the ROIs. A grid search was performed to find the optimal L1 and L2 norm regularization parameters to maximize 10-fold cross-validation accuracy across 50 iterations.

RESULTS

BEHAVIORAL PROFILE

Table 2 shows demographic and neuropsychological profiles of the DD and the TD groups. The two groups differed significantly on mathematical abilities as assessed by the WIAT-II, but not on IQ, age, reading ability, or working memory.

GREY MATTER VOLUME

Compared to TD children, children with DD showed decreased GM volume in several posterior brain regions (**Figure 1**, **Table 3**). Within the ventral visual stream, differences were found bilaterally in the cuneus/precuneus, lateral occipital cortex, lingual gyrus, and the fusiform gyrus. Within the dorsal visual stream, children with DD showed GM deficits bilaterally in the superior parietal lobule (SPL) and right posterior IPS. They also showed deficits in the medial temporal lobe, within the right entorhinal cortex and, bilaterally, within the parahippocampal gyrus, and the hippocampus. Peak differences were observed in the right fusiform gyrus ($t = 4.03$; MNI coordinates: 24, –80, 0), right anterior temporal cortex ($t = 4.01$; 46, –2, –32), right posterior IPS ($t = 3.93$; 24, –74, 24), the precuneus ($t = 3.86$; 12, –50, 36), left fusiform gyrus ($t = 3.83$; –18, –82, –4), left parahippocampal gyrus ($t = 2.83$; –32, –38, –18), right parahippocampal gyrus ($t = 2.92$; 26, –40, –12) and the left superior parietal lobule ($t = 2.7$; –16, –70, 46). Prominent GM differences were also found bilaterally in the vermis and cerebellar regions 8, 9, 10. Compared to TD children, children with DD did not show increased GM volume in any brain region.

WHITE MATTER VOLUME

Children with DD showed reduced WM in the posterior brain, primarily within WM regions adjacent to the right temporal-parietal cortex (**Figure 2**, **Table 4**). Roughly two-thirds of the voxels within

Table 2 | Demographic and neuropsychological characteristics of participants.

Characteristics	DD (*N* = 23)	TD (*N* = 24)
DEMOGRAPHIC		
Age	8.8 (0.7)	8.9 (0.7)
Sex (F/M)	13/10	12/12
Grade	2.4 (0.5)	2.6 (0.5)
WASI		
Full scale IQ	111 (11.8)	111.8 (11.3)
WMTBC		
Working memory composite	98.27 (11.63)	101.31 (7.96)
WIAT II		
Word reading	107.2 (15)	111.9 (9.7)
Reading comprehension	106.8 (11)	107.5 (8.5)
Numerical operations	88.8 (6.2)**	112.3 (9.3)
Math reasoning	103.3 (13.7)*	111 (11.4)
Math composite	94.9 (8.9)**	113 (10)

Mean and standard deviation are shown for each measure. Significant differences between the DD and TD groups and indicated with single (, $p < 0.05$) and double (**, $p < 0.01$) asterisks. WASI = Wechsler Abbreviated Scale of Intelligence; WMTBC = Working Memory Test Battery for Children; WIAT-II = Wechsler Individual Achievement Test, Second Edition.*

this WM region overlapped with WM tracts included in the JHU white matter tract atlas (**Figure 3**). Prominent right hemisphere WM tracts included the inferior fronto-occipital fasciculus ($t = 4.16$; 30, –26, –4), the forceps major of the splenium of the corpus collossum ($t = 3.76$; 6, –36, 14), the inferior longitudinal fasciculus ($t = 4.16$; 30, –28, 4), the corticospinal tract ($t = 3.56$; 20, 36, 46), the superior longitudinal fasciculus ($t = 3.90$; 22, 36, 42), and the anterior thalamic radiation ($t = 3.54$; 22, –32, 32), as shown in **Figure 4**. In the left hemisphere, deficits were observed in the superior longitudinal fasciculus ($t = 3.40$; –36, –30, 44), forceps major ($t = 3.39$; –16, –46, 8), and the anterior thalamic radiation ($t = 3.42$; –18, –40, 2). No clusters showed greater WM volume in DD compared to TD children.

FRACTIONAL ANISOTROPY

Analysis of the region contained within the WM ROI revealed significantly reduced FA in the DD group ($p = 0.009$, $df = 45$), as shown in **Figure 5A**. FA in this region was significantly correlated with Numerical Operations ($r = 0.35, p = 0.007$), as shown in **Figure 5B**, but not the Mathematical Reasoning ($r = 0.002, p = 0.50$) or the Word Reading ($r = 0.03, p = 0.42$) subscores of the WIAT-II. FA did not correlate significantly with IQ ($r = -0.18, p = 0.23$) or the composite working memory score ($r = -0.16, p = 0.28$).

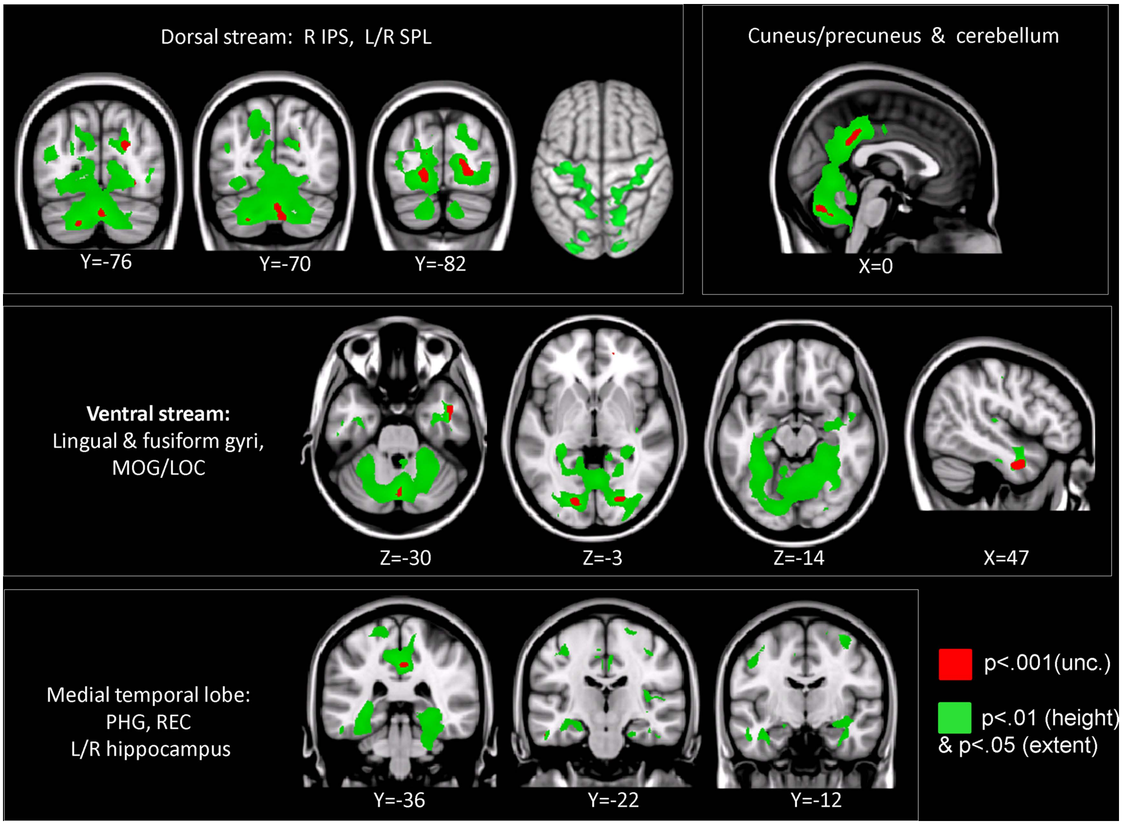

FIGURE 1 | Brain regions where children with DD showed significant gray matter deficits, compared to TD children (two-sample *t*-test results for TD > DD contrast). Red: $p < 0.001$; Green: height threshold $p < 0.01$, extent threshold $p < 0.05$ with family-wise error correction for multiple comparisons and correction for non-isotropic smoothness. Abbreviations: IPS = intraparietal sulcus; LOC = lateral occipital complex; MOG = medial occipital gyrus; PHG = parahippocampal gyrus; REC = right enthorinal cortex; SPL = superior parietal lobule.

Table 3 | MNI coordinates of brain areas that showed significant gray matter deficits in children with DD.

Brain area	*t*-score	Peak MNI coordinate		
		x	*y*	*z*
Right fusiform gyrus**	4.03	24	–80	0
Right anterior temporal cortex**	4.01	46	–2	–32
Right posterior IPS**	3.93	24	–74	24
Right precuneus**	3.86	12	–50	36
Left fusiform gyrus**	3.83	–18	–82	–4
Right cerebellum**	3.23	22	–71	–44
Left parahippocampal gyrus*	2.83	–32	–38	–18
Right parahippocampal gyrus*	2.92	26	–40	–12
Left superior parietal lobule*	2.7	–16	–70	46

Both at $p < 0.001$ (uncorrected) and height threshold $p < 0.01$, extent threshold $p < 0.05$ FWE corrected.

***Height threshold $p < 0 .001$ (uncorrected).*

PROBABILISITIC TRACTOGRAPHY

We used probabilistic tractography to examine structural connections linking specific seed and target regions. Seed regions consisted of voxels that showed lower WM volume in children with DD, and target regions consisted of voxels that showed lower GM in the DD group. This analysis revealed a cluster of voxels in the right inferior temporal gyrus with significantly lower projection density in the DD group, compared to TD children (**Figure 6**). The peak difference was in the right fusiform gyrus (38, –42, –18). No voxels showed greater projection density in children with DD.

NETWORK ANALYSIS

We used deterministic tractography to examine cortical connectivity of the WM region that showed significant differences between TD and DD children. Whole brain tractography from, on average, 92420 seed voxels (those with FA > 0.25, of 172180 brain voxels) resulted in 43000 to 86000 fiber tracts per participants, of which 9000 to 14000 pathways were found to pass through the

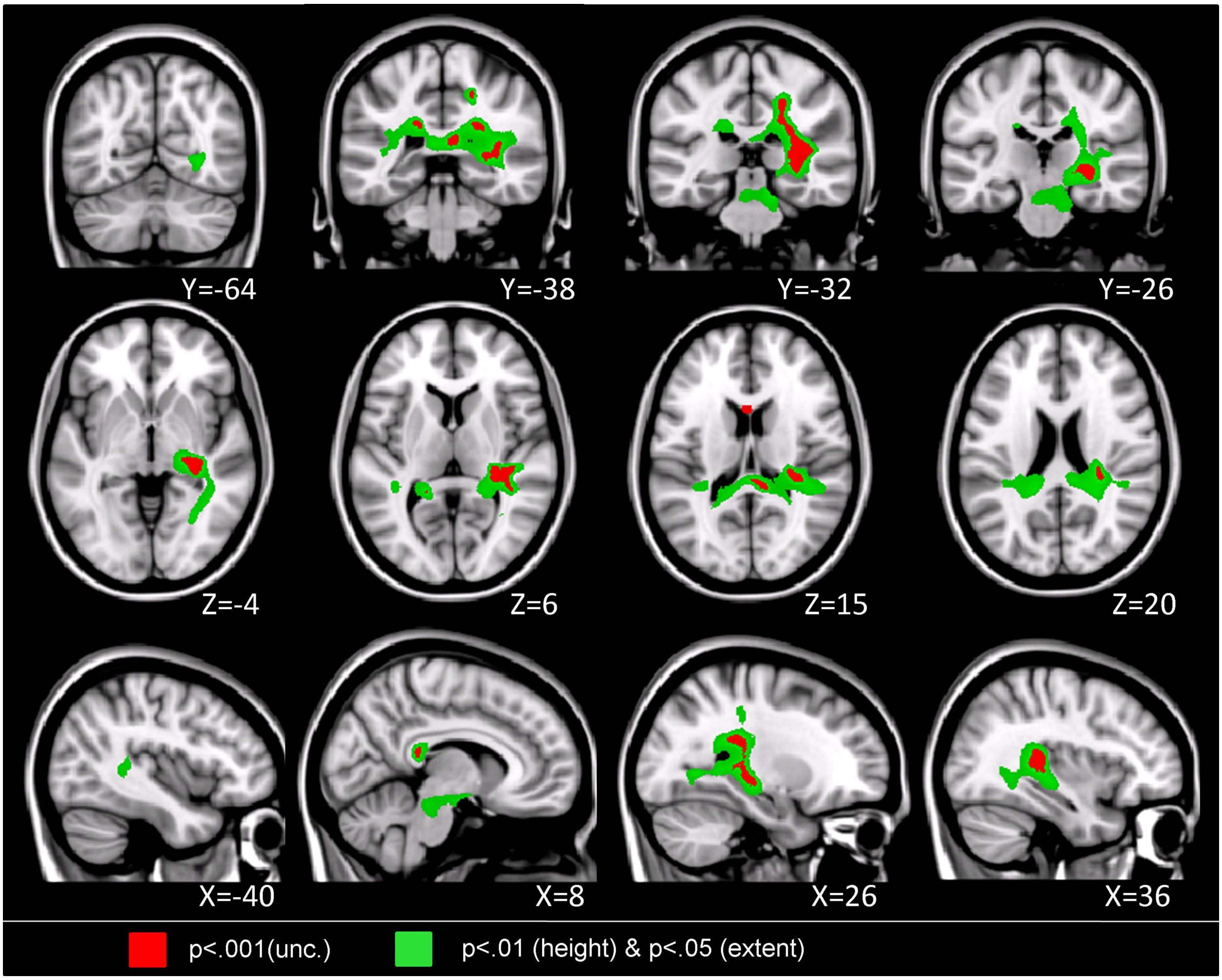

FIGURE 2 | Brain regions where children with DD showed significant white matter deficits, compared to TD children (two-sample *t*-test results for TD > DD contrast). Red: $p < 0.001$; Green: height threshold $p < 0.01$, extent threshold $p < 0.05$ with family-wise error correction for multiple comparisons and correction for non-isotropic smoothness.

VBM-defined WM ROI. Fiber density regularization, performed by removing redundant fibers (see Materials and Methods), resulted in 1500 to 2300 fibers of diameter 2 mm per participant. More than 50% of these fibers originated and terminated within cortical regions in the dorsal and ventral visual stream areas identified using the AAL atlas (**Table 1**). SVM-based classification analysis revealed that connectivity patterns in children with DD could be distinguished from those in TD children with a cross-validation accuracy of 70%, which is considerably higher than the 50% chance level. See Supplementary Material for visualization of brain connectivity patterns by group.

Table 4 | MNI coordinates of brain areas that showed significant* white matter deficits in children with DD.

WM tract	*t*-score	Peak MNI coordinate		
		x	*y*	*z*
Right inferior fronto-occipital fasciculus	4.16	30	–26	–4
Right inferior longitudinal fasciculus	4.16	30	–28	4
Right superior longitudinal fasciculus	3.90	22	36	42
Right forceps major	3.76	6	–36	14
Right corticospinal tract	3.56	20	36	46
Right anterior thalamic radiation	3.54	22	–32	32
Left anterior thalamic radiation	3.42	–18	–40	2
Left superior longitudinal fasciculus	3.40	–36	–30	44
Left forceps major	3.39	–16	–46	8

**Significance reached at both $p < 0.001$ (uncorr), as well as at height threshold $p < 0.01$, extent threshold $p < 0.05$ FWE corrected.*

DISCUSSION

The study of developmental dyscalculia is complicated by heterogeneity with respect to severity, specificity, and extent of mathematical difficulties. Mathematical performance can be influenced by nonspecific deficits such as slow speed of processing, poor working memory, inattention, and deficits in long-term storage of arithmetic facts (Temple, 2002). Additionally, mathematical difficulties can often be accompanied by low IQ and other cognitive deficits (von Aster and Shalev, 2007). Our study overcomes these issues by using a carefully selected sample of children who scored within the normal range on standardized measures of IQ, reading, and working memory, but had a specific deficit in standardized measures of mathematical ability. Our multimodal structural imaging approach revealed prominent deficits in both GM and WM integrity in this

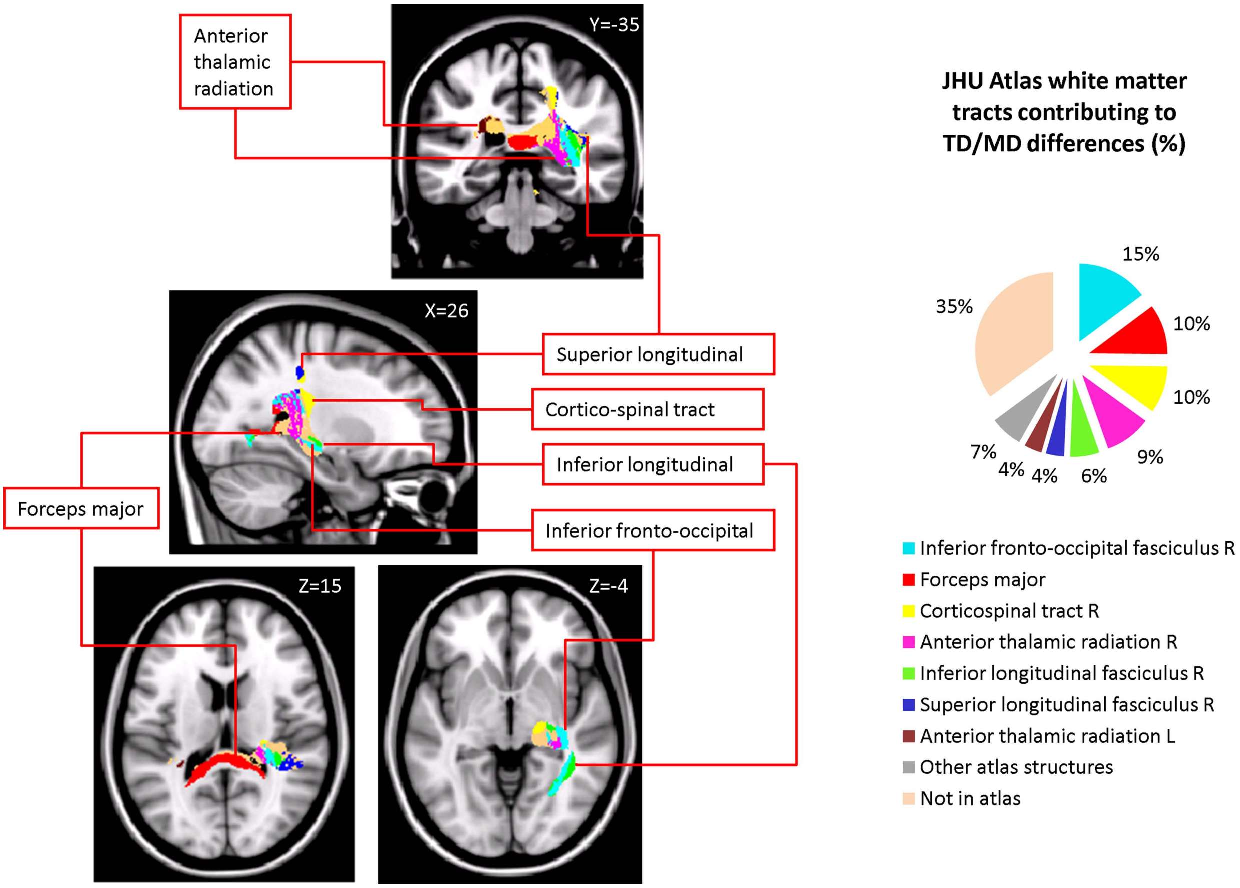

FIGURE 3 | White matter tracts in brain regions where children with DD showed significant gray matter deficits, compared to TD children. Tracts were labeled using the JHU white matter atlas.

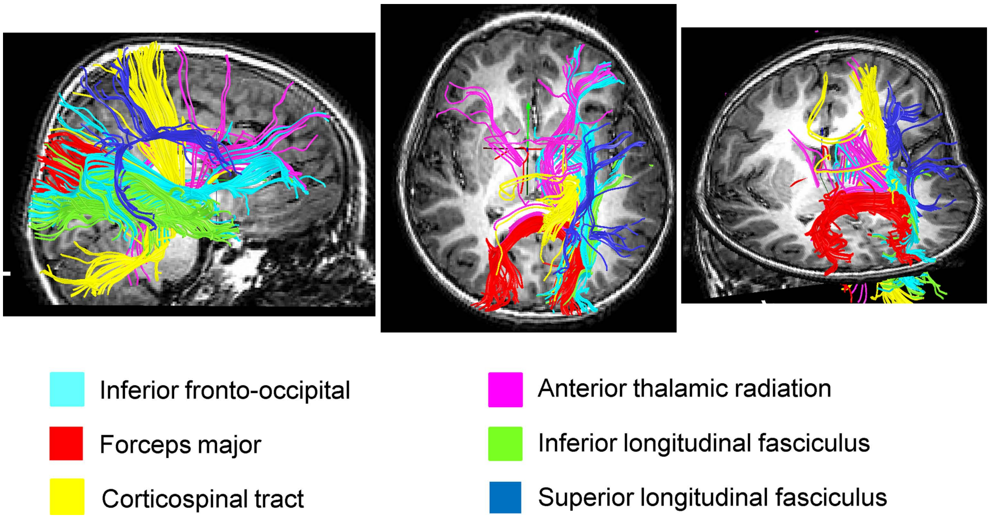

FIGURE 4 | Streamlined reconstruction of white matter pathways passing through the right temporal-parietal regions where children with DD showed significant white matter volumetric deficits, compared to TD children. Data is from a representative TD child. Tracts labels are obtained using the probabilistic JHU white matter tractography atlas.

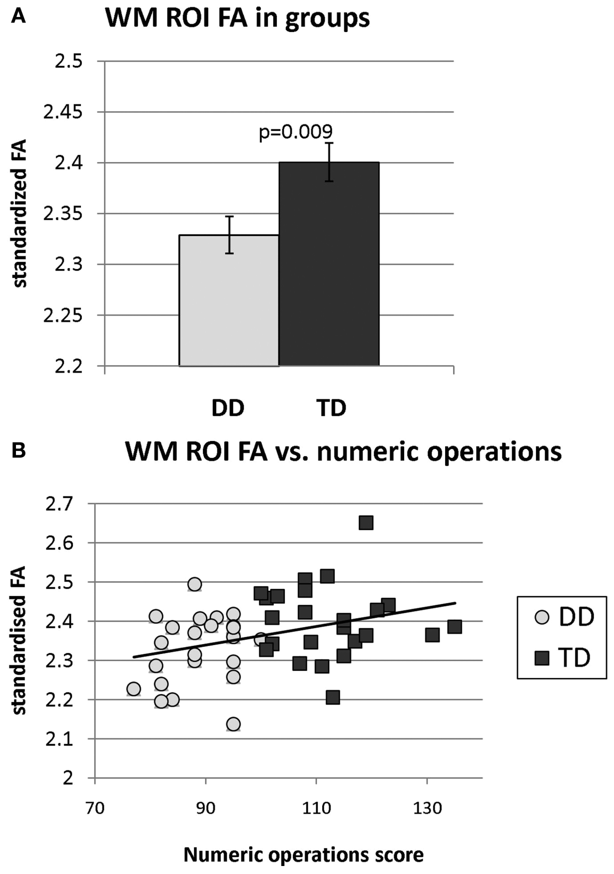

FIGURE 5 | (A) Fractional anisotropy (FA) within right temporal-parietal regions that showed white matter deficits in DD children. FA was significantly reduced in children with DD, compared to TD children. **(B)** Across the two groups, FA was significantly correlated with the Numerical Operations subscore of the WIAT-II.

well-characterized sample of young children with DD. In addition to identifying the anatomical location of GM and WM abnormalities underlying DD in young children, our data also points to WM microstructural and connectivity deficits underlying DD.

GREY MATTER DEFICITS IN CHILDREN WITH DD

Children with DD showed prominent GM deficits in both dorsal and ventral visual streams as well as the anterior temporal cortex. Unlike Rotzer et al. (2008) we found no evidence for deficits in the anterior cingulate cortex, the inferior frontal gyrus and the dorsolateral prefrontal cortex, perhaps because of our use of more stringent criteria for matching the groups on IQ, working memory and reading.

The dorsal visual stream regions that showed GM deficits included most prominently the posterior aspects of the IPS and the SPL. Similar to Rotzer et al. (2008), we found that children with DD showed deficits in the right IPS, but we also observed differences in the adjoining SPL as well as the left IPS and left SPL. The IPS is a core parietal lobe region implicated in the development of number sense and magnitude judgment (Ansari, 2008). Functional imaging studies have also shown that the IPS region plays a crucial role in arithmetic calculations, independent of other processing demands such as working memory (Gruber et al., 2001; Zago and Tzourio-Mazoyer, 2002; Delazer et al., 2003; Rivera et al., 2005; Wu et al., 2009).

Children with DD also showed significant deficits in the ventral visual stream, within the medial aspects of the inferior temporal gyrus. Deficits were most prominent in the fusiform gyrus and lingual gyrus, extending anteriorly into the parahippocampal gyrus and the hippocampus. These latter regions form part of a hierarchically organized processing stream for encoding complex visual stimuli (Menon et al., 2000). Reduced GM in the fusiform gyrus (Molko et al., 2003) and functional deficits (Kesler et al., 2006) have previously been reported in Turner syndrome, although it should

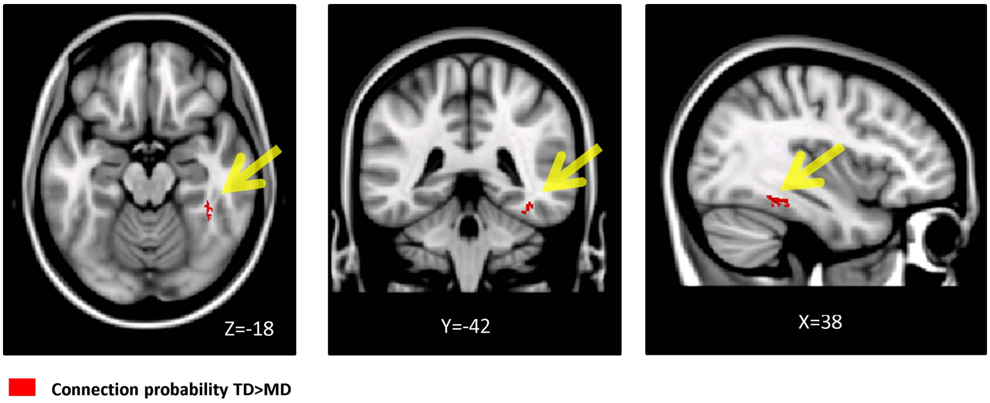

FIGURE 6 | The fusiform gyrus showed significantly reduced connection probability in children with DD. Results of probabilistic tractography from right temporal-parietal WM regions that showed deficits in children with DD as seeds.

be noted that DD is not the only cognitive deficit that exists in this syndrome. Less prominent deficits were also observed in the lateral occipital complex, a region that subserves processing of complex visual objects (Grill-Spector et al., 2008). Convergent with our findings, an fMRI study of DD in third graders found deficits in the right parahippocampal gyrus during calculation (Kucian et al., 2006). Critically, prominent deficits were also observed in the hippocampus and the entorhinal cortex, two regions critical for memory and learning. Previous studies on mathematical cognition in adults have not detected activation in the hippocampus even during tasks that involve learning (Ischebeck et al., 2006, 2007), presumably because of greater reliance on long-term neocortical memory representations. Consistent with this view, emerging evidence suggests that the hippocampus plays a greater role in facilitating learning and retrieval in children, compared to adults (Rivera et al., 2005). Our findings provide the first evidence suggesting that medial temporal lobe deficits may contribute to DD in young children.

While previous studies have emphasized the IPS as the neural substrate most closely associated with DD (Price et al., 2007), more recent reports have emphasized deficits in a more distributed network (Rubinsten and Henik, 2009). Functional neuroimaging studies have consistently reported involvement of both dorsal and ventral visual streams in basic number sense (Ansari, 2008) as well as simple and complex mental arithmetic tasks in children and adults (Zago et al., 2008). Although initially believed to be distinct and non-overlapping streams, there is growing evidence from both numerical and non-numerical domains that these two streams interact during complex visuo-spatial processing (Konen and Kastner, 2008). Reduced GM volume in both ventral and dorsal visuo-spatial streams may underlie difficulties in mapping visual numerical symbols to their semantic representations in children with DD (Rosenberg-Lee et al., 2009). Finally, the novel finding of our study is the identification of right anterior temporal cortex abnormalities in DD. This result is intriguing because the known role of the anterior temporal cortex in semantic processing (Visser et al., 2009). Functional imaging studies of number processing have generally implicated the IPS in semantic processing of numbers. Beyond this, the role of the IPS in semantic cognition of mathematical information more broadly has been less clear. Our findings suggest that the anterior temporal cortex may be an additional locus within a network of deficits that impair the ability of children with DD to develop semantic memory representations important for rapid fact retrieval.

WHITE MATTER MORPHOMETRIC AND MICROSTRUCTURE DEFICITS IN CHILDREN WITH DD

VBM analysis revealed reduced WM volume in the right temporal-parietal region and the splenium of the corpus callosum. The WM region isolated in our study is significantly different from the subgenual aspects of the medial prefrontal cortex and the bilateral parahippocampal gyrus regions reported by Rotzer et al. (2008). A close reading of their data, however, suggests that WM differences were primarily localized to the hippocampal region, rather than the paraphippocamapal gyrus. Apart from differences in anatomical location, another key difference is that the WM differences detected in our study overlap with long association fibers in the temporal-parietal cortex, rather than along short-range U-shaped tracts that connect neighboring sulci.

Reduced WM volume can arise from either decreased myelination, which results in slower signal transfer speeds, or smaller number of axons which results in lower signal transfer capacity, causing impaired connectivity among the GM regions connected by the fiber tracts that travel through regions with lower WM volume (Dougherty et al., 2007). To address this question, we used DTI data to examine microstructural differences within the WM cluster identified by the VBM analysis. Children with DD showed significantly reduced fractional anisotropy (FA) in this right hemisphere WM cluster. Moreover, in the combined DD and TD sample, FA was significantly correlated with the Numerical Operations but not the Mathematical Reasoning subscore of the WIAT-II, suggesting that WM deficits are primarily related to fact retrieval and computation, rather than verbal problem solving skills in young children with DD (Meyer et al., 2009). This is consistent with the view that the ability to rapidly retrieve and perform exact calculations is a core deficit of children with DD (Jordan et al., 2003; Fuchs et al., 2006). Additional analyses confirmed that WM volume and FA in this region were not related to reading scores or working memory, pointing to the specificity of our findings with respect to

DD. In spite of differences in FA, mean diffusivity was, however, not significantly different, suggesting that abnormalities in orientation, number and connectivity of axonal pathways rather than overall myelination may be the major source of WM deficits in children with DD (Dougherty et al., 2007).

The prominent right hemisphere deficits observed in our study may reflect the importance of visuo-spatial processes during the initial stages of mathematical skill acquisition. Behavioral studies have reported that children with mathematical disabilities manifest problems in non-verbal tasks involving visuo-perceptual organization (Rourke, 1993). Consistent with this view, a recent fMRI study reported weaker activation in the right parahippocampal gyrus in children with DD, compared to TD children, during an approximate calculation task (Kucian et al., 2006). Deficits in basic number processing skills are thought to be the core abnormality in DD (Landerl et al., 2004; Butterworth, 2005; Jordan et al., 2009). Although the differential contribution of the left and right PPC to basic number processing and calculation is still not well understood, the emerging literature is beginning to point to a critical role for the right IPS in number processing. Using fMRI-guided TMS in healthy adults, Cohen Kadosh et al. (2007) found that automatic magnitude processing was impaired only during disruption of right-IPS activation clusters. Furthermore, an identical paradigm with dyscalculic participants reproduced a pattern similar to that obtained with nondyscalculic volunteers during right-IPS disruption. Consistent with these results, children with DD appear not to modulate right IPS during non-symbolic (Price et al., 2007) and symbolic number comparison tasks (Mussolin et al., 2009). Taken together, these findings suggest that automatic magnitude processing abnormalities within the right hemisphere temporal-parietal WM may adversely impact visuo-spatial operations crucial for numerical and mathematical cognition.

NETWORK ANALYSIS: LINKING WM AND GM DEFICITS

We used both deterministic and probabilistic tractography to examine WM pathways that are potentially abnormal in children with DD. Atlas-based tract mapping provided new and detailed information about the major tracts that run through the WM cluster where children with DD showed deficits. Based on the JHU atlas, we identified the inferior fronto-occipital fasciculus, the forceps major of the splenium of the corpus collossum, the inferior longitudinal fasciculus, the corticospinal tract, the superior longitudinal fasciculus, and the anterior thalamic radiation as key fiber tracts that run through this region. Deterministic tractography of DTI data confirmed that children in our age group had prominent tracts in this region. Three major projection fibers are noteworthy here based on the known functional neuronatomy of mathematical cognition (Dehaene et al., 2003; Wu et al., 2009) – the superior longitudinal fasciculus emanating from the parietal lobe, the inferior fronto-occipital fasciculus emanating from the occipital-parietal region and the inferior longitudinal fasciculus emanating from the occipital-temporal region (Schmahmann et al., 2007). Together, these axonal pathways help to link the lingual, fusiform and the parahippocampal gyrus regions in the ventral visual stream with the IPS and superior parietal lobule in the dorsal visual stream. Finally, the caudal most part of the splenium of the corpus collosum which links the left and right inferior temporal gyri (Schmahmann et al., 2007) also showed significant deficits. This result parallels our finding of both left and right GM deficits in the fusiform gyrus.

In order to relate white and grey matter abnormalities, we next compared WM tract projections from the WM to GM ROIs that showed deficits in children with DD. Differences in tract projections could not be addressed with deterministic tractography, because fiber density regularization essentially eliminates projection density information. Probabilistic tractography with seeds in the temporal-parietal WM ROI and targets in the GM ROI revealed that children with DD had significantly lower density of projections to the right fusiform gyrus in the inferior temporal cortex. This finding, together with concurrent analysis of fiber tracts emanating from the WM ROI, helps to link WM and GM deficits and point to tracts connecting the fusiform gyrus with temporal-parietal WM, most likely via the inferior longitudinal fasciculus, as a major locus of neuroanatomical abnormalities in DD. An intriguing question here is whether there are significant differences in axonal pathways linking the fusiform gyrus with the IPS, given the critical role of the latter in numerical information processing (Cohen Kadosh et al., 2007). While probabilistic tractography is well suited for tracking medium-range fibers, its main limitation is that it produces accumulating errors when computing long-range fiber pathways, and it is currently difficult to apply distance-corrected measures of connectivity to examine potential abnormalities with connections to the posterior parietal cortex. Further studies with high-resolution diffusion spectrum imaging (Schmahmann et al., 2007) are necessary to test the hypothesis that fibers linking the inferior temporal cortex with the posterior parietal cortex are a specific source of vulnerability in DD.

Finally, we examined whether connectivity within more extended posterior cortical and related subcortical networks is impaired in children with DD. We used a coarse-grained anatomical parcellation (Tzourio-Mazoyer et al., 2002) and deterministic tractography to examine network-level abnormalities in WM tracts arising from the WM ROI that showed deficits in children with DD. We found strong overlap between target sites of the WM pathways and regions with reduced GM volume in the DD group. This finding provides additional evidence for links between WM and GM deficits in children with DD. Classification analysis further revealed that connectivity of fibers emanating from the WM ROI is significantly different in children with DD. The precise nature of additional potential deficits in inter-hemispheric connectivity, as well as deficits in connectivity along additional nodes of the dorsal-ventral visual pathways, and the manner in which each contributes to specific behavioral deficits in children with DD remains to be investigated (Rusconi et al., 2009).

Previous studies of developmental disabilities have in general focused on identification of specific brain regions that may contribute to overall deficits. In most of these studies, brain regions are treated independently, and network connectivity is typically not examined. Our data suggests that analysis of deficits in network connectivity arising from macrostructural WM deficits may prove useful in explaining individual differences in number sense and calculation previously attributed to localized differences in cortical structure and function.

CONCLUSION

The neurobiological deficits detected in our DD group are particularly noteworthy, given that the children are closely matched to the control group in terms of age, IQ, reading ability, and working memory capacity. Our results provide strong evidence that pure DD is characterized by robust GM and WM deficits in key brain areas that have previously between implicated in mathematical cognition. Integrated analyses of brain structure using a combination of VBM and DTI provide converging evidence for deficits in the ventral and dorsal visual stream. Critically, our findings point to right hemisphere temporal-parietal WM, its microstructure and pathways associated with it, including most notably, the inferior fronto-occipital fasciculus and the inferior longitudinal fasciculus, as key anatomical correlates of DD. Our network analysis further suggests the possibility of multiple dysfunctional circuits arising from a core WM deficit, and lead to testable hypothesis that DD may, at its core, be a disconnection syndrome. We propose that, just as findings from structural neuroimaging studies of dyslexia have paved the way for targeted intervention, the current findings may eventually aid the development of remediation programs designed to enhance mathematical skills in young children with DD.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/humanneuroscience/paper/10.3389/neuro.09/051.2009/

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Neurophysiological correlates of executive function: a comparison of European-Canadian and Chinese-Canadian 5-year-old children

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This study explored the neurophysiological correlates of executive function (EF) in young children from two different cultural backgrounds. Twenty European-Canadian and 17 Chinese-Canadian 5-year-olds participated in a go/no-go task, during which high-density electroencephalographic (EEG) data were recorded. No cultural group differences were observed in children's behavioral performance on the task, but marked differences were revealed by ERP analyses, which focused on the amplitude and latency of the N2 waveform. Chinese-Canadian children showed larger (i.e., more negative) N2 amplitudes than European-Canadian children on the right side of the scalp on no-go trials, as well as on the left side of the scalp on go trials, and for all children, larger N2 amplitudes were associated with faster median reaction times. Source analyses of the N2 were consistent with the hypothesis that compared to European-Canadian children, Chinese-Canadian children showed more activation in dorsomedial, ventromedial, and (bilateral) ventrolateral prefrontal cortex. These findings reveal that EEG can provide a measure of cultural differences in neurocognitive function that is more sensitive than behavioral data alone; that Chinese-Canadian children show a pattern of hemispheric differentiation in the context of this task than that is more pronounced than that of age-matched European-Canadian children; that the asymmetrically lateralized N2 may be a reliable marker of both effortful inhibition (on the right) and effortful approach (on the left); and that the neural correlates of EF may vary across samples of healthy participants, even in children.

Keywords: executive function, culture, EEG/ERP, children, go/no-go, N2

INTRODUCTION

Executive function (EF) – the conscious control of thought and action – develops markedly during the preschool years, in parallel with maturation of prefrontal cortex (see Zelazo et al., 2008 for review). Most research on EF in children has been conducted with Western samples, but an emerging corpus of cross-cultural comparisons suggests that Asian children may perform better than Western children on measures of EF despite comparable, or worse, performance on other measures of cognitive function, such as theory of mind (e.g., Sabbagh et al., 2006; Oh and Lewis, 2008).

Correlations with culture are always difficult to interpret because of the numerous, often unmeasured, differences between groups, but the finding that Asian children sometimes display better EF than age-matched Western children is consistent with reports that Chinese parents expect their children to master impulse control at a relatively young age (e.g., Ho, 1994; Wu, 1996; Chen et al., 1998), and that impulse control in daycare settings is more highly valued in China than it is in North America (Tobin et al., 1989). It is also consistent with the finding that the 7-repeat allele of the dopamine D4 receptor gene (DRD4), which has been linked to behavior problems involving EF (e.g., Swanson et al., 1998; Faraone et al., 2001), is extremely rare in individuals from East and South Asia (Chang et al., 1996).

The observation that children from different cultures may show differences in EF abilities raises the question of whether children's brain function varies in systematic ways across cultures, and if so, to what extent. There is currently considerable interest in the specific neural correlates of EF (e.g., Bunge and Crone, 2009), but as with behavioral research on this topic, nearly all of this work has been conducted with Western samples, and indeed, to date, there have been no published cross-cultural comparisons of these neural correlates. It seems plausible, however, that genetic and/or environmental influences associated with culture may be reflected not only in children's behavior, but also in their neural function. Recent research on neural plasticity, for example, supports the suggestion that neural development is experience dependent (e.g., Neville, 1993; Huttenlocher, 2002), and indeed, interventions designed to promote EF have been found to produce changes in neural function related to EF (Olesen et al., 2004; Rueda et al., 2005).

One task that has been used to examine the neural correlates of EF in young children is the go/no-go task, in which children must respond on the majority of trials (go stimuli) but inhibit responding on certain trials (no-go stimuli) (e.g., Davis et al., 2003). Event-related fMRI research comparing adults and school-age children on go/no-go-type tasks indicates that several regions of prefrontal cortex, including ventrolateral prefrontal cortex and anterior cingulate cortex (e.g., Bunge et al., 2002; Durston et al., 2002a,b), play an important role in successful performance on this task. Electroencephalographic (EEG) recordings during performance on the task yield a well-studied ERP component, the N2, which in children is usually observed at medial-frontal sites between 250 and 500 ms following stimulus presentation (e.g., Davis et al., 2003; Lamm et al., 2006; Todd et al., 2008). N2 amplitude has been found to be larger on successful no-go trials than on go trials in both adults (e.g., Eimer, 1993; Falkenstein et al., 1999) and children (e.g., Johnstone et al., 2005; Lewis et al., 2006) although this is not always the case (e.g., see Nieuwenhuis et al., 2003 for adults, and Davis et al., 2003 for children). Consistent with fMRI results, source analyses of the N2 in adults have identified cortical generators in both dorsomedial prefrontal cortex (e.g., dorsal anterior cingulate cortex Nieuwenhuis et al., 2003) and ventral prefrontal cortex (Bokura et al., 2001), and similar results have been obtained with children (e.g., Lamm et al., 2006). In children, the location and activation strength of these sources appears to be related to individual differences in EF (Lamm et al., 2006) and emotion regulation (Lewis et al., 2006, 2008).

An important consideration in neurocognitive research with children is hemispheric laterality. Bunge et al. (2002) found that interference suppression was related to prefrontal activation in the opposite hemisphere in adults as in 8- to 12-year-old children. Moreover, ERP studies of the N2 component on No-go trials have revealed a right-lateralized topography in both young children (Todd et al., 2008; see also Perez-Edgar and Fox, 2007) and adults (Bokura et al., 2001), although this pattern has not usually been reported in adults (e.g., Nieuwenhuis et al., 2003).

In the present study, we used high-density (128-channel) EEG to compare the neural correlates of EF in young European-Canadian and Chinese-Canadian children as they performed a go/no-go task. We examined 5-year-old children because Western children at this age typically display considerable advances in EF and related skills (Zelazo et al., 2008 for review), and because the acquisition of these skills at this age appears to be critical for the transition to school, where demands on EF may be increased (e.g., Blair and Razza, 2007). We focused on the N2 waveform and its estimated underlying sources, and expected to find evidence of cultural differences in neurocognitive function. As children from a Chinese background have been found to perform better on measures of EF than North American children, and may be more conscientious, we expected to find larger N2 (i.e., more negative) amplitudes for the Chinese-Canadian children than the same-age European-Canadian children. Although studies with school-age children generally report that N2 amplitude decreases with age across the school-age years and into adolescence, and that these decreases co-occur with continued improvements in EF (e.g., Lamm et al., 2006), our prediction is based on studies showing larger N2 amplitudes to be associated with better EF within a single age group (e.g., Overtoom et al., 1998, 2002; Pliszka et al., 2000; Liotti et al., 2005). For example, Pliszka et al. (2000) found larger N2 amplitudes for controls, as compared to children with ADHD, over right inferior frontal cortex on a go/no-go-type task. In addition, in light of evidence for the recruitment of right prefrontal cortex on the go/no-go task and other EF tasks, we expected to find a right-lateralized N2 on no-go trials, as well as right-lateralized source activation.

MATERIALS AND METHODS

PARTICIPANTS

Participants were 37 English-speaking 5-year-olds (*M* age = 5.28 years, SD = 0.54, Range = 4.10–6.00), including 20 children (9 boys and 11 girls) from a European-Canadian background (*M* age = 5.38 years, SD = 0.41) and 17 children (5 boys and 12 girls) from a Chinese-Canadian background (*M* age = 5.18 years, SD = 0.65), who were recruited through a computerized database containing names of parents who have expressed interest in their child's participation in research. Children from the two cultural groups did not differ in age, $t(35) = 1.13$, $p > 0.26$, or in the proportion of boys in the group, $\chi^2(1, N = 37) = 0.36$, $p > 0.55$. Chinese-Canadian children were second-generation immigrants from China and European-Canadian children were second-generation (or more) immigrants from Europe. Five of the European-Canadian parents and 15 of the Chinese-Canadian parents indicated that more than one language was spoken in the home. Most European-Canadian parents had post-secondary education (mothers 85%; fathers 65%) and were mostly employed in managerial and professional, clerical, sales, and entertainment occupations. All of the Chinese-Canadian parents had post-secondary education and were mostly employed in managerial and professional, sales, and industrial processing occupations. In addition, parents were administered an individualism–collectivism questionnaire (Triandis, 1995), and no significant difference was found between the two cultural groups on either the individualism, $F(1, 33) = 2.89$, $p < 0.10$, $\eta_p^2 = 0.08$, or collectivism, $F(1, 33) = 0.00$, $p < 0.98$, $\eta_p^2 = 0.00$, dimension. All children had normal or corrected-to-normal vision, and were free of any psychiatric diagnoses or medication. An additional 17 participants were tested but eliminated from the final analysis because (a) they refused to wear the EEG net (see below), $n = 7$, (b) they had fewer than 11 correct no-go trials that that were free of eye blinks or movement artifacts, $n = 9$, or (c) their data were not recorded due to technical difficulties, $n = 1$. Recruitment and all procedures were approved by the appropriate Research Ethics Board at the University of Toronto, in accord with the Canadian Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans.

PROCEDURE

Children were given a brief introduction to the testing environment and the EEG system, and parental informed consent (and child assent) was obtained for all participants. The experimenter then applied a 128-channel HydroCel Geodesic Sensor Net to the child's head, the child was seated in front of a computer monitor with the distance and alignment to the monitor controlled, and the go/no-go task was administered. This task, which was adapted from Durston et al. (2002b), was presented using E-Prime Version 1.2 (PST, Pittsburgh, PA, USA). On each trial, an animal stimulus (cow, horse, bear, pig, or dog) was presented at a central location on the screen. During a block of 10 practice trials (6 go, 4 no-go), the child was instructed to press a sticker-covered key on a keyboard as soon as they saw each animal (go stimuli) except for the dog (no-go stimulus). They were told not to press when they saw the dog. The practice block was repeated until children were correct on at least 9/10 trials. The task itself consisted of 144 trials divided into four blocks. In each block, 27 (75%) of the trials were go trials and 9 (25%) were no-go trials. This ratio encouraged a pre-potent tendency to respond. No-go trials were preceded by two, three, or four go trials. On each trial, a fixation point in the form of a "pokeball" appeared at a central location on the screen, along with a "ding" sound, and lasted 1500 ms. Next, an animal stimulus was presented for 1500 ms. In order to increase children's motivation to complete the task, feedback was given following each response. Positive feedback following correct responses was provided by a bright yellow smiley face, and negative feedback following incorrect responses, omitted responses, and responses that occurred after the 1500 ms stimulus window, was provided by a red frowning face. Feedback stimuli were shown for 500 ms (see **Figure 1** for trial structure). Accuracy on go and no-go trials was recorded, as was reaction time (RT) on correct go trials.

During the go/no-go task, EEG data were sampled at 1000 Hz using EGI Netstation 4.1.2 software (EGI, Eugene, OR, USA), and impedances were maintained below 40 kΩ. Electrodes were referenced to Cz during recording. Editing of the EEG for eye blinks, eye movements, movement artifacts, signals exceeding 200 μV, and fast transits exceeding 100 μV was carried out offline, and all trials containing more than 20% artifacts were eliminated from analysis. During averaging, all data were re-referenced to the average reference of all 128 sites (Tucker et al., 1993). Data were filtered using a Finite Impulse Response 1–30 Hz bandpass filter. Stimulus-locked data were segmented into epochs ranging from 200 ms prior to stimulus onset to 1000 ms after onset. ERP data from correct trials (go and no-go) were baseline corrected using the first 200 ms of each segment. The N2 was then coded as the largest negative deflection after the P1 with a fronto-central topography and a latency of 250–500 ms post-stimulus. N2 latency was recorded as the latency from stimulus onset to the peak identified in the amplitude analysis. The mean number (and SD) of trials contributing to the N2 was 70.92 (18.93) for go trials and 23.81 (5.98) for no-go trials, and there were no differences in trial count between cultural groups for either go trials, $t(35) = -1.02$, *ns*, or no-go trials, $t(35) = -0.07$, *ns*.

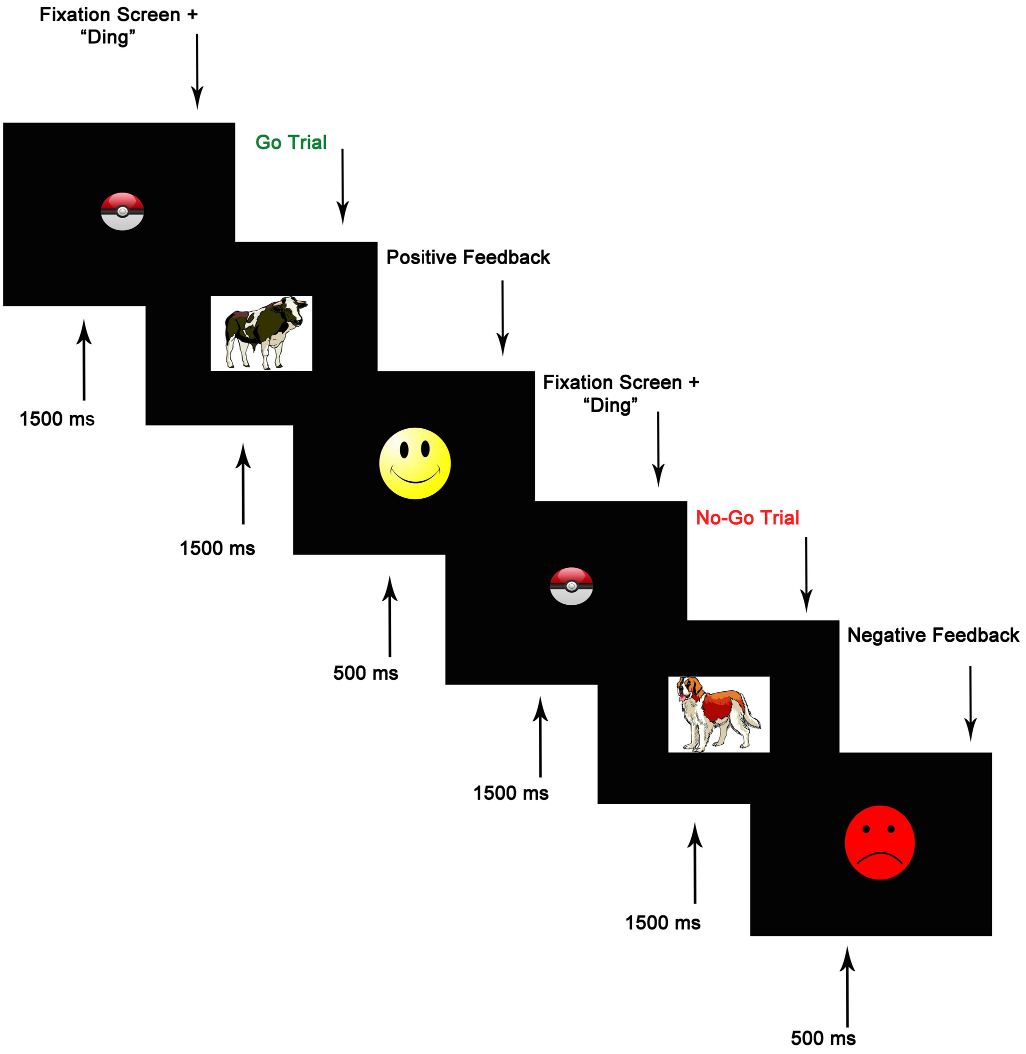

FIGURE 1 | Go/no-go task trial structure.

RESULTS

For all analyses reported below, the same pattern of results was obtained when gender was included as a variable, and there were no effects of (or interactions involving) gender. For all ERP analyses, including source analyses, the same pattern of results was obtained when behavioral performance (no-go accuracy cost and median RT) and trial count were included as covariates. In addition, a regression, with number of languages spoken in the home as a predictor, was conducted on the major variables of interest and no significant results were obtained ($0.26 < p < 0.96$).

BEHAVIORAL RESULTS

To account for possible group and individual differences in aspects of performance not specific to EF, we created a baseline-adjusted measure of the extent to which individuals erred on no-go relative to go trials. This measure, no-go accuracy cost, was calculated as the difference between the proportion of correct go trials and the proportion of correct no-go trials, divided by the proportion of correct go trials. No-go accuracy cost provides a more pure measure of EF than does simple no-go accuracy, but the same pattern of results was obtained using no-go accuracy, except that no-go accuracy cost scores were negatively correlated with median RT ($r = -0.42$, $p < 0.01$), indicating a speed-accuracy tradeoff in this sample, whereas there was no relation between simple no-go accuracy and median RT, $r = 0.12$. Separate one-way ANOVAs found no cultural group differences in no-go accuracy cost scores, $F(1, 35) = 0.84$, *ns*, $\eta_p^2 = 0.02$ (Chinese $M = 0.01$, SE = 0.03; European $M = 0.04$, SE = 0.02) or median RT, $F(1, 35) = 0.16$, *ns*, $\eta_p^2 = 0.01$ (Chinese $M = 744.38$ ms, SE = 25.47; European $M = 758.20$, SE = 23.48). The absence of cultural group differences in behavioral performance facilitates interpretation of any differences to emerge from ERP analyses.

ERP ANALYSES

An examination of the scalp topo-maps of the grand-averaged data revealed that the N2 component was right-lateralized on no-go trials, consistent with previous studies with children and adults (e.g., Bokura et al., 2001; Todd et al., 2008), but also that N2 amplitudes were larger for go trials than for no-go trials over the left frontal part of the scalp. Therefore, rather than create no-go minus go difference waveforms, we compared N2 waveforms for go and no-go trials as a function of laterality. Data were averaged across a right-hemisphere fronto-central cluster of electrodes that included HydroCel electrodes 112, 111, 118, 117, and 124 (encompassing F4 in the 10–20 System), and across a left-hemisphere fronto-central cluster that included electrodes 13, 29, 20, 28, and 24 (encompassing F3); see **Figure 2**.

N2 results

N2 amplitudes and latency were analyzed using an ANOVA with cultural group as a between-subjects variable and go/no-go condition (go trials, no-go trials) and laterality (right, left) as within-subjects variables. *Post hoc* pairwise comparisons with Bonferroni adjustments were carried out for all significant interactions. **Figure 3** presents the grand-averaged waveforms of the N2 component at the corresponding pair of left-right electrode sites that best illustrate the effects reported below: electrode site 112 (on the right) for the no-go condition and electrode site 13 (on the left) for the go condition. Waveforms are also shown for all other sites in the montage.

No significant effects or interactions were found for N2 latencies ($\eta_p^2 < 0.07$ in all cases). The ANOVA on N2 amplitudes revealed a marginally significant main effect for cultural group, $F(1, 35) = 3.78$, $p < 0.06$, $\eta_p^2 = 0.10$ (Chinese $M = -6.01$ μV, SE = 0.89; European $M = -3.67$ μV, SE = 0.82), and a significant effect of laterality, $F(1, 35) = 15.05$, $p < 0.001$, $\eta_p^2 = 0.30$ (Right $M = -5.72$ μV, SE = 0.65; Left $M = -3.95$ μV, SE = 0.64). These effects were qualified by a significant Go/no-go condition × Laterality interaction, $F(1, 35) = 110.64$, $p < 0.001$, $\eta_p^2 = 0.76$, and a significant Cultural group × Go/no-go condition × Laterality interaction, $F(1, 35) = 5.40$, $p < 0.05$, $\eta_p^2 = 0.13$. *Post hoc* tests examining the effect of cultural group revealed that Chinese-Canadian children showed larger (i.e., more negative) N2 amplitudes than European-Canadian children on no-go trials on the right side of the scalp, $p < 0.05$ (Chinese $M = -9.13$ μV, SE = 1.14; European $M = -5.79$ μV, SE = 1.05; **Figure 4A**), as well as on go trials on the left side of the scalp, $p < 0.05$ (Chinese $M = -7.66$ μV, SE = 0.96; European $M = -4.59$ μV, SE = 0.89; **Figure 4B**), but that there were no group differences on no-go trials on the left or on go trials on the right. The three-way interaction reflected the fact that Chinese-Canadian children showed a stronger effect of laterality on no-go trials ($\eta_p^2 = 0.59$) than did European-Canadian children on go trials ($\eta_p^2 = 0.16$).

N2 amplitude lateralization scores

To assess the degree of lateralization of the N2, right-hemisphere N2 amplitudes were subtracted from left-hemisphere N2 amplitudes, and then these difference scores were analyzed using an ANOVA with cultural group as a between-subjects variable and go/no-go condition as a within-subjects variable. There was a main effect of go/no-go condition, $F(1, 35) = 110.64$, $p < 0.001$, $\eta_p^2 = 0.76$, and a Cultural group × Go/no-go condition interaction, $F(1, 35) = 5.4$, $p < 0.05$, $\eta_p^2 = 0.13$. Examination of means revealed that for both cultural groups, left-minus-right difference scores were positive for no-go trials (Chinese $M = 6.98$, SE = 0.98; European $M = 4.39$, SE = 0.91), reflecting larger (i.e., more negative) amplitudes on the right, and negative for go trials (Chinese $M = -2.57$, SE = 0.72; European $M = -1.72$, SE = 0.67), reflecting larger (i.e., more negative) amplitudes on the left. Pairwise comparisons indicated that the effect of go/no-go condition was significant for both cultural groups, but that the size of this effect was larger for the Chinese-Canadian participants, $\eta_p^2 = 0.69$ than for the European-Canadian participants, $\eta_p^2 = 0.51$. These findings suggest that the N2 was more strongly lateralized (to the left for go trials and to the right for no-go trials) for the Chinese-Canadian participants.

N2 amplitudes: relations with behavioral performance

A total of eight Pearson correlations were conducted to examine relations among mean N2 amplitude for the left- and right-hemisphere electrode clusters, median RTs, and no-go accuracy cost. Significant correlations were found between median RTs on the go/no-go task and the following: right-hemisphere N2 amplitudes on no-go trials, $r = 0.30$, $p < 0.05$, left-hemisphere N2 amplitudes on go trials, $r = 0.28$, $p < 0.05$, and left-hemisphere N2 amplitude on no-go trials, $r = 0.35$, $p < 0.05$. Larger (i.e., more

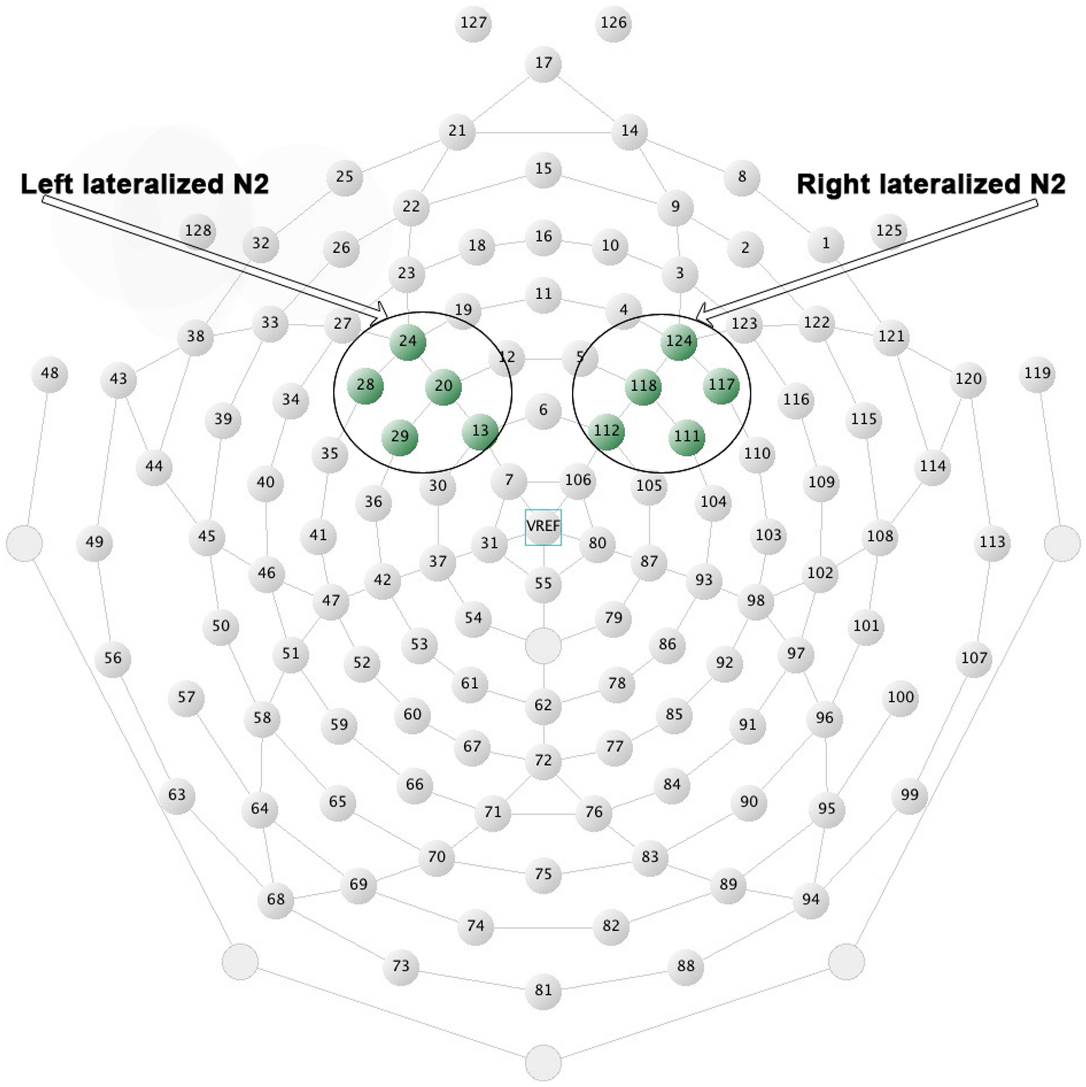

FIGURE 2 | Hydrocel electrode sites contributing to right- and left-lateralized N2 waveforms and data.

negative) N2 amplitudes were associated with faster responding on go trials. Correlations involving no-go accuracy cost were not significant ($-0.13 < r < 0.11$). These correlations indicate that larger N2 amplitudes are related to better EF performance.

Source analysis

We used a minimum norm method with the local autoregressive average (LAURA) to create a source model of the grand-averaged scalp data. Modeled source activation was then examined using GeoSource (EGI) for latencies between 250 and 500 ms post-stimulus. Given that relatively little is known about the sources underlying the N2 in young children, an underdetermined model was used (Michel et al., 2004; Luck, 2005). Regions of interest (ROIs) were defined functionally around the voxels of peak activation in the model, and also in light of source analyses of the N2 in studies conducted with North American children (Lamm et al., 2006; Lewis et al., 2006, 2008; Todd et al., 2008). Activation was then averaged across all voxels in each ROI for each participant in each go/no-go condition, and a single-source waveform was extracted for each ROI. This process yielded source activation waveforms for four hypothetical generators of scalp activation located generally in ventromedial prefrontal cortex, right ventrolateral prefrontal cortex, left ventrolateral prefrontal cortex, and dorsomedial prefrontal cortex. **Figure 5** shows means and standard deviations of activation levels (in *nA*) extracted from these modeled sources for the two cultural groups for the peak N2 interval between 300 and 350 ms post-stimulus. To investigate cultural group differences in extracted activation levels for each of the four modeled sources, separate ANOVAs were carried out with cultural group as a between-subjects variable, and go/no-go condition and 50-ms interval as within-subjects variables.

For the VMPFC modeled source, there were significant main effects of cultural group, $F(1, 35) = 23.79$, $p < 0.001$, $\eta_p^2 = 0.41$ (Chinese $M = 0.45$, SE = 0.027; European $M = 0.28$, SE = 0.025) and go/no-go condition, $F(1, 35) = 48.23$, $p < 0.001$, $\eta_p^2 = 0.58$

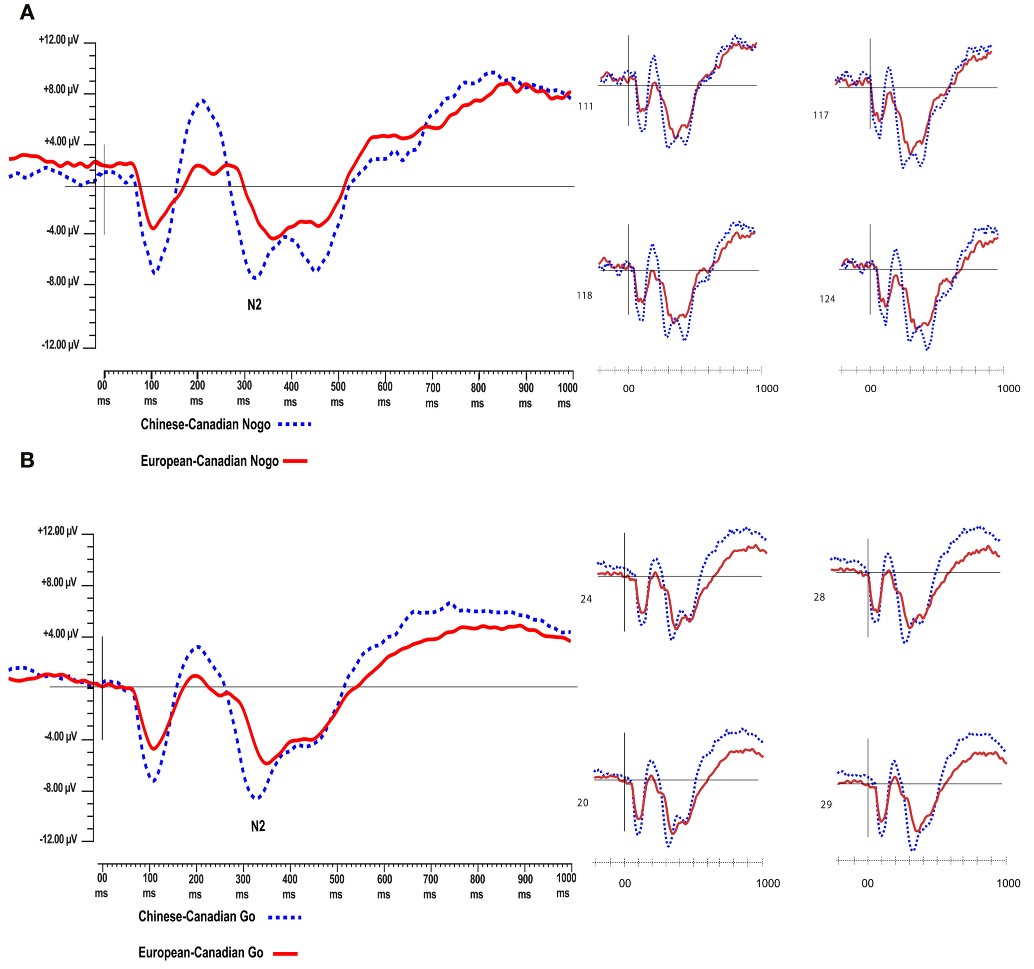

FIGURE 3 | Stimulus-locked grand-averaged ERP waveforms for the two cultural groups. (A) Data from fronto-central electrode site 112 (right-hemisphere) for the no-go condition, accompanied by waveforms from electrode sites 111, 117, 118, 124 (right-hemisphere) for the no-go condition. **(B)** Data from fronto-central electrode site 13 (left-hemisphere) for the go condition, accompanied by waveforms from electrode sites 24, 28, 20, 29 (left-hemisphere) for the go condition.

(go $M = 0.28$, SE = 0.015; no-go $M = 0.45$, SE = 0.027), with greater overall activation on no-go trials and for Chinese children. There were no interactions.

For the right VLPFC source, there were significant main effects of cultural group, $F(1, 35) = 13.96$, $p < 0.001$, $\eta_p^2 = 0.29$ (Chinese $M = 0.27$, SE = 0.016; European $M = 0.19$, SE = 0.014), go/no-go condition, $F(1, 35) = 197.52$, $p < 0.001$, $\eta_p^2 = 0.90$ (go $M = 0.12$, SE = 0.007; no-go $M = 0.33$, SE = 0.017), and interval, $F(4, 140) = 21.08$, $p < 0.001$, $\eta_p^2 = 0.38$ (250–300 ms $M = 0.19$, SE = 0.011; 300–350 ms $M = 0.23$, SE = 0.012; 350–400 ms $M = 0.24$, SE = 0.011; 400–450 ms $M = 0.23$, SE = 0.010; 450–500 ms $M = 0.24$, SE = 0.010) with greater activation for Chinese children; no-go trials; and the interval of 250–300 ms vs. all others. These main effects were qualified by a Cultural group × Go/no-go condition interaction, $F(1,35) = 6.91$, $p < 0.05$, $\eta_p^2 = 0.17$, a Go/no-go condition × Interval interaction, $F(4, 140) = 25.28$, $p < 0.001$, $\eta_p^2 = 0.42$, and a Cultural group × Go/no-go condition × Interval interaction, $F(4, 132) = 3.10$, $p < 0.05$, $\eta_p^2 = 0.09$. *Post hoc* tests revealed that the difference between go and no-go trials (i.e., no-go > go) for this right VLPFC source was larger for Chinese-Canadian children than for European-Canadian children, that the difference between go and no-go trials was largest between 350 and 400 ms, and that the Cultural group × Go/no-go condition interaction was especially pronounced for the two intervals between 300 and 400 ms.

For the left VLPFC source, there were main effects of cultural group, $F(1, 35) = 22.49$, $p < 0.001$, $\eta_p^2 = 0.39$ (Chinese $M = 0.26$, SE = 0.015; European $M = 0.17$, SE = 0.013), go/no-go condition, $F(1, 35) = 22.39$, $p < 0.001$, $\eta_p^2 = 0.39$ (go $M = 0.24$, SE = 0.012; no-go $M = 0.18$, SE = 0.012), and interval, $F(4, 140) = 10.70$, $p < 0.001$, $\eta_p^2 = 0.23$ (250–300 ms $M = 0.19$, SE = 0.011; 300–350 ms $M = 0.21$, SE = 0.011; 350–400 ms $M = 0.22$, SE = 0.011; 400–450 ms $M = 0.22$, SE = 0.010; 450–500 ms $M = 0.23$, SE = 0.010) with greater activation for Chinese children and for go trials, and differences between 250–300 ms vs. all other intervals, and between 400–450 ms and 450–500 ms). These were qualified by a Go/no-go condition × Interval interaction, $F(4, 140) = 42.50$, $p < 0.001$, $\eta_p^2 = 0.55$, and a Cultural group × Go/no-go condition × Interval interaction, $F(4, 140) = 2.63$, $p < 0.05$, $\eta_p^2 = 0.07$, reflecting the fact that the difference between go and

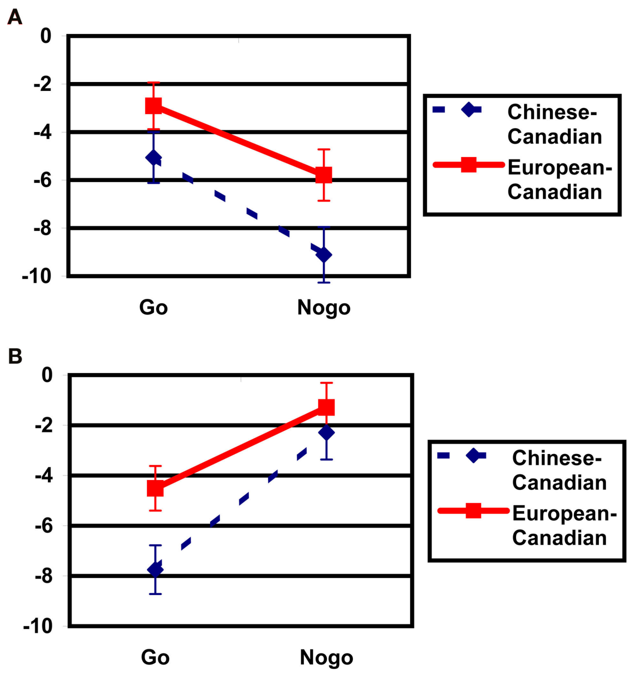

FIGURE 4 | Cultural group differences in N2 amplitudes (μV) by go/no-go condition. (A) Mean amplitudes for the right-hemisphere fronto-central cluster of electrodes (Hydrocel electrodes 112, 111, 118, 117, and 124). **(B)** Mean amplitudes for the left-hemisphere fronto-central cluster of electrodes (Hydrocel electrodes 13, 29, 20, 28, and 24).

no-go trials (i.e., go > no-go) for this left VLPFC source was larger for Chinese-Canadian children, and that this two-way interaction was especially pronounced for the two intervals between 300 and 400 ms.

Finally, for the DMPFC source, there were main effects of cultural group, $F(1,35) = 14.73, p < 0.001, \eta_p^2 = 0.30$ (Chinese $M = 0.34$, $SE = 0.020$; European $M = 0.24$, $SE = 0.019$), go/no-go condition, $F(1, 35) = 7.72$, $p < 0.01$, $\eta_p^2 = 0.18$ (go $M = 0.27$, $SE = 0.013$; no-go $M = 0.31$, $SE = 0.019$), and interval, $F(4, 140) = 6.38$, $p < 0.001$, $\eta_p^2 = 0.15$ (250–300 ms $M = 0.27$, $SE = 0.014$; 300–350 ms $M = 0.30$, $SE = 0.015$; 350–400 ms $M = 0.30$, $SE = 0.016$; 400–450 ms $M = 0.29$, $SE = 0.014$; 450–500 ms $M = 0.28$, $SE = 0.014$) with greater activation for Chinese children and for no-go trials, and a difference between 250–300 ms vs. both 300–350 ms, 350–400 ms. These were qualified by a Go/no-go condition × Interval interaction, $F(4,140) = 4.40$, $p < 0.01$, $\eta_p^2 = 0.11$, and a Cultural group × Interval interaction, $F(4, 140) = 4.87$, $p < 0.001$, $\eta_p^2 = 0.12$. *Post hoc* tests revealed that Chinese-Canadians showed more activation than European-Canadians especially between 250 and 350 ms, and that the differences between go and no-go trials (i.e., no-go > go) for this dorsomedial prefrontal region of interest was largest between 250 and 300 ms.

DISCUSSION

Recent research suggests that Asian preschoolers may perform better than age-matched North American or British children on measures of EF (e.g., Sabbagh et al., 2006; Oh and Lewis, 2008), consistent with reported cultural differences in the emphasis placed on impulse control (e.g., Chen et al., 1998). This study provides a

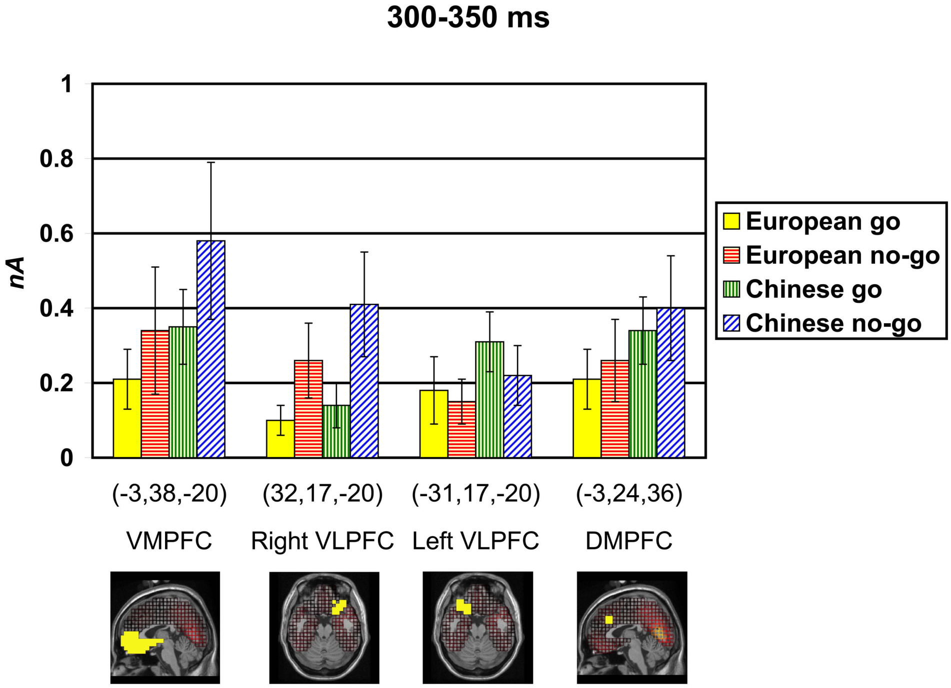

FIGURE 5 | Modeled source activations (in *nA*) and ROIs displayed using the Montreal Neurological Institute (MNI) average adult MRI scan for the peak N2 interval of 300–350 ms in ventromedial prefrontal cortex (VMPFC; –3, 38, –20), right ventrolateral prefrontal cortex (VLPFC: 32, 17, –20), left VLPFC (–31, 17, –20), and dorsomedial prefrontal cortex (DMPFC: –3, 24, 36), as a function of cultural group and go/no-go condition. GeoSource voxel locations (*x, y, z*) in Talairach space are provided for the peak activation in each ROI.

first look at whether the neural correlates of EF, measured in the context of a go/no-go task, differ systematically between European-Canadian and Chinese-Canadian 5-year-olds. Although no cultural group differences were observed in children's behavioral performance on the go/no-go task, substantial differences were revealed by ERP analyses, with Chinese-Canadian children showing larger N2 amplitudes than European-Canadian children. In studies comparing participants within a single age, N2 amplitude is often larger in groups performing better on EF (e.g., Pliszka et al., 2000), and in the current study, larger N2 amplitudes were associated with faster RTs (i.e., better performance).

N2 amplitude is usually observed to be larger on successful no-go trials than on go trials (e.g., Eimer, 1993; Falkenstein et al., 1999). In the present study, N2 amplitudes were indeed larger on no-go trials than on go trials over the right frontal part of the scalp, a pattern of lateralization that has sometimes been observed in previous research with both children and adults (e.g., Todd et al., 2008; see also Lavric et al., 2004, who localized sources of N2 difference scores in right ventral and dorsolateral prefrontal cortex). In contrast, however, over the left frontal part of the scalp, N2 amplitudes were larger on go trials than on no-go trials. This asymmetrical pattern of scalp lateralization (to the right for no-go trials and to the left for go trials) was reflected in the results of the source analysis of the N2 in the current study, which suggested that a cortical generator in left VLPFC may contribute more to go N2s whereas a cortical generator in right VLPFC may contribute more to no-go N2s. Together, these findings are in line with a large body of research on EF tasks that show a lateralized pattern (e.g., Konishi et al., 1998, 1999; Garavan et al., 1999, 2002; Bokura et al., 2001; Menon et al., 2001; Bunge et al., 2002; Rubia et al., 2003; Aron et al., 2004; Perez-Edgar and Fox, 2007; Todd et al., 2008; Madsen et al., 2009) as well as with research revealing other lateralized ERP components implicated in emotion regulation (e.g., the late positive potential, LPP; Cunningham et al., 2005), and research on EEG alpha power asymmetry, indicating a greater role for left PFC in approach behavior and a greater role for right PFC in withdrawal or inhibition (e.g., Davidson, 1992; Davidson and Fox, 1989; Harmon-Jones and Allen, 1997). At least in young children in the context of this task, therefore, the amplitude of the N2 may provide an index not only of the inhibitory aspects of EF (over right PFC) but also of effective goal-directed approach behavior (over left PFC).

In general, the prefrontal sources modeled in the present study fit well with previous research on the regions implicated in the go/no-go task with children (e.g., Durston et al., 2002b; Lamm et al., 2006; Lewis et al., 2006, 2008). In addition, the regions modeled in the VMPFC and DMPFC are roughly consistent with studies modeling sources of the N2 that are centrally localized (e.g., Nieuwenhuis et al., 2003), although it should be noted that these studies used different approaches to source modeling than the present study and modeled the N2 difference between no-go and go trials (i.e., the N2 effect).

The asymmetric pattern of lateralization to emerge in this study was more pronounced for Chinese-Canadian children than it was for European-Canadian children. It was not simply that Chinese-Canadian children showed more activation overall than European-Canadian children; rather, they showed a more differentiated pattern, congruent with the different task demands inherent in the go/no-go task: Chinese-Canadian children showed larger N2 amplitudes than European-Canadian children on the right side of the scalp on no-go trials, as well as on the left side of the scalp on go trials. In addition, left-minus-right difference scores, which were positive for no-go trials and negative for go trials for all children, were larger in absolute magnitude for the Chinese-Canadian children. Further work is needed to examine the origin and implications of this group difference in lateralization.

The lack of behavioral differences between the cultural groups in this study differs from previous findings that Chinese children outperformed North American children on behavioral measures of EF (e.g., Sabbagh et al., 2006), although this earlier research focused on younger children and did not examine go/no-go performance. In addition, the Chinese-Canadians in the current study grew up in Canada, whereas previous research has examined Asian children living in Asia. In any case, it would appear that at least in some situations, EEG can provide a measure of cultural differences in neurocognitive function that is more sensitive than behavioral data alone.

Further work remains to be done to clarify the nature of the group differences in neurocognitive function observed in the current study. Neurocognitive abilities are undoubtedly influenced by both genes and environment, and both types of influence are likely to vary across cultures. For example, cultural differences in the importance that parents place on impulse control could affect children's motivation to succeed on a task like the go/no-go task, resulting in greater PFC activation. Neither number of languages spoken in the home nor parent-reported individualism–collectivism accounted for the observed differences, but there are several possible factors that may contribute to them, including genetic differences related to EF and possible group differences in skull density, thickness, and shape (Zilles et al., 2001; Knott et al., 2004), as well as any number of other unmeasured variables. Regardless of the origin of the observed group differences, however, the findings underscore the fact that the neural correlates of EF may vary across samples of healthy participants, even in children. One simply cannot assume that neural function is the same in all samples of healthy children – cultural background, and whatever may be correlated with cultural background, needs to be taken into consideration.

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Testing for a cultural influence on reading for meaning in the developing brain: the neural basis of semantic processing in Chinese children

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Functional magnetic resonance imaging was used to explore the neural correlates of semantic judgments in a group of 8- to 15-year-old Chinese children. Participants were asked to indicate if pairs of Chinese characters presented visually were related in meaning. The related pairs were arranged in a continuous variable according to association strength. Pairs of characters with weaker semantic association elicited greater activation in the mid ventral region (BA 45) of left inferior frontal gyrus, suggesting increased demands on the process of selecting appropriate semantic features. By contrast, characters with stronger semantic association elicited greater activation in left inferior parietal lobule (BA 39), suggesting stronger integration of highly related features. In addition, there was a developmental increase, similar to previously reported findings in English, in left posterior middle temporal gyrus (BA 21), suggesting that older children have more elaborated semantic representations. There were additional age-related increases in the posterior region of left inferior parietal lobule and in the ventral regions of left inferior frontal gyrus, suggesting that reading acquisition relies more on the mapping from orthography to semantics in Chinese children as compared to previously reported findings in English.

Keywords: functional magnetic resonance imaging, semantic, association strength, developmental, children

INTRODUCTION

Language is acquired without much effort and is associated with maturational changes in the brain (Friederici, 2006). The nature of developmental changes in human cerebral functional organization for language is a core issue in cognitive neuroscience (Brown et al., 2005). This paper focuses on the developmental changes in the neural substrate for semantic processing in reading, which may be somewhat different than spoken language. Emerging evidence of the neural correlates of semantic development mainly comes from English and other alphabetic languages. This paper uses a semantic association task to explore the age effects on the neural correlates of semantic processing in a logographic language, Chinese. The semantic association task has been used to understand the functional architecture of word recognition, identifying brain regions for semantic processing in English in left inferior frontal gyrus (BA 45, 47), left posterior middle temporal gyrus (BA 21), and left inferior parietal lobule (BA 39, 40) in adults (Fletcher et al., 2000; Booth et al., 2002) and in children (Chou et al., 2006a,b).

The role of inferior frontal gyrus in semantic processing has been explored in English by comparing activation to semantic association judgments involving strongly related pairs (e.g., *king–queen*) versus weakly related pairs (e.g., *net–ship*). Weaker association produced greater activation in left inferior frontal gyrus as compared to stronger association. Greater activation in left inferior frontal gyrus was suggested to result from the difficulty of selecting appropriate semantic features, as weakly related pairs share few semantic features (Fletcher et al., 2000). In support of this, many studies show greater inferior frontal gyrus activation in more difficult semantic tasks and in tasks with increased retrieval or selection demands. These studies include high vs. low requirement for selection among alternatives (Thompson-Schill et al., 1997, 1999), weak vs. strong association strength (Wagner et al., 2001; Chou et al., 2006a,b), naming low vs. high familiarity objects (Whatmough et al., 2002), generating novel vs. repeated base nouns (Seger et al., 2000), naming low vs. high agreement pictures (Kan and Thompson-Schill, 2004), deep vs. shallow processing of words (Fujii et al., 2002) and producing words for pre-specified semantic categories vs. over-learned letter sequences (Gurd et al., 2002).

A review article recently proposed different cognitive functions for sub-regions of the inferior frontal gyrus (Badre and Wagner, 2007). The anterior ventral region of left inferior frontal gyrus (BA47) may support controlled retrieval of stored semantic representations, whereas the mid-ventral region of left inferior frontal gyrus (BA 45) may support increased selection demands to process active representations. Previous studies using association strength have found greater activation for weaker association pairs in both the anterior ventral region and the mid-ventral region of left inferior frontal gyrus (BA 45, 47) (Badre et al., 2005; Chou et al., 2006a,b). For weaker association pairs, participants may need controlled access to stored conceptual representations to seek for existing associations in verbal semantic memory. Moreover, participants may require a selection process that operates post-retrieval to resolve competition among active representations during association judgments.

Studies in English have also shown that weaker association strength between words results in greater activation in left middle temporal gyrus (Chou et al., 2006a; Wible et al., 2006). This region has been implicated in the representation of verbal semantic information (Booth et al., 2002; Blumenfeld et al., 2006). Greater activation in this region for weaker association pairs may result from a more extensive access to semantic representations in order to identify distant relationships (Booth et al., 2007). In contrast, studies in English have shown that word pairs with stronger association result in greater activation in left inferior parietal lobule (Chou et al., 2006a,b; Raposo et al., 2006). Some studies have interpreted the left inferior parietal lobule as involved in feature integration and semantic categorization to form a coherent concept so that relationships between words can be determined (Smith, 1995; Grossman et al., 2003). Left inferior parietal cortex has also been suggested to support the integration of lexical input into the larger units during semantic processing (Lau et al., 2008). Stronger association pairs may involve greater integration because there are more overlapping features between the words or because the shared features are more characteristic of each of the words (Chou et al., 2006a,b). Thus, if there are several overlapping features between words, then semantic integration processes are effectively engaged in the inferior parietal lobule and this results in greater activation in this region. However, if there are few or no overlapping features, then the inferior parietal lobule will not be engaged because it is less or not possible to integrate these features.

Chinese is different from English in the nature of the mapping between orthography and semantics as well as the mapping between orthography and phonology. English has an arbitrary relationship between orthography and semantics at a mono-morphemic level, whereas many Chinese characters are derived from pictures representing meanings or encode meanings by including a semantic radical. There are approximately 200 semantic radicals in Chinese and these units of characters give a clue to the meaning of the character (e.g., category). Thus, Chinese has a more direct mapping between orthography and semantics than English. In support of this, evidence from event-related potential measures suggests earlier involvement of semantics in Chinese compared to English (Zhang et al., 2006). Moreover, spoken Chinese is highly homophonic, with a single syllable shared by many characters. When learning to read, a Chinese child is confronted with the fact that a large number of written characters correspond to the same syllable, and phonological information is insufficient to access semantics of a printed character. There are phonetic radicals, but most of them (about 60%) provide inconsistent information regarding pronunciation. It is important to explore whether the unique linguistic features of Chinese influence developmental changes in the neural substrate for semantic processing. Based on the more systematic relationship between orthography and semantics and less systematic relationship between orthography and phonology as compared to English, one may expect a larger role for semantics in processing Chinese characters.

Previous English studies examining developmental differences at word level during semantic processing tasks have shown age-related increases in the dorsal region of left inferior frontal gyrus (Schapiro et al., 2004; Brown et al., 2005; Schmithorst et al., 2006; Szaflarski et al., 2006), the anterior region of left inferior parietal lobule (Chou et al., 2006a), and the posterior region of left middle temporal gyrys (Chou et al., 2006a,b; Szaflarski et al., 2006). Limited neuroimaging studies have examined developmental differences in the neural correlates of Chinese language processing (Cao et al., 2009, in press), showing increasing reliance on brain areas involved in visuo-orthographic processing and concomitant decreases in reliance on phonology in spelling and rhyming tasks in the visual modality. Reading for meaning places greater demands on the mapping from orthography to semantics at word level in Chinese (Leck et al., 1995; Zhou and Marslen-Wilson, 2000; Feng et al., 2001), and therefore one may expect that the unique structure of Chinese influences developmental trajectories. Reading development of Chinese may be characterized by decreased reliance on phonology due to many homophones in Chinese, making it more efficient to access meaning through the connection between orthography and semantics rather than the connection of orthography to phonology to semantics (Peng et al., 1985; Song et al., 1995; Meng et al., 2007).

The current study examined the neural substrate of developmental changes during semantic processing in Chinese children (8-to 15-year olds). We manipulated semantic relatedness by varying the 'free association strength' between pairs of visually presented Chinese characters (Hue et al., 2005). From aforementioned studies, we expected weaker association to produce greater activation in the ventral region of left inferior frontal gyrus, and stronger association to produce greater activation in left inferior parietal lobule. Moreover, we investigated whether there were similarities and differences on reading for meaning in the developing brain in Chinese as compared to those reported in English, with an attempt to differentiate between brain processes inherent across languages from those specific to the unique structure of a language.

MATERIALS AND METHODS

PARTICIPANTS

Thirty-three native speakers of Chinese (mean age = 12.3, standard deviation = 1.8, 16 females) in Taiwan participated in the functional magnetic resonance imaging (fMRI) study. The participants were all monolingual. The distribution of ages and genders is shown in **Table 1**. Parents were given an informal interview to insure that their children met the following inclusionary criteria: (1) right-handedness, (2) normal hearing and normal or corrected-to-normal vision after an examination of vision by experimenters, (3) free of neurological disease or psychiatric disorders, (4) not taking medication affecting the central nervous system, (5) no history of intelligence, reading, or oral-language deficits, and (6) no learning disability or attention deficit hyperactivity disorder. After the administration of the interview, informed consent was obtained. Informed consent procedures were approved by the Institutional Review Board at the National Taiwan University Hospital. Standardized intelligence testing was then administered, using the Wechsler Intelligence Scale for Children

Table 1 | The distribution of ages and genders.

	Ages 8–9	Ages 10–11	Ages 12–13	Ages 14–15
Female	2	6	4	4
Male	2	4	7	4

(WISC-III) Chinese version (The Psychological Corporation, 1999). Participants' standard scores (mean ± SD) were 113 ± 10 on the verbal scale and 114 ± 11 on the performance scale.

FUNCTIONAL ACTIVATION TASKS

The children were given two practice sessions, one outside the scanner and the other in the scanner, to make sure that they understood the task. The practice items were different stimuli than those used in fMRI sessions. Each participant was at least 80% correct for each condition separately for both practice sessions. In the meaning judgment task, two visual Chinese characters were presented sequentially and the participant had to determine whether the character pair was related in meaning. Trials lasted 4500 ms and consisted of a solid square (500 ms), followed by the first character (800 ms), a 200-ms blank interval, and the second character for 3000 ms. The duration of 800 ms for the first word and a 200-ms ISI was the same as our previous English study (Chou et al., 2006a). The duration of 3 s for the second word was based on our previous Chinese study testing adults, healthy children, and children with smaller vocabulary sizes (Lee et al., in press). The upper bound of the mean + 2.5 SD of reaction time for children with smaller vocabulary sizes was close to 3 s. Thus, the duration of 3 s for the second word was chosen for a previous adult study (Chou et al., in press) and for this child study. The participant was instructed to make a response during the presentation of the second character.

Forty-eight character pairs were semantically related according to their free association values (mean = 0.14, SD = 0.13, ranging from 0.73 to 0.01) (Hue et al., 2005). The Chinese norms (Hue et al., 2005) were created by presenting 100 native Mandarin-Chinese speakers with a list of target words and asking them to generate the first word that came to mind. For example, if 40 out of the 100 participants generated the same word to a given target, the association strength was 0.4 for the word pair. This same procedure has been used in English (Nelson et al., 1998). Even though some of the word pairs had low association values in the related pairs, on average, the participants in our study were able to determine they were related. For example, the average accuracy for the word pair with association strength 0.01 was above 70%. Character pairs were arranged in a continuous variable according to association values. The distribution of association values was positively skewed so a logarithmic transformation was used (Howell, 2006). When values are near-zero, one can use $\log(X + 1)$ to perform a logarithmic transformation, but we used $\log(100X + 1)$ in order to get a positive number after transformation (mean = 1.04, SD = 0.36, ranging from 1.86 to 0.30). Such a logarithmic transformation has been shown to be suitable for item-level parametric analyses in developmental studies (Bolger et al., 2008). In addition, 24 word pairs were semantically unrelated with zero association values. The participants were instructed to quickly and accurately press with their right hand the yes button to the related pairs and the no button to the unrelated pairs.

The perceptual control condition had 24 pairs of non-characters. Non-characters were created by replacing radicals of real characters with other radicals that did not form real Chinese characters. Non-characters were larger (50 font size) than real characters (40 font size) in order to encourage participants to perform the task based on the recognition of low level visual similarity and not on the extraction of semantic information. The size was a cue for participants to judge the stimuli perceptually. For the perceptual control condition, trials consisted of a solid square (500 ms), followed by the first non-character (800 ms), a 200-ms blank interval, and the second non-character for 3000 ms. Participants determined whether the pair of stimuli were identical or not by pressing a yes or no button with their right hand. There were also 24 baseline events as 'null' trials so that we could better deconvolve the response to the lexical and perceptual trials. The participant was instructed to press a button when a solid square (1300 ms) at the center of the visual field turned to a hollow square (3000 ms) after a blank interval (200 ms). In order to control for visual-orthographic information between real characters and non-characters, we compared the related or unrelated conditions to the perceptual control condition for the fMRI analyses.

STIMULUS CHARACTERISTICS

Several lexical variables were controlled across the related and unrelated conditions. First, all Chinese characters were monosyllabic. Second, the first character and the second character did not share radicals. Third, the first character and the second character did not form a word (Wu and Liu, 1987; Sinica Corpus, 1998). Fourth, the number of nouns, verbs, adjectives, adverbs was similar for strong (48%, 23%, 29%, and 0%), weak (48%, 23%, 25%, and 4%) and unrelated pairs (50%, 23%, 21%, and 6%), based on their most frequent usage in Academia Sinica balanced corpus (Sinica Corpus, 1998). In addition, we split the related condition into strong and weak pairs, each pair with 24 trials, and calculated two word (first, second) by three relatedness (strong, weak, unrelated) ANOVAs on stimulus characteristics. First, characters were matched for visual complexity (in terms of strokes per character) across conditions. The main effect of relatedness, $F(2,138) = 0.68$, $p > 0.05$, nor its interaction with word was significant, $F(2,138) = 2.85$, $p > 0.05$. In addition, the correlation of visual complexity (first or second words) with association strength was not significant, $r = -0.20$, $p > 0.05$. Second, characters were matched for written frequency for adults (Wu and Liu, 1987) and written familiarity for children across conditions. For written frequency, the main effect of relatedness, $F(2,138) = 1.00$, $p > 0.05$, nor its interaction with word was significant, $F(2,138) = 2.41$, $p > 0.05$. In addition, the correlation of frequency (first or second words) with association strength was not significant, $r = -0.02$, $p > 0.05$. Familiarity scores were obtained from pre-tests in which all the characters were rated on a 7-point scale by 30 age-matched children who were native Mandarin speakers from Taiwan. The instruction for written familiarity asked the children how often they saw the word in books, newspaper, and magazines. The 30 children were equally distributed across the 8- to 15-year-old age range. For written familiarity, the main effect of relatedness, $F(2,138) = 0.7$, $p > 0.05$, nor its interaction with word was significant, $F(2,138) = 2.62$, $p > 0.05$. Finally, the semantic relation for related pairs (Lee et al., in press) was a dichotomous scale (categorical coding:1, functional coding:0). The point-biserial correlation of the measure of semantic relation with association strength was not significant, $r = -0.05$, $p > 0.05$, indicating that association effects should not be due to semantic relation differences.

MRI DATA ACQUISITION

Participants lay in the scanner with their head position secured. An optical response box was placed in the participants' right hand. The head coil was positioned over the participants' head. Participants viewed visual stimuli projected onto a screen via a mirror attached to the inside of the head coil. This study adopted an event-related design. Each participant performed two functional runs. Each run took 4.7 min.

All images were acquired using a 3 Tesla Siemens scanner. Gradient-echo localizer images were acquired to determine the placement of the functional slices. For the functional imaging studies, a susceptibility weighted single-shot EPI (echo planar imaging) method with BOLD (blood oxygenation level-dependent) was used. Functional images were interleaved from bottom to top collected parallel to the AC-PC plane. The following scan parameters were used: TE = 24 ms, flip angle = 90°, matrix size = 64 × 64, field of view = 25.6 cm, slice thickness = 3 mm, number of slices = 34; TR = 2000 ms. Each participant performed two 4.7-min functional runs. Each functional run had 4 dummy volumes discarded from fMRI analysis and 136 image volumes used for fMRI analysis. In addition, a high resolution, T1 weighted 3D image was acquired (TR = 1560 ms, TE = 3.68 ms, flip angle = 15°, matrix size = 256 × 256, field of view = 25.6 cm, slice thickness = 1 mm, number of slices = 192). The orientation of the 3D image was identical to the functional slices. The task was administered in a pseudorandom order for all subjects, in which the order of related, unrelated, perceptual, and baseline trials was optimized for event-related design (Burock et al., 1998). We used the Optseq script for randomized event-related design (http://surfer.nmr.mgh.harvard.edu/optseq, written by D. Greve, Charlestown, MA, USA) that implemented Burock et al. (1998)'s approach.

IMAGE ANALYSIS

Data analysis was performed using SPM2 (Statistical Parametric Mapping). The functional images were corrected for differences in slice-acquisition time to the middle volume and were realigned to the first volume in the scanning session using affine transformations. No participant had more than 3 mm of movement in any plane. We judged a 3-mm criterion to be appropriate because this was less than the in plane resolution of acquisition (4 mm). The average estimated motion over the whole run was small in all directions ($x = 0.02$ mm; $y = 0.07$ mm; $z = 0.04$ mm). In addition, the maximum displacement in any direction was small for each trial type (related = 0.019 mm, perceptual = 0.021 mm, baseline = 0.021 mm, unrelated = 0.021 mm). There were no significant differences in maximum movement across trial types and there was not correlation of x, y or z movement with age. Co-registered images were normalized to the MNI (Montreal Neurological Institute) average template (12 linear affine parameters for brain size and position, 8 non-linear iterations and 2 × 2 × 2 nonlinear basis functions). We did not use a child template because given the age of our participants and the voxel size it was appropriate to use an adult template (Burgund et al., 2002). This also allowed for comparison of the results of the present study with other published adult studies. Statistical analyses were calculated on the smoothed data (10 mm isotropic Gaussian kernel), with a high pass filter (128 s cutoff period).

Data from each participant was entered into a general linear model using an event-related analysis procedure (Penny and Holmes, 2003). Character pairs were treated as individual events for analysis and modeled using a canonical HRF (Hemodynamic Response Function). There were four event types: related, unrelated, perceptual, and baseline. For the related pairs, association strength was an item-level parametric modulator in order to differentiate semantic relatedness as a continuous variable according to log transformed free association strength (Hue et al., 2005). The analysis of association strength was based on the related pairs and was not in comparison to baseline (also see Bolger et al., 2008). The resulting model coefficients for individual subjects were entered into subsequent second-order random-effects analyses in a whole brain analysis. All reported areas of activation were significant using $p < 0.05$ corrected for FDR (false discovery rate) at the voxel level with a cluster size greater than or equal to 10 voxels.

Random-effects analysis using one-sample *t*-tests across all participants was used to determine whether activation during a contrast was significant (i.e., parameter estimates were reliably greater than zero). First, we compared the related and unrelated pairs separately to the perceptual control condition, and the related to the unrelated pairs in a whole brain analysis. Second, we examined the effects of association strength for the related pairs, including reaction times as an item-based (within-subject) covariate. This allowed us to examine the association effects that were independent of reaction time differences. Positive effects indicated greater activation for related pairs with stronger association strength, whereas negative effects indicated greater activation for related pairs with weaker association strength. For the effects of association strength, we used the adult study (Chou et al., in press) to determine if a similar effect of association strength was present in children. An inclusive mask ($p < 0.005$ uncorrected) of left inferior parietal activation from the adult study was used to test for stronger association in this child study. An inclusive mask ($p < 0.005$ uncorrected) of left inferior frontal activation from the adult study was used to test for weaker association in this child study. Third, we used multiple regression to correlate the continuous variable of age in months with signal intensity for the related minus unrelated contrast, including accuracy or reaction time as a between-subject covariate. This allowed us to examine age-related increases or decreases in activation that were independent of accuracy or reaction time differences. We extracted the beta values from the peak voxels of brain regions to visualize correlations for these age analyses.

RESULTS

BEHAVIORAL PERFORMANCE

Because 6% of accuracy values were located outside 2.5 SD from the group mean for each condition, trimming these outliers was applied to accuracy analysis. The subject-based accuracy (mean ± SD) for the related and unrelated conditions was 88 ± 10% and 96 ± 6%, respectively, with the related condition being less accurate than the unrelated condition, a paired $t(32) = 4.31$, $p < 0.01$. The subject-based reaction times (mean ± SD) measured from the onset of the second stimulus for the related and unrelated conditions were 1012 ± 244 ms and 992 ± 221 ms, respectively, with no significant difference, a paired $t(32) = 1.11$, $p = 0.28$. The correlations of age with accuracy and reaction times were not significant for the related pairs [$r(33) = 0.216$, $p = 0.228$; and $r(33) = -0.338$, $p = 0.054$, respectively]. Because character pairs were arranged in a continuous variable according to association strength, we calculated correlations between association strength for related pairs and behavioral performance. The item-based correlation between accuracy and association strength was not significant, $r(48) = 0.27$, $p = 0.06$; and the item-based correlation between reaction times and association strength was negative, $r(48) = –0.39$, $p < 0.01$. The subject-based accuracy and reaction times (mean ± SD) for the perceptual control were 99 ± 1% and 687 ± 152 ms, respectively. The subject-based accuracy and reaction times (mean ± SD) for the baseline were 99 ± 2% and 615 ± 155 ms, respectively.

BRAIN ACTIVATION PATTERNS

The presentation of the results will focus on brain regions that have been implicated in previous studies of semantic processing, namely left inferior frontal gyrus, posterior middle temporal gyrus, and inferior parietal lobule. All activation differences are reported in the tables. Because no significant differences were found between the analysis of correct responses alone and the analysis that includes all responses, only results from the analysis with all responses are presented to equate the statistical power between conditions with different accuracies (Bitan et al., 2007). This analysis was done by separately modeling correct and incorrect responses, and only examining condition differences for correct responses. We examined condition differences using correct responses for related versus perceptual, unrelated versus perceptual and related versus unrelated. The peak coordinates and corresponding *Z* values were similar for all responses and for correct responses only.

Table 2 shows greater activation for the related or unrelated pairs compared to the perceptual control condition, and for the related compared to the unrelated pairs. Both related and unrelated pairs produced greater activation in left inferior frontal gyrus (IFG, BA 47, 45) and left posterior middle temporal gyrus (MTG, BA 21) as compared to the perceptual control condition. The direct comparison of the related and unrelated pairs produced greater activation in left inferior frontal gyrus (BA 47, 45), left posterior middle temporal gyrus (BA 21), and left inferior parietal lobule (IPL, BA 39).

The effects of semantic association strength for the related pairs, partialing out the effect of reaction times in the scanner as a within-subject covariate, are shown in **Table 3**. Stronger association

Table 2 | Greater activation for the related or unrelated conditions compared to the perceptual control condition. The direct comparison between the related and unrelated conditions is also presented.

Condition	Regions	H	BA	*z*-test	Voxels	*x*	*y*	*z*
Related–Perceptual	Inferior frontal gyrus	L	47	7.81	1677	–42	26	0
	Inferior frontal gyrus	L	45			–48	21	12
	Middle frontal gyrus	L	46			–45	21	24
	Medial frontal gyrus	L	6	6.66	1308	–6	24	42
	Caudate	L	–	5.73	1602	–12	6	9
	Middle temporal gyrus	L	21	5.38	383	–63	–48	0
	Inferior frontal gyrus	R	47	5.24	242	36	18	–3
Unrelated–Perceptual	Inferior frontal gyrus	L	47	5.78	1000	–42	24	0
	Inferior frontal gyrus	L	45			–48	24	10
	Middle frontal gyrus	L	46			–48	21	27
	Medial frontal gyrus	L	6	4.91	253	–3	9	63
	Middle temporal gyrus	L	21	3.44	19	–57	–48	3
	Inferior frontal gyrus	R	47	3.42	14	33	21	0
Related–Unrelated	Inferior frontal gyrus	L	45	5.17	365	–50	21	10
	Inferior frontal gyrus	L	47			–48	24	–3
	Caudate	L	–	4.40	552	–9	9	9
	Medial frontal gyrus	L	6	4.38	358	–9	6	72
	Middle temporal gyrus	L	21	3.75	56	–60	–42	–3
	Middle frontal gyrus	L	6	3.74	49	–42	–3	54
	Inferior parietal lobule	L	39	3.47	27	–42	–66	36

Note. H: hemisphere, L: left, R: right, BA: Brodmann's Area. Coordinates of activation peak(s) within a region based on a z-test are given in the MNI stereotactic space (x, y, z). Voxels: number of voxels in cluster at p < 0.05 FDR (false discovery rate) corrected, only clusters greater than or equal to 10 are presented.

Table 3 | The effects of semantic association for related pairs.

Modulation	Regions	H	BA	*z*-test	Voxels	*x*	*y*	*z*
Stronger Association	Inferior parietal lobule	L	39	4.22	65	–42	–64	33
	Inferior parietal lobule	L	40			–44	–59	30
Weaker Association	Inferior frontal gyrus	L	45	3.45	14	–48	22	18

Note. See Table 2 note. The activation maps of stronger and weaker association (Chou et al., in press) were used as inclusive masks (p < 0.005 uncorrected) for the stronger and weaker association analyses in this study, respectively.

produced greater activation in left inferior parietal lobule (BA 39, 40) (see **Figure 1A**). Weaker association produced greater activation in left mid ventral inferior frontal gyrus (BA 45) (see **Figure 1B**).

The correlations between age in months and activation, partialing out the effect of accuracy or reaction time in the scanner as a between-subject covariate, are shown in **Table 4**. Both correlations show that increasing age was correlated with greater activation for the related pairs compared to the unrelated pairs in the anterior and mid ventral regions of left inferior frontal gyrus (BA 45, 47), left posterior middle temporal gyrus (BA 21), and left inferior parietal lobule (BA 39) (**Figure 2**). For descriptive purposes, we visualize the age effects by the correlations between age in months and beta values from the peak voxels of these three regions for the related pairs (correlations in IFG, MTG, and IPL were $r = 0.44$; $r = 0.41$; $r = 0.43$, respectively), as well as for the unrelated pairs (correlations in IFG, MTG, and IPL were $r = 0.31$; $r = 0.05$; $r = 0.07$, respectively). There were no negative age effects for the related minus unrelated pairs.

DISCUSSION

The neural correlates of semantic processing in children (8- to 15-year olds) to visually presented Chinese characters were examined with a task requiring association judgments as to whether character pairs were related in meaning. In order to more effectively measure activation within the semantic system, we manipulated the strength of association between the words in related pairs. Similar to previous child studies on English (Chou et al., 2006a,b), in Chinese stronger semantic association produced greater activation in left inferior parietal lobule (BA 39, 40), whereas weaker semantic association produced greater activation in the mid ventral region of left inferior frontal gyrus (BA 45). Regarding age effects on semantic processing, developmental increases in left posterior middle temporal gyrus were similar to those previously reported for English (BA 21) (Chou et al., 2006a,b; Szaflarski et al., 2006). However, developmental increases in left inferior parietal activation were more posterior in Chinese (BA 39) than in previous studies on English (BA 40) (Chou et al., 2006a), and left inferior frontal activation were more ventral in Chinese (BA 45, 47) than in previous studies on English (BA 6, 9) (Schapiro et al., 2004; Brown et al., 2005; Schmithorst et al., 2006; Szaflarski et al., 2006). After a discussion of the association strength effects, we will turn to a consideration of the developmental differences.

Stronger semantic association produced greater activation in left inferior parietal lobule (BA 39, 40). Activation in this region has previously been identified in semantic association tasks in English children and Chinese adults (Chou et al., 2006a,b, in press). Greater activation in left inferior parietal lobule has been interpreted as evidence of semantic integration (Thompson et al., 2007). Left inferior parietal cortex has also been suggested to support the integration of lexical input into the larger units during semantic processing (Lau et al., 2008). Stronger association pairs may allow for greater integration because there are more overlapping features between the words or because the shared features are more characteristic of each of the words (Chou et al., 2006a,b). Greater integration for stronger association pairs may account for the increase in left inferior parietal lobule activation with increasing association strength in Chinese. Thus, the stronger association effect was similar between Chinese and English as well as between adults and children.

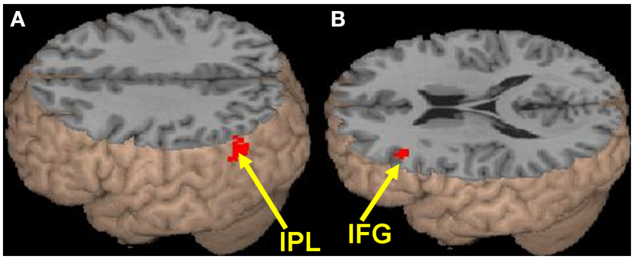

FIGURE 1 | The effects of semantic association strength. (A) Stronger association produced greater activation for the related pairs in left inferior parietal lobule (IPL, BA 39, 40). **(B)** Weaker association produced greater activation for the related pairs in the mid ventral portion of left inferior frontal gyrus (IFG, BA 45).

Table 4 | Increasing activation with age for the related pairs compared to the unrelated pairs, partialing out the effect of accuracy (ACC) or reaction time (RT).

Increase with age	Regions	H	BA	*z*-test	Voxels	*x*	*y*	*z*
Partial for ACC	Caudate	L	–	4.51	546	–9	6	9
	Inferior frontal gyrus	L	45	4.31	259	–51	27	10
	Inferior frontal gyrus	L	47			–48	21	–3
	Inferior parietal lobule	L	39	4.02	52	–40	–69	34
	Middle temporal gyrus	L	21	3.44	19	–58	–39	–3
Partial for RT	Inferior frontal gyrus	L	45	4.94	302	–54	21	10
	Inferior frontal gyrus	L	47			–48	28	–3
	Caudate	L	–	4.47	313	–9	9	9
	Medial frontal gyrus	L	6	4.26	206	–9	9	72
	Middle frontal gyrus	L	6	3.62	25	–42	–3	54
	Middle temporal gyrus	L	21	3.57	32	–58	–42	–1
	Inferior parietal lobule	L	39	3.54	19	–36	–69	36

Note. See Table 2 note.

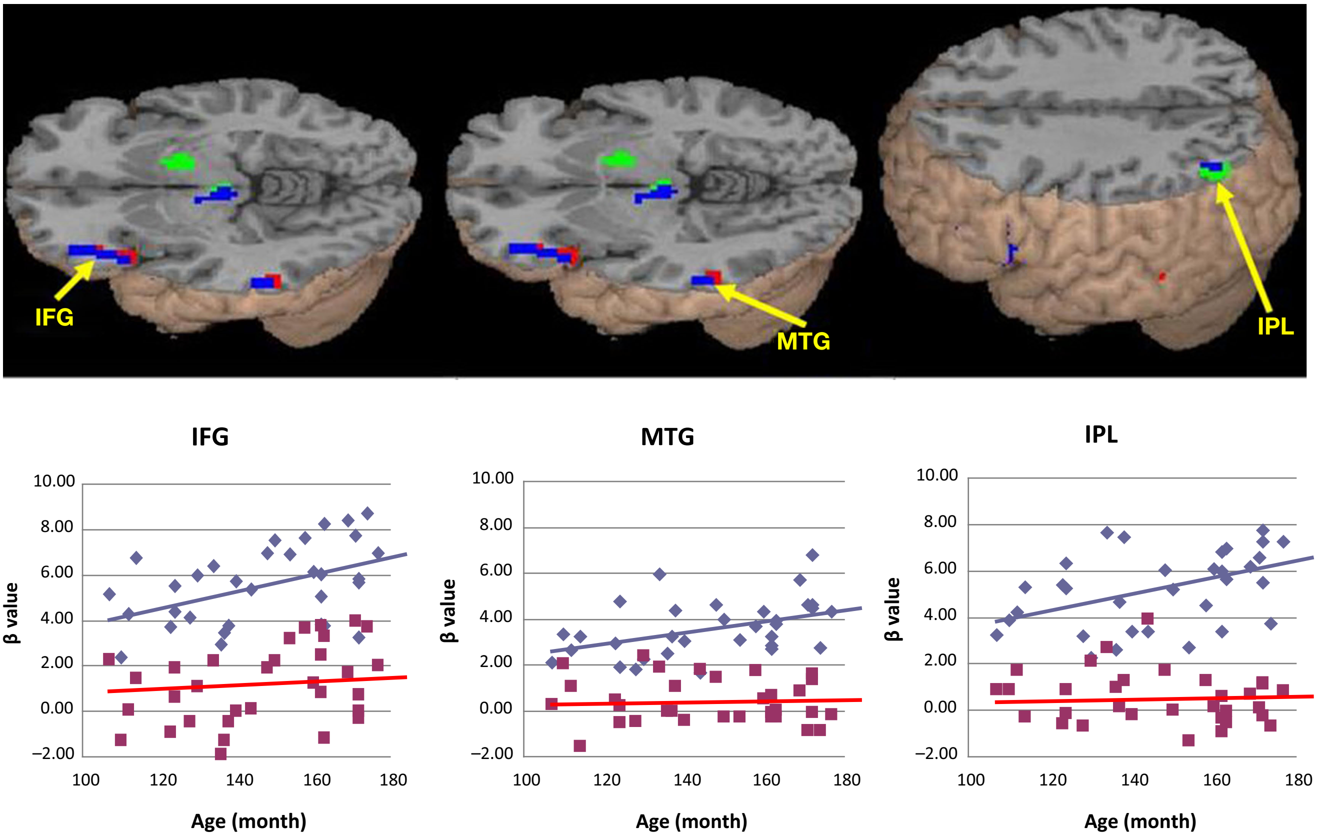

FIGURE 2 | The effects of age, partialed for accuracy (green) or reaction time (red), for the related minus unrelated pairs. The overlap between the two maps is represented in blue, showing that increasing age was correlated with greater activation in the anterior and mid ventral regions of left inferior frontal gyrus (IFG), left posterior middle temporal gyrus (MTG), and the posterior region of left inferior parietal lobule (IPL). For descriptive purposes, the graphs below are used to visualize the whole brain data that are shown above. The scatterplots of the correlation of age in months with the related (blue) and unrelated (red) conditions in these three regions are presented below the brain images. Beta values were taken from the peak voxel of the related minus unrelated contrast partialed for reaction time.

We also found that weaker semantic association produced greater activation in the mid-ventral region of left inferior frontal gyrus (BA 45), similar to those reported in English children (Chou et al., 2006a) and Chinese adults (Chou et al., in press). Previous studies in English suggest that this region is involved in effortful semantic processing, particularly when there is increased demands on the process of selecting relevant semantic knowledge or when comparing words along semantic features (Thompson-Schill et al., 1997, 1999; Fletcher et al., 1998; Whatmough et al., 2002; Blumenfeld et al., 2006). Badre and Wagner (2007) propose that the anterior ventral region of left inferior frontal gyrus (BA 47) may support controlled access to stored semantic representations, whereas the mid-ventral region of left inferior frontal gyrus (BA 45) may support increased selection demands to process active representations. Of particular relevance to the current study, semantic judgments to weaker association pairs produced greater activation in the mid-ventral region of left inferior frontal gyrus as compared to stronger association pairs. Greater activation for weaker association pairs could result from increased demands on the selection of appropriate semantic features in Chinese. Thus, the weaker association effect was similar between Chinese and English as well as between adults and children.

Developmental increases in left posterior middle temporal gyrus in Chinese are consistent with English studies showing age-related increases in this region (Chou et al., 2006a,b; Szaflarski et al., 2006). Previous child studies in English and Chinese implicate left posterior middle temporal gyrus in semantic processes (Chou et al., 2006a; Cao et al., 2009). Several studies suggest that the best candidate for the storage of lexical representations is in left posterior middle temporal gyrus (Hickok and Poeppel, 2004, 2007; Martin, 2007). Greater activation over age in this region may be associated with increasing elaboration of semantic representations, i.e. a greater number of semantic representations with more interconnections between these representations. Behavioral research shows that as vocabulary knowledge increases, the child's semantic system is gradually elaborated due to a greater number of conceptual links in Chinese and English (McGregor and Appel, 2002; McGregor et al., 2002; Lee et al., in press). Therefore, the developmental increase in left posterior middle temporal gyrus may reflect a general process that is universal across languages.

Developmental increases in left inferior parietal lobule [−40, −69, 34] in the present Chinese study are more posterior than developmental effects in this region [−45, −33, 40] reported in previous studies of English using the same visual semantic task (Chou et al., 2006a). The anterior region of the left inferior parietal lobule has been implicated as part of the phonological loop (Paulesu et al., 1993) or the phonological store (Chen and Desmond, 2005), whereas the posterior region of left inferior parietal lobule has been suggested to support the integration of lexical input into larger units for semantic processing (Lau et al., 2008). Because reading for meaning places greater demands on the mapping from orthography to semantics at word level in Chinese (Leck et al., 1995; Zhou and Marslen-Wilson, 2000; Feng et al., 2001), developmental increases in the posterior region of left inferior parietal lobule may be related to more extensive semantic integration in older children. This suggestion is supported by our finding reported above that stronger association pairs, which can be more thoroughly integrated, also produced greater activation than weaker association pairs in the posterior region of left inferior parietal lobule. The developmental increases in the anterior region of left inferior parietal lobule in English may be due to greater involvement of phonological processing when reading in an alphabetic language.

In contrast to developmental studies of English, our study showed an age-related increase in ventral regions of left inferior frontal gyrus for Chinese children. Previous studies in English have shown developmental increases in more dorsal frontal regions in auditory narrative comprehension at [−42, 7, 30] (Schmithorst et al., 2006) and in verb generation in response to a noun at [−49, 3, 39] (Brown et al., 2005), at [−46, 7, 25] (Schapiro et al., 2004), and at [−47, 3, 30] (Szaflarski et al., 2006). Our coordinates in left inferior frontal gyrus at [−51, 27, 10] (BA 45) and at [−48, 21,−3] (BA 47) showing developmental effects in Chinese are substantially ventral to the coordinates reported in English studies. The subparts of left inferior frontal gyrus have been associated with distinct cognitive processes involved in language tasks. In general, the dorsal region of left inferior frontal gyrus is thought to be specialized for processing phonological representations, while the ventral region of left inferior frontal gyrus is proposed to be specialized for manipulating semantic representations (Poldrack et al., 1999), including controlled retrieval and selection mechanisms in ventral prefrontal regions (Badre, et al., 2005). Because reading for meaning places greater demands on mapping from orthography to semantics in Chinese (Leck et al., 1995; Zhou and Marslen-Wilson, 2000; Feng et al., 2001), older children may have learned to engage more thoroughly in controlled retrieval and selection of semantic knowledge at word level. The developmental increases in dorsal regions of left inferior frontal gyrus in English may reflect greater phonological involvement in alphabetic languages in older children.

There are two potential limitations of the study. First, the related condition had twice as many trials as all other conditions, possibly resulting in higher signal-to-noise ratio in the related condition. We checked that in three critical regions (left inferior frontal gyrus, left middle temporal gyrus, and left inferior parietal lobule) signal was greater for the related than the unrelated condition. However, this difference should not have affected the main results of the paper as the strength of association effect was examined within the related trials, and age effects were examined for the related condition. Second, it is possible that the non-characters for the perceptual control condition could have been in part processed linguistically because radicals of real characters were replaced with other radicals to form non-characters. However, the comparison of the related or unrelated to the perceptual condition revealed activation in both left inferior frontal gyrus and left middle temporal gyrus, suggesting that real characters more robustly activated the semantic system than non-characters. Moreover, our association and age analyses examined only the related characters. Thus, the non-characters would be unlikely to have influenced the results of these analyses.

In conclusion, our study of Chinese children showed association strength effects in left inferior parietal lobule and in mid-ventral regions of left inferior frontal gyrus. These brain regions have been implicated in semantic integration and selection, respectively. We additionally showed that there were age-related increases in left posterior middle temporal gyrus, similar to those reported in previous studies of English, suggesting that older children have more elaborated semantic representations across scripts. Because Chinese has a more consistent relationship in the mapping between orthography and semantics and a less consistent relationship in mapping between orthography and phonology, the developmental increases in the posterior region of left inferior parietal lobule and in the ventral regions of left inferior frontal gyrus suggests that reading acquisition relies more on the mapping from orthography to semantics in Chinese children at word level.

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A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing

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A review of the human developmental neuroimaging literature that investigates outcomes following exposure to psychosocial adversity is presented with a focus on two subcortical structures – the hippocampus and the amygdala. Throughout this review, we discuss how a consideration of developmental timing of adverse experiences and age at measurement might provide insight into the seemingly discrepant findings across studies. We use findings from animal studies to suggest some mechanisms through which timing of experiences may result in differences across time and studies. The literature suggests that early life may be a time of heightened susceptibility to environmental stressors, but that expression of these effects will vary by age at measurement.

Keywords: amygdala, hippocampus, human development, stress, adversity, HPA axis

INTRODUCTION

Early adverse social environments such as abuse and neglect have been associated with a wide range of negative outcomes, including a dramatically increased risk for a variety of mental disorders (Breslau et al., 1999; Brewin et al., 2000). These often include, but are not limited to, anxiety, depression, ADHD, substance use disorders, and tobacco dependence. The link between childhood trauma and adult risk for mental health disorders has been described in a variety of ways but fundamentally, this link is biological in nature. These negative social environments become biologically embedded as changes in neural structure and function and, ultimately, the behaviors that lead to mental illness. Although initial susceptibilities for exposure to adversity may contribute to this association, pressures from the environment can alter neural development leading to negative outcomes. Describing the mechanisms by which adverse experiences during childhood lead to changes in neural development is an important step for understanding both brain development and ultimately for developing tools for clinical intervention.

In this review we will attempt to link the timing of negative childhood psychosocial stress exposure to differences in neural structure and function during childhood and adolescence. We restrict this review to empirical articles that address psychosocial trauma of abuse and neglect. A paper by Raizada & Kishiyama (this issue) reviews the literature on cognitive deprivation (e.g., low socio-economic status of the family), and therefore, this manuscript will not focus on early adversity of that type. We further limit our review to the neural development of two subcortical structures: the hippocampus and amygdala. We focus on these two regions because, based on a large adult human and non-human animal literature, we would expect significant environmental influence on these structures. The hippocampus and amygdala are important for socio-emotional functioning throughout development and are closely linked with the activity of the hypothalamic pituitary adrenocortical (HPA) axis, a significant neuroendocrine mediator of stressful events for humans. We purposefully narrow our focus to provide a richer discussion of the amygdala and hippocampus and how developmental timing interacts with environmental influences. Because the systemic output of the HPA axis, glucocorticoids (cortisol in humans), can pass through the blood-brain barrier, the HPA axis is one of the major pathways through which the effects of stress can shape brain development. The amygdala and hippocampus are rich with receptors for cortisol and are therefore major targets of the HPA axis. Thus, we see narrowing our review to the amygdala and hippocampus as one reasonable way to limit the scope of the neural effects of adversity that we examine here. We will describe some specific examples of when amygdala and hippocampal development are disrupted by negative psychosocial environments. By describing these associations, we hope to distill potential mechanisms by which exposure to adversity could become biologically embedded resulting in increased susceptibility to mental illness. Finally we describe potential future directions for research. Throughout the review, we will be making the argument that the effects of adversity will vary as a function of developmental timing, such that regionally defined effects will depend on the age at which exposures occurred and when neural outcomes were measured.

This focus on timing is consistent with the notion of sensitive periods identified in other developmental processes, such as vision or language, where developmental timing modifies the environment's impact on neural development. A fundamental precept of developmental studies is that the timing of a particular exposure matters for the expression of a phenotype. Not all neural regions follow the same developmental trajectory (Giedd et al., 1996; Bourgeois, 1997; Huttenlocher and Dabholkar, 1997). For example, primary sensory cortex such as V1 (visual cortex) appears to undergo important structural changes in the first year leading to life-long differences in visual perception, whereas other cortical regions (e.g., prefrontal cortex) continue to show structural development into adulthood. In the case of binocular vision or phoneme perception, timing of environmental exposure and timing of measurement is critical for observation of normal perceptual development (Hubel and Wiesel, 1970; Kuhl, 2004). These are powerful examples of the concept of a developmental sensitive period, which are periods of life when a system exhibits increased plasticity and therefore, susceptibility, to environmental influences. Although the effects of sensitive periods are observed in behavior, they are properties of neural circuits (see Knudsen, 2004). Central to this concept is the notion that the process of development itself may increase the system's likelihood of being shaped by the environment (Casey et al., 2000). These periods often coincide with rapid development of a brain system, and therefore, individual neural systems will have their own sensitive periods (Lupien et al., 2009). Once environmental exposure occurs, it modifies the architecture of the circuit in such a way that certain patterns of future activity are preferred (Knudsen, 2004). Therefore, knowing the developmental timing of environmental exposures is critical when evaluating its effects. Beyond the timing of exposure, the timing of measurement can influence how we interpret the effects of environmental exposures, like adversity. Because compensatory neural mechanisms, which were not present during the stressor, may emerge once the adverse experience has terminated, timing should be well-characterized to disambiguate the effects of stress versus the effects of recovery. Moreover, as will be discussed below, the effects of an environmental exposure may not emerge for some time after the termination of the exposure. In the case of adversity we predict that the timing of exposure (i.e., age at which exposure occurred) and the timing of measurement (time since stressor) will matter for neural structure and function. Therefore, we will present human developmental neuroimaging studies to support this hypothesis and use findings from the animal literature to provide some description of mechanisms for developmental timing (literature summarized in **Table 1**).

PSYCHOSOCIAL STRESS EXPOSURE: HPA AXIS FUNCTION

Psychosocial stress can adversely impact brain development, and the literature on stress suggests that these changes occur largely through the HPA axis (reviewed in Loman and Gunnar, 2010). We will begin by briefly reviewing the structure of the HPA axis. A stressor sufficiently strong will elicit a full stress response (Kemeny, 2009) which includes activation of both the sympathetic nervous system and activation of the HPA axis. The latter, which produces a longer-term response to a stressor than the former (hours rather than seconds to minutes), begins with signals from the amygdala (reviewed in Herman and Cullinan, 1997), which lead to peripheral (systemic) glucocorticoid increases via hypothalamus, pituitary, and adrenal gland activity and increases in corticotropin-releasing hormone in the brain (CRH; including in the amygdala; Makino et al., 1994a). Eventually peripheral glucocorticoids make their way to the brain. Glucocorticoids easily pass through the blood-brain barrier (Zarrow et al., 1970), and because the amygdala and hippocampus have a high density of receptors for unbound glucocorticoids, they are regions that are highly susceptible to the products of the HPA axis. When the stressor is removed and high circulating glucocorticoids are no longer necessary, glucocorticoids suppress HPA axis activation by occupying receptors in the hippocampus eventually inhibiting activity of the HPA axis (van Haarst et al., 1997). In contrast, glucocorticoid occupation of amygdala receptors can have a facilitating effect on the activity of the HPA axis, often increasing CRH production within the amygdala. Therefore, circulating glucocorticoids can have contrasting effects in the amygdala and hippocampus, and these two structures can play contrasting roles in the activity of the HPA axis.

As discussed in Gunnar and Quevedo (2007), stress is a psychological condition in which the individual experiences challenges to their well-being that overwhelm their resources for coping. Although this construct can be studied behaviorally and biologically (Dantzer, 1991), behavioral distress does not always mirror physiological stress reactions. For example, infants' physiological (cortisol) response to inoculation is not perfectly correlated with amount or intensity of crying (Gunnar et al., 1989). However, elevated HPA axis activity can provide one biological index of stress, and importantly, one that can shape brain development.

There is much evidence that children who are exposed to early adverse experiences, such as poverty (Lupien et al., 2000), abuse (Tarullo and Gunnar, 2006), or orphanage rearing (Gunnar et al., 2001; Dobrova-Krol et al., 2008), have increased stress reactivity and corresponding increased GR exposure. Adverse caregiving is a type of stress also used in animal models. These models provide the opportunity, usually not available in humans, for examination of stress effects at the cellular level. A variety of stressors (Bonaz and Rivest, 1998), as well as administration of high levels of glucocorticoids (Makino et al., 1994a), result in increased mRNA for CRH receptors hypothalamus and amygdala. This upregulation can lead to an increased fear response and/or a lowered threshold for the fear response to occur. High levels of glucocorticoids will also result in CRH mRNA level increases in the amygdala (Makino et al., 1994a), thereby potentiating the fear response. The amygdala has been understood to be functionally dormant in the rat neonatal period. However, significant stressors and/or GR administration can precociously activate the amygdala (Moriceau et al., 2004), indicating that the amygdala is biologically prepared to be activated early in life under the right conditions. In addition, CRH receptors are maximally expressed in the amygdala and hippocampus early in development (reviewed in Baram and Hatalski, 1998), a finding that may provide insight into why young animals are especially vulnerable to adversity. Moreover, high elevations of glucocorticoids can downregulate hippocampal receptors that normally aid in the negative feedback to the HPA axis (van Haarst et al., 1997), thereby resulting in a dysregulated axis. The process of glucocorticoids increasing hippocampal receptors occurs throughout development, including early in life (Vazquez, 1998). We will discuss in greater detail below how the products of the HPA axis specifically affects both the hippocampus and the amygdala.

HIPPOCAMPUS

The hippocampus has been implicated in learning and memory in adults and children. In adults, when the hippocampus is removed surgically, encoding of long term memories is disrupted resulting in anterograde amnesia: new memories cannot be formed (Markowitsch and Pritzel, 1985). Initial findings such as these in neuropsychological research were the result of bilateral hippocampal resection as a treatment for epilepsy (including the

Table 1 | Summary of findings on hippocampus and amygdala.

Article	N	Human/ Animal	Sex	Age of adversity	Test during or post adversity	Age at test	Adversity	Findings
Admon et al. (2009)	50	Human adults	25M/25F	18 years	Within 6 months post stress	19 years	Combat exposure	1. Amygdala Reactivity pre stress predicted increase in stress-related symptomology. 2. Increases in Hippocampal reactivity predicted increases in stress-related symptomology
Gilbertson et al. (2002)	35	Human adult	12M Combat exposed Twin pairs/23 M noncombat exposed twin pairs	Adulthood	Post	52.8/51.8	Combat exposure	1. Small hippocampal volume is a risk factor for PTSD. No effect of combat stress on volume
Kasai et al. (2008)	41	Human adult	18M Combat exposed Twin pairs/23 M noncombat exposed twin pairs	Adulthood	Post	52.8/51.8	Combat exposure	1. Combat exposed twin with PTSD showed decreased hippocampal activity relative to combat exposed twin without PTSD
Liberzon et al. (1999)	39	Human adult	14M Combat Exposure and PTSD/11M Combat only/ 14M Control	Adulthood	Post	45–52	Combat exposure/current anxiety disorder	1. PTSD patients exhibited elevated activation of left amygdala to combat sounds for
Rauch et al. (2000)	16	Human adult	8M Combat Veterans with PTSD/8 Controls	Adulthood	Post	48–61 years	Combat exposure/current anxiety disorder	1. Combat Exposed Veterans with PTSD had exaggerated amygdala responses to faces
Shin et al. (2004)	36	Human adult	17 (7M) Combat Exposed Veterans with PTSD/19(9M) Combat Exposed Veterans without PTSD	Adulthood	Post	adulthood	Combat exposure/current anxiety disorder	1. Male Combat Veterans with PTSD exhibited elevated amygdala activity
Shin et al. (2004)	16	Human adult	8M Firefighters with PTSD/8M Controls	Adulthood	Post	adulthood	Fire fighting exposure/ current anxiety disorder	1. Participants with PTSD showed decreased hippocampal activation during memory task. 2. Participants with PTSD had smaller hippocampal volume than controls
Gianaros et al. (2007)	48	Human adults	48 F	Adulthood	During	48 years	Self report of stress on perceived stress scale for 20 years	1. Decreases hippocampal volume associated with increased life stress
Armony et al. (2005)	13	Human adults	9F/4M	Adulthood	Post (within past 4–6 weeks)	19–57 years	Motor vehicle accidents	1. Increased amygdala activation to masked fearful faces > masked happy faces
Andersen et al. (2008)	43	Human adults	26F with abuse; 17F healthy controls	Childhood	Post	18–22 years	Retrospective sexual abuse reporting	1. Sexual abuse at 3–5 or 11–13 years of age was associated with reductions in hippocampal volume. 2. Sexual abuse at 9–10 and 14–16 years was not
Bremner et al. (1997)	34	Human adult	12M/5F with child abuse; 12M/5F matched controls	Childhood	Post	30–50 years	Chronic child abuse (at least once a month for a year)	1. PTSD patients have smaller hippocampus and unchanged amygdala

(Continued)

Table 1 | *(Continued)*

Article	N	Human/Animal	Sex	Age of adversity	Test during or post adversity	Age at test	Adversity	Findings
Cohen et al. (2006)	250	Human adult	Sex unreported; 100 with 2 or more early negative events/150 with less than 2 negative events	Childhood	Post	18–70 years; mean age 40 years	Retrospective reporting of early life stressors (various)	1. Differences in hippocampal volume were marginally significant and amygdala were nonsignificant between groups
Driessen et al. (2000)	42	Human adults	21F patients with borderline personality disorder/21 age, gender, handedness matched contents	Childhood	Post	21–40 years	Childhood trauma/current borderline personality disorder	1. Patients had 16% smaller hippocampal volume and 8% smaller amygdala than controls
Schmahl et al. (2003)	33	Human adult	10F with Borderline Personality Disorder and childhood trauma/10F controls with childhood trauma/13F controls with no trauma	Childhood	Post	20–39 years	Childhood trauma/ Borderline Personality Disorder (BPD)	1. Patients with BPD had smaller amygdala (~22%) and hippocampal (~14%) volumes; Trauma exposure only was not tested
Carrion et al. (2001)	24	Human children	14M/10F with trauma and PTSD or subthreshold PTSD; 14M/10 Fage matched controls	Childhood	Post	7–14 years	Various traumatic exposures	No differences in hippocampal volume
DeBellis et al. (1999)	52	Human children	44 Children with PTSD (25M)/61 Control (36M)	Childhood	Post	8–13 years	Childhood maltreatment/ current anxiety disorder	1. Decreased intercranial and cerebral volume, increased ventricle size. 2. No differences in hippocampal volume
DeBellis et al. (2001)	18	Human children	9 Children with PTSD(5M)/9 age, gender, Tanner Stage matched Controls	Childhood/ pre-puberty	Post	Pre (10 years)/post (13 years) puberty; longitudinal design	Childhood maitreatment/ current anxiety disorder	1. No significant decrease in hippocampal volume for children with PTSD across adolescence in a longitudinal design
Tottenham et al. (2009b)	62	Human children	8M/26F previously institutionalized; 5M/23F comparison	Infancy	Post	5–12 years	Adverse caregiving	1. Larger amygdala volume in previously institutionalized group. 2. No differences in hippocampal volume
Mehta et al. (2009)	25	Human children	6M/8F previously institutionalized; 6M/5F comparison	Infancy	Post	16 years	Adverse caregiving	1. Larger amygdala volume in previously institutionalized group. 2. No differences in hippocampal volume
Conrad et al. (1999)	72	Sprague-Dawley rat	36M chronically stressed/ 36M no stress	Adulthood	0, 10, and 20-days post stress termination	Adulthood	6 h of restraint stress for 21 consecutive days	1. Decreased hippocampal CA3 apical dendrite morphology. 2. 10 or 20 days of recovery eliminated changes in dendritic morphology caused by stress
Honkaniemi et al. (1992)	24	Sprague-Dawley rat	20 Capsaicin/4 Control	Adulthood	Post	Adulthood	Capsaicin	1. Expression of c-fos, c-jun, and junB in the PVN and central nucleus of the amygdala

(Continued)

Table 1 | *(Continued)*

Article	N	Human/ Animal	Sex	Age of adversity	Test during or post adversity	Age at test	Adversity	Findings
Kim et al. (2001)	28	Long-Evans rats	28M	Adulthood	Post	Adulthood	Restraint/tail shock	1. Lesioning the amygdala pre-stress protected hippocampal structure (LTP) and function (Morris Water Maze) effects of stress exposure
Vyas et al. (2002)	3 groups	Adult Wistar rats	Male Wistar Rats (Chronic Immobilization Stress/ Chronic Variable Stress/ Control	2-months old (adulthood)	Directly after stress	3-months old (adulthood)	Chronic immobilization stress/chronic variable stress	1. Accelerated dendritic arborization in the amygdala. 2. Dendritic atrophy in CA3 (hippocampus)
Vyas et al. (2004)	3 groups	Adult Wistar rats	Male Wistar Rats (Chronic Immobilization Stress/ Chronic Variable Stress/ Control	2.5–3 months old (adulthood)	Post	3.5–4 months old (adulthood)	Chronic immobilization stress	1. Anxiety behavior and amygdala dendtric arborization persisted in stressed rats compared to control. 2. Hippocampal dendritic atrophy did not persist after 21 days of recovery
Liu et al. (1997)	–	Norway rat	Several large liters	PND 1–10	Post	Adulthood	Reduced maternal care	1. Increased adrenocorticotropin and corticosterone to stress, decreased hippocampal GR mRNA
Plotsky et al. (2005)	20	Long evans hooded rats	5M non-handled/ 5M handled/5M maternal separation/5 M control	PND2–14	Post	PND 100–120	Maternal separation	1. Elevated CRH mRNA in Amygdala of Maternally separated and non-handled rats
Brunson et al. (2005)		Sprague-Dawley rat	Several male litters	PND2-weaning	Post	4-and 12-months old	Poor maternal care	1. Impaired hippocampal function appeared only in middle aged adults
Tsoory et al. (2008)	104	Sprague-Dawley rat	56M exposed to juvenile stress/48M control	PND 27–29	Post	PND 33 - juvenile/9-week-old adult	3-day variable stress exposure	1. Increased neural cell adhesion molecule in basolateral amygdala, hippocampus CA1, dentate gyrus, entorhinal cortex measured at adulthood but not during the juvenile period
Andersen and Teicher (2004)	100	Sprague-Dawley rat	50M/50F	PND 2–20	Post	PND 25, 40, 60, 80, 100	Maternal separation	1. Synaptophysin OD decreases in hippocampal CA1 and CA3 at postnatal day 60 (not before). 2. No effect on Synaptophysin OD in the amygdala
Ono et al. (2008)	148	Balb/c mice	76 (33M) early weaned/ 72 (34M) typically weaned	PND 14	Post	3, 5, and 8 weeks of age	Early weaning (PND 14)	1. Precocious development of amygdala, not hippocampus (accumulation of galactosylcera-mide) at 5 weeks of age
Chen et al. (2006)	55	Sprague-Dawley rat	55 stressed	PND 18 and Adult (3 months old)	Directly after stress	PND 18 and Adult	Restraint/restraint-noise	1. Hippocampal response (FOS) to stress was more rapid and robust at PND 18 than adulthood
Kikusui and Mori (2009)	129	Balb/c mice	63(28M) early weaned/66 (32M) typically weaned	PND 14	Post	8–22 weeks	Early weaning (PND 14)	1. Accelerated amygdala development

(Continued)

Table 1 | *(Continued)*

Article	N	Human/ Animal	Sex	Age of adversity	Test during or post adversity	Age at test	Adversity	Findings
Salzberg et al. (2007)	29	Wistar rats	16 (9M) maternal separation/13 (6 M) early handling	PND 2–14	Post	7 weeks	Maternal Separation	1. Amygdala sensitization following maternal separation
Isgor et al. (2004)	156	Juvenile Sprague Dawley rats	156M	PND 28	24 h post stress/ 3 weeks post stress	PND 57/77	28 days of variable chronic stress	1. 3 weeks post stress when rodents were adults hippocampal volume (CA1 and CA3) was decreased due to inhibited growth. 2. 24-h post stress when rodents were still juvenile hippocampal volume differences were absent or reversed
Becker et al. (2007)	20	Octodon Degus	10M stressed/10M non-stressed	PND 1–21	Post stress	PND 22 (post weaning/pre reproduction)	PND1–21 separated 1x per day for 1 h	1. Higher levels of CRF neurons in basolateral amygdala. 2. Lower levels of corticotropin releasing factor (CRF) fibers/ neurons in central amygdala and hippocampal dentate gyrus and CA1
Vazquez et al. (2006)	300	Hybrid Sprague-Dawley/ Long-Kvans	80 (40M) stress at PND 6/ 80(40M) stress at PND 12/80(40M) stress at PND 18/ 60(30M) controls	PND 6, 12, or 18	Directly after, 1 h after, or 4 h after	PND 6, 12, or 18	Maternal separation and restraint stress	1. Basal CRH gene expression is higher at earlier ages in amygdala than hippocampus. 2. Deprivation had site-specific effects on the temporal response to restraint stress
Vazquez et al. (1996)	12 groups	Juvenile Wistar rats	Stressed at PND 6, 9, or 18/ challenged at PND 6, 9, 18/ control PND 6, 9,18	PND 6/9/18	1-h post stress	PND 6/9/18	Maternal deprivation/saline injection	1. Mineral corticoid receptor (MR) and glucocorticoid receptor (GR) mRNA decreases in hippocampus of deprived pups
Moriceau et al. (2004)	108	Juvenile Long Evans rats	Approximately 1/2 M	PND 8 or 12	During stress	PND 8 or 12	Predator odor	1. Exogenously administered Cortisol increased amygdala activation and fear responding in young rats
Hatalski et al. (1998)	2 litters	Juvenile Sprauge Dawley	Mixed litters	PND 9/10	4-h post stress	PND 9/10	Single exposure to cold stress/3 exposures to cold	1. Increased CRF-mRNA in the central nucleus of the amygdala
Sabatini et al. (2007)	12	Juvenile Rhesus Macaque	4 F 1 week maternally seperated/4F 1 month maternally separated/ 4F controls	Infancy equivalent	Post stress	3 months	1 week maternally seperated/1 month maternally separated	1. Early separation, more than later separation, decreased amygdala gene expression (guanylate cyclase 1 α 3)
Spinelli et al. (2009)	28	Rhesus monkeys	15 (7M) mother reared/13 (6M) peer reared	0–6 months old	Post stress	23–32 Months (age equivalent to childhood)	Maternal deprivation	1. No difference in hippocampal volume measurements

famously described patient HM; Zola-Morgan et al., 1982). When the hippocampus is lesioned in children, a similar specific deficit in episodic memory is observed (Vargha-Khadem et al., 1997; Brizzolara et al., 2003; Temple and Richardson, 2004).

As described above, in the context of stress exposure, the hippocampus has another important role; it provides a negative feedback mechanism, which modifies the HPA axis response (as reviewed in Kim and Yoon, 1998). This negative feedback mechanism is accomplished via activation of glucocorticoid (GR) and mineralocorticoid receptors (MR) by circulating levels of glucocorticoids. Within the brain, MR have a 90% higher affinity for glucocorticoids than GR. At basal (non-stress) levels, the majority of MR are occupied by circulating levels of glucocorticoids. During a typical HPA axis stress response, increased availability of glucocorticoids leads to GR occupation, activating a negative feedback loop and decreasing the HPA axis response. Early in life, the MR/GR ratio is lower than later in life, with MR mRNA increasing with age and GR mRNA decreasing with age (Vazquez et al., 1996). The immature ratio of MR to GR may result in unique hippocampal vulnerability to stress early in life. As has been demonstrated in older animals, chronic occupation of GR may impair the hippocampally-mediated negative feedback process resulting in extended HPA axis activation following stressful events and dysregulation of the HPA axis. Down-regulation of hippocampal MR has been identified in very young rats as well (reviewed in Vazquez, 1998), which may increase the likelihood of GR occupation for young animals.

HIPPOCAMPAL DEVELOPMENT

In developmental neuroimaging studies, there is some evidence for age-related change (increases) in recruitment of the hippocampus during long-term memory tasks across late childhood (Paz-Alonso et al., 2008). However, other studies observed unchanging hippocampal recruitment across 8–24 years of age during scene memory encoding (Ofen et al., 2007), supporting the idea that hippocampal function is intact at least by childhood. Structural studies using magnetic resonance imaging (MRI) have revealed developmental differences in the volume of the hippocampus from birth through young adulthood. From birth to year 2, the hippocampus shows relatively little growth (Knickmeyer et al., 2008). More substantial structural changes tend to be observed later in development. In an initial cross-sectional study of children it was determined that the hippocampus showed protracted volumetric growth across childhood for girls but not boys (Giedd et al., 1996). In subsequent studies, however, this gender difference was not replicated, and instead, a longitudinal analysis of hippocampal growth between the ages of 4–25 years has shown continued developmental change into adulthood, where that the anterior hippocampus decreases in volume across childhood while the posterior hippocampus increased in volume (Gogtay et al., 2006).

As will be discussed further below, the animal literature suggests that hippocampal development lags slightly behind amygdala development. For example, whereas learning to pair a cue with a shock (cued fear conditioning – an amygdala-dependent function) is present by postnatal day 18 in the rat, the same aged rats are unable to pair a context to a shock (contextual fear conditioning – a hippocampus dependent function; Rudy, 1993). These findings have been interpreted as occurring because of the relative immaturity of the hippocampus (Cotman et al., 1973; Wilson, 1984; Rudy and Morledge, 1994) Similarly, a recent non-human primate finding obtained with longitudinal structural MRI showed that, although both structures showed early development during an age-equivalent to early childhood, hippocampal development lagged behind the amygdala as indexed by age at which the slope of change leveled off (Payne et al., 2010).

Despite the protracted development of the hippocampus, behavioral evidence suggests that some aspects of hippocampal function are present early in life. Across the first year of life, memory becomes increasingly context-independent, which is evidence of increased relational memory and involvement of the hippocampus (Robinson and Pascalis, 2004). Extending putative hippocampal development into early childhood, Sluzenski and colleagues demonstrated that 4-year olds were not able to perform a relational memory task binding together pictures of animals and backgrounds whereas 6-year olds could and showed adult-like performance (Sluzenski et al., 2006). However, when using more familiar objects (faces and scenes), 9-month-old infants showed evidence of intact relational memory (Richmond and Nelson, 2009). Taken together, these findings are consistent with the notion that the basic relational function of the hippocampus is present early in life, although the hippocampus and its connections continue to show developmental change into adulthood.

HIPPOCAMPUS AND STRESS

Evidence from adult rodent models shows that stress exposure alters hippocampal volume and function in adulthood (McEwen, 1999, 2007). At baseline levels, glucocorticoids appear to aid memory formation by enhancing hippocampal excitability (Diamond et al., 1992; Pavlides et al., 1993, 1994). However, during stress-induced HPA axis activation, hippocampal function is disrupted (Diamond et al., 1992; Pavlides et al., 1993), and prolonged exposure to glucocorticoids from chronic stress is harmful to the hippocampus, resulting in reduced dendritic spines and eventually apoptosis of hippocampal neurons (Sapolsky, 1996; Kim and Yoon, 1998). Rodents exposed to early stress also demonstrate dendritic atrophy in hippocampal cells and decreased amplitude of long term potentiation in the CA3 area of the hippocampus, leading to deficits in memory formation (Brunson et al., 2005).

Most animal studies of stress exposure examine pre- and early postnatal stress exposure or chronic stress exposure in the mature animal. To keep the parallels to human psychosocial trauma exposure as consistent as possible, we will not review the effects of prenatal stress here. Poor or absent maternal care has lasting effects on the hippocampus. Early stress exposure of this variety is associated with decreased hippocampal volume and function and dysregulated HPA function in adulthood (Liu et al., 1997; for review, see Sanchez et al., 2001). Few rodent studies have examined the effect of stress experienced or measured during the child/adolescent equivalent of the juvenile phase. In one study of juvenile rodents, early exposure to chronic stress did not result in differences in the hippocampus 24-h post stress but did 3-weeks post exposure when these rodents had reached adulthood (Isgor et al., 2004), suggesting that hippocampal effects may not be readily apparent until animals are mature. In studies of mature rodents, chronic stress exposure is followed by hippocampal volume reductions 24-h post stress exposure. Adult rodent stress exposure confers risk for short-term differences in hippocampal structure, which within ten stress-free days, reverse (Conrad et al., 1999). Intervention during the adolescent phase of development may modify the behavioral effects of juvenile stress exposures in rodents (Francis et al., 2002), but does not alter the hippocampal pathology in adulthood associated with exposure to stress during the juvenile phase. Thus, it appears that stress-related reductions in hippocampal volume and changes in HPA axis activation incurred as a result of juvenile stress exposure are more permanent than those incurred during adulthood (Seckl and Meaney, 2004). This may be the result of a greater number of stress hormone receptors early in life (reviewed in Baram and Hatalski, 1998) and/or decreased MR (higher affinity)/GR(lower affinity) ratios early in life (Vazquez et al., 1996).

In human adults, stress-related pathologies, such as major depressive disorder and post-traumatic stress disorder (PTSD), correlate with decreased hippocampal volume (Sheline et al., 2003; Campbell et al., 2004; Geuze et al., 2005; Kitayama et al., 2005; Smith, 2005) and altered activity (Bremner, 2006; often decreased activity Etkin and Wager, 2007, but increased activity has also been identified – Shin et al., 2004). Even in healthy middle-aged adults, self-reported stress over 12 years in a longitudinal study was associated with decreases in hippocampal grey matter volume acquired at year 13 in women aged 54 years (Gianaros et al., 2007). In aging populations (60–90 years), high baseline and chronically increasing exposure to glucocorticoids measured yearly across a 6-year period predicted decreases in hippocampal volume relative to moderate baseline and decreasing glucocorticoids across the same time period (Lupien et al., 2005). Patients taking high doses of corticosteroids for long periods demonstrate hippocampally-mediated memory deficits (Keenan et al., 1996), and healthy volunteers given GR agonists perform more poorly on memory tasks within a few days (Wolkowitz et al., 1990; Newcomer et al., 1994). Finally, studies of adults who were exposed to abuse during childhood reveal decreases in hippocampal volume (Bremner et al., 1997) and increased stress hormone production (Heim et al., 2002).

Because toxic levels of stress cannot be experimentally manipulated in humans, these studies cannot causally link stress exposure to hippocampal volume. Recent data has called into question the direction or timing of the association between adult stress exposure and hippocampal volume; for instance small hippocampal volume may confer risk for acquiring PTSD or reporting greater stress instead of vice-versa. One study attempted to address this problem of directionality by showing that for monozygotic twins one of whom was in combat and one who was not, volume of the hippocampus in the non-combat exposed twin predicted PTSD symptomology in the individual exposed to combat (Gilbertson et al., 2002; Kasai et al., 2008). This finding points to the potential importance of early-shared environment and/or genetics in determining hippocampal volume separate from chronic exposure to toxic levels of stress in adulthood. Early environment in the form of childhood trauma exposure is a predictor of PTSD risk in combat exposed veterans (Brewin et al., 2000; Dedert et al., 2009). How early stress predisposes an individual to hippocampal alterations and increases stress reactivity is unclear, but a recent study showed that the association between hippocampus and emotional reactivity is *predicted* by pre-existing amygdala reactivity (Admon et al., 2009). This prospective study suggests a specified temporal relationship between amygdala and hippocampus – one potential mechanism by which hippocampal volume indexes risk for PTSD.

Despite the numerous studies showing reduced hippocampal volume in adulthood following stress exposure in either childhood or adulthood, there is no evidence of hippocampal volume differences during childhood caused by stress. In fact, it has been concluded that adversity exposed individuals studied during childhood do not differ in hippocampal volume from their peers (De Bellis et al., 2001; Woon and Hedges, 2008), despite evidence that children who experience trauma have increased GR exposure (De Bellis et al., 1999a). While it is possible that developmental timing of stress exposure is responsible for this lack of effect in the hippocampus, it is also possible that this discordance in findings is the result of timing of measurement. That is, hippocampal impacts could emerge late in life even when exposure to stressful life circumstances occurred early. Only one longitudinal study has attempted to directly test this hypothesis. De Bellis and colleagues imaged children before puberty (Tanner stage I or II) and 2 to 3 years later (Tanner stage III or IV) to determine if childhood stress-induced changes in hippocampal volume become evident during adolescence. This investigation did not support the hypothesis that early abuse exposure might 'emerge' as differences in hippocampal volume during adolescence (De Bellis et al., 2001); however, in the final stage of this longitudinal study, these participants were not yet adults. A more recent study that extends the age at test has shown evidence of hippocampal changes that emerge in adulthood from childhood stress (Andersen et al., 2008). Taken together, these data suggest that early-life stress results in hippocampal volume decreases and functional alterations when measured in adulthood, but these effects are difficult to observe or are not observable during childhood. The conclusion that childhood stress exposure leads to adult but not childhood differences in hippocampal volume leaves open certain possibilities. For instance, early stress exposure, while unrelated to childhood hippocampal volume, may result in other neurobiological changes (for example, in the amygdala) during childhood, which subsequently result in adulthood differences in hippocampal volume. We will discuss the impact of adversity on the amygdala during development in the subsequent section.

AMYGDALA

The amygdala has been implicated in learning about the emotional significance of stimuli (Davis and Whalen, 2001). Having a mechanism to determine the relative safety or danger of situations is adaptive at any age. However, when less is known about the environment (e.g., early in life), the need to learn about the safety or danger of novel events will be greater (Tottenham et al. 2009a), and this type of learning is heavily dependent on the amygdala (reviewed in Davis and Whalen, 2001; LeDoux, 1993). Neuroimaging studies have confirmed that, like in rodents and non-human primates, the human amygdala responds to negative as well as positively valenced stimuli (Breiter et al., 1996; Somerville et al., 2004; Hennenlotter et al., 2005), suggesting it supports learning about the emotional significance of the environment in general.

AMYGDALA DEVELOPMENT

The basic architecture of the human amygdala is present at birth (Humphrey, 1968; Ulfig et al., 2003). Nonetheless, the amygdala undergoes structural and functional change across an extended developmental period (reviewed in Tottenham et al., 2009a). Structurally, the amygdala exhibits a protracted period of development, extending from year one through late childhood. In an initial study, the amygdala was observed to grow in volume across 4–18 years only for male subjects (Giedd et al., 1996); structurally, the female amygdala reached adult-like volume by age 4-years old. This growth trajectory was confirmed in subsequent studies (Schumann et al., 2004) and observed developmental change even when children 2 years of age were included (Mosconi et al., 2009). Notably, many studies of amygdala volume in children have focused on autism, therefore limiting the control sample to male children, which may be problematic given the sexual dimorphism identified in structural studies (Giedd et al., 1996). Amygdala volume development for female children is understudied, and sex-hormones influence amygdala development in human populations (Rose et al., 2004), indicating that future research should carefully consider the role of sex in modifying differences in amygdala function and structure in response to stress. Despite these structural changes observed during childhood, a recent longitudinal non-human study showed that the most rapid *rate* of primate amygdala development occurs during the early postnatal period, leveling off soon after (Payne et al., 2010). This rapid rate of change may heighten the vulnerability of the amygdala to environmental influence early in life.

Similar to structural development, the amygdala shows early functionality that is followed by developmental change across childhood and adolescence. Like adults (e.g., Breiter et al., 1996), children and adolescents reliably recruit the amygdala when processing emotion from facial expressions. Greater amygdala activity for fearful faces relative to fixation is observed in both adolescents (Baird et al., 1999; Killgore et al., 2001) and children (Thomas et al., 2001). Adolescents also demonstrate greater activity in response to these faces than adults, activity that is not generated by differential visual scanning of the faces (Guyer et al., 2008). However, the response pattern differs for younger subjects where children, unlike adults, exhibit greater amygdala recruitment for neutral faces than fearful (Thomas et al., 2001) and other facial expressions (Lobaugh et al., 2006), suggesting that the amygdala (and most likely its connections with cortical regions) undergoes refinement over childhood and adolescence.

Fear conditioning experiments with adolescents (e.g., pairing a neutral cue with an air blast directed at the larynx) have shown that before adulthood, individuals can learn to associate a neutral stimulus with a negative one via increased amygdala activity to the conditioned stimulus (Monk et al., 2003a). This type of amygdala-dependent cued fear conditioning is similar to the process identified in adults (LaBar et al., 1998; Critchley et al., 2002; Knight et al., 2005; Delgado et al., 2006). In adults, both positively valenced (e.g., reward, happy and/or attractive faces) and negatively valenced events recruit amygdala activity (Breiter et al., 1996; LaBar et al., 1998; Baxter and Murray, 2002; Hamann et al., 2002; Somerville et al., 2004; Hennenlotter et al., 2005; Belova et al., 2007). However, many studies with adults find that negative events activate amygdala response above and beyond positive events (Morris et al., 1998; Hamann et al., 2002; Whalen et al., 2004) – suggesting that the adult amygdala discriminates based not only on arousal properties but also on the valence of stimuli. It is not yet established whether early in life the amygdala discriminates stimuli of different valence as well as it does to arousal properties.

The amygdala appears to be more reactive earlier in life than in adulthood. This claim is supported by the repeated findings that amygdala activity continues to change from childhood to adulthood, peaking in activity during adolescence before it declines in adulthood (Monk et al., 2003b; Killgore and Yurgelun-Todd, 2007; Guyer et al., 2008; Hare et al., 2008) and this activity is tightly coupled to the modulatory connections of the ventromedial prefrontal cortex (vmPFC) (Hare et al., 2008). This increased susceptibility to emotionally-relevant events would aid in learning about the environment, at a time in life when relatively little is known about the world. Moreover, the functional precocity of the amygdala may reflect the relative importance of establishing competence in recognizing and learning about emotionally-relevant stimuli prior to establishing competence in other developmental domains (e.g., cognitive development).

Consistent with the notion that the amygdala is highly active early in life, lesion studies that have isolated the timing of lesions to discrete periods of life suggest that lesions early in life often have a more dramatic effect on behavior than those that occur later. Naturally occurring lesion studies in humans show that early amygdala lesions (i.e., congenital) significantly impair processing of facial expressions, particularly fearful ones (Adolphs et al., 1994). However, amygdala lesions occurring later in life (i.e., adulthood) appear to have less of an effect on processing these expressions (Hamann and Adolphs, 1999). These developmental differences are most apparent when the task is non-verbal (e.g., judgments of perceived similarity between expressions). Presumably, the amygdala is important during developmental periods when learning about the meaning of relevant social stimuli, like facial expressions, is occurring, but may be less critical once these associations have been formed. More precise lesion studies in non-human primates when the effects of timing can be manipulated also lend support to the idea that the amygdala of young animals is uniquely important in learning about the environment. Amygdala lesions that occur both in neonatal or adult macaques result in the animals showing less fear of non-social items. However, these lesions produce distinct responses to social stimuli that vary as a function of the timing of the lesion. Amygdala lesions in adult animals result in an increase in affiliative social behaviors (e.g., less distance from peers, more affiliative vocalization coos, more walk bys; Emery and Amaral, 1999), but when they occur in infancy, these lesions result in exaggerated fear responses during social interactions (e.g., decreased exploration, increased fear grimaces and screams; Bauman et al., 2004). Prather et al. (2001) suggest that the exaggerated social fear in monkeys with early amygdala lesions is the result of these monkeys never having the capacity to appropriately learn any social signal from conspecifics, and therefore, are left unable to recognize social cues that signal safety. To summarize, the literature suggests that the amygdala becomes functionally active early in life although it demonstrates continued refinement (largely through increased cortical connections) throughout childhood and adolescence. How this trajectory interacts with exposure to stress will be discussed in the next section.

AMYGDALA AND STRESS

The amygdala and the extended amygdala (the bed nucleus of the stria terminalis; Davis, 1994), appear to be critical in activating the HPA axis in response to cognitive-emotional challenge and threat (reviewed in Dedovic et al., 2009). Stress and glucocorticoids have been found to increase CRH levels and upregulate CRH receptors in the amygdala of both mature (Makino et al., 1994b) and developing rodents (Hatalski et al., 1998) lowering the threshold for the fear response to occur. Chronic GR administration acts to augment the amygdala-mediated startle response in rats (Lee et al., 1994) and CRH antagonist administration (Swerdlow et al., 1989) suppresses the fear response in primates. Lesions of the amygdala prevent elevations in glucocorticoids in response to psychological stressors, such as restraint in rats, but do not prevent elevations in response to physiological stressors, such as illness or injury (Feldman et al., 1994; Herman and Cullinan, 1997). There are a large number of CRH producing neurons and CRH receptors in the amygdala, and infusion of CRH to the amygdala (Rosen and Schulkin, 1998), amygdala stimulation (Mason, 1959), and stress (Baram and Hatalski, 1998; Bonaz and Rivest, 1998) produce large increases in glucocorticoids and corresponding increases in behaviors indicative of fear and anxiety. Developmental human neuroimaging studies have also shown that naturally occurring elevations in glucocorticoids are associated with amygdala hyperactivity (Maheu et al., 2008). Taken together, these findings strongly support the notion that the HPA axis and amygdala CRH are involved in potentiated fear responses following stress. Adverse experiences produce long-term changes in the amygdala structurally and functionally via high levels of circulating glucocorticoids and endogenously produced CRH, decreasing an individual's threshold for reacting to emotional events. Animal models have provided evidence for this type of stress-induced kindling of the amygdala (where continual stimulation produces greater future excitability; Adamec and Shallow, 2000).

Numerous neuroimaging studies have demonstrated that the amygdala is altered structurally and functionally by psychosocial stress in humans as well. Many of these studies have been with adults who have experienced extremely traumatic events (e.g., combat, physical assault) and show that in adults the amygdala is both smaller (Driessen et al., 2000; Schmahl et al., 2003) and more reactive to emotional stimuli (Liberzon et al., 1999; Rauch et al., 2000; Shin et al., 2004; Armony et al., 2005; reviewed in Shin et al., 2006). The amygdala is influenced by trauma of less intensity as well. In one study, first and second year undergraduates showed a correlation between their parent's social status, linked in previous studies to stress reactivity (Lupien et al., 2000; Evans and Kim, 2007) and their amygdala activation while viewing angry faces (Gianaros et al., 2008). Although it is difficult to draw conclusions about causality from most human studies, directionality between adversity and amygdala structure and function has been confirmed with animal models of stress, which have routinely identified changes in the amygdala following stress administration (reviewed in Roozendaal et al., 2009).

Developmentally, there are some similarities and some differences in the associations between amygdala and stress. Studies that have manipulated the timing of stress, typically in non-human animals, have found that the amygdala is particularly sensitive to stress early in life. Poor caregiving in rodents results in increased anxiety- and aggressive-behaviors in adulthood, which is associated with acceleration of amygdala development (Kikusui and Mori, 2009), including early myelination (Ono et al., 2008), increases in CRH-containing neurons (Becker et al., 2007), and sensitization of the amygdala in adulthood (Salzberg et al., 2007). Although the amygdala is functionally dormant in the rat neonatal period, significantly stressful events and/or GR administration can precociously activate the amygdala (Moriceau et al., 2004), perhaps because of the early presence of CRH mRNA [present in day 2 (Avishai-Eliner et al., 1996; Fenoglio et al., 2004; Vazquez et al., 2006)], and such amygdala effects can last until adulthood (elevated CRH mRNA; Plotsky et al., 2005; reviewed in Caldji et al., 2000). Non-human primate work shows that maternal deprivation stress also alters amygdala development. These effects can be more devastating the earlier they in life they occur, and they include deficits in socio-emotional behaviors (e.g., decreased social behavior and increased self-comforting), which are mediated by stress-induced changes in amygdala gene expression (Sabatini et al., 2007). These stress-related timing effects coincide with the early development of the amygdala (Payne et al., 2010), and suggests that amygdala vulnerability to stressors may be at a peak during the early postnatal period primates. This position is supported as well by rat models of early stress, which find that the amount of CRH required to produce amygdala-originating seizures in developing animals is 200 times smaller than required for adult animals (reviewded in Baram and Hatalski, 1998) – another suggestion that the juvenile amygdala has heightened susceptibility to environmental pressure.

While there are a growing number of studies that have examined the role of stress on amygdala structure and function during development, most in humans have not been able to isolate the timing of stress to a discrete period in development (the way that animal models can). This difficulty is common in human studies since adversity that occurs at one point in development is rarely isolated and typically is accompanied by a lifetime of adverse rearing environment, making temporally-defined periods of stress difficult to identify. Studies in adults have often found decreased amygdala volumes in individuals with a history of stressful childhoods (Driessen et al., 2000; Schmahl et al., 2003 – note: these individuals also had borderline personality disorder). This type of finding seems to contradict animal studies, which have identified larger amygdala cell size in addition to exaggerated amygdala activity (Vyas et al., 2002, 2004). In one human study that examined the effects of adverse caregiving restricted to the postnatal period (i.e., children reared in and then removed from orphanages) also found amygdala volume to be larger (once controlling for total brain volume), and did not find hippocampal differences (Tottenham et al., 2009b). A second independent study replicated these findings of amygdala volume increases and a lack of hippocampal differences in adolescents who experienced institutional rearing in infancy (Mehta et al., 2009). Importantly, these populations of previously institutionalized children were studied years after the adversity ended, and children were adopted into families of very high socio-economic status. These data suggest that the amygdala can change as a function of early adverse experiences, but once it does it is resistant to ameliorative environmental influences – an interpretation supported by animal work showing that cellular growth in the amygdala following adult stress, unlike the hippocampus, fails to reverse during a recovery period (Vyas et al., 2004; Yang et al., 2007). Perhaps this heightened and biased sensitivity to emotionally-relevant events is one way the organism ensures that it is prepared for future adversity, in an environment that has already proved to be threatening. Longitudinal studies that vary the timing of stress in humans to include the neonatal period, childhood, adolescence, and adulthood are required to fully understand sensitive periods for environmental stress.

The amygdala is part of an extended neural network. In particular, it has rich connections with the vmPFC (Amaral et al., 1992; Milad and Quirk, 2002; Ghashghaei et al., 2007) and hippocampus (Ikegaya et al., 1996a,b). The vmPFC can modulate the activity of the amygdala through descending projections, perhaps via afferents to the intercalated cells of the amygdala that inhibit its own activity. Secondly, the amygdala and hippocampus co-modulate each other such that the amygdala can influence hippocampally-mediated memory formation and the hippocampus influences amygdala response when emotional stimuli are encountered (Phelps, 2004). The three structures (amygdala, hippocampus, and vmPFC) coordinate during emotional learning. In new environmental contexts, the hippocampus inhibits the vmPFC, which releases the amygdala from vmPFC inhibition (reviewed in Kim and Richardson, 2010). Like the amygdala and hippocampus, stress alters vmPFC activity in adults, where significantly stressful events leads to impaired vmPFC-dependent fear extinction learning (Milad et al., 2009). It has been demonstrated that chronic stress impairs extinction learning (Miracle et al., 2006) possibly via blockage of long-term potentiation (Maroun and Richter-Levin, 2003) and dendritic reorganization (Wellman, 2001; Izquierdo et al., 2006), including shortening of dendritic length caused by stress-induced reduction of the growth factor brain-derived neurotrophic factor (reviewed in Czeh et al., 2008). Also, chronic stress weakens vmPFC regulation over amygdala and results in enhanced amygdala sensitivity to footshock (Correll et al., 2005). Additionally, the bidirectional and direct connections between amygdala and vmPFC suggest that stress-induced changes in the amygdala may, over the course of development, impair amygdala-vmPFC connectivity. Much less is known about the development of the vmPFC following early adversity, although a small number of rodent studies shows that early-life adversity alters vmPFC development both structurally (higher synaptic densities compared to controls – Ovtscharoff and Braun, 2001) and functionally (reduced tyrosine hydroxylase-positive fiber innervation – Braun et al., 1999). Because of its rich interconnectedness, early-life stress may act on this system through multiple pathways and future investigations will benefit from a systems-level approach. At the current time, the literature has primarily focused on the effects of stress on each area.

Taken together, the developmental data and the human adult data suggest that early in life, the amygdala undergoes expansive growth following a stressor. This growth is accompanied by hyperactivity, which after a prolonged period results in cellular atrophy and/or death (and smaller volumes as measured by MRI). Such a trajectory would explain why many studies in adults who experienced years of adversity show decreased amygdala volume. This hypothesized trajectory has been identified in studies with depressed patients, who after the initial depressive episode show enlarged amygdala volume, but after living with depression for an extended period of time, show amygdala volume decreases (Frodl et al., 2002; Lange and Irle, 2004). These findings point to the hypothesis that alterations of amygdala following neonatal stress are apparent early in life because of the amygdala's early functional development, its early exposure to stress hormones (those associated with the HPA stress response), and its maximal expression of stress hormone (CRH) receptors early in life (reviewed in Baram and Hatalski, 1998).

STRESS AND A CONSIDERATION OF TIMING

Stressful events do not impact the whole brain in a uniform fashion, but instead the effects are region specific, exhibiting some of the largest effects in the amygdala and hippocampus. The amygdala and the hippocampus exhibit differential effects of stress that occurs in adulthood, and often contrasting, such that stress decreases size, complexity, and activity of the hippocampus (reviewed in Lupien et al., 2007; Bremner et al., 2008) and shows the opposite effects in the amygdala (larger, more reactive amygdala) (Liberzon et al., 1999; Rauch et al., 2000; Armony et al., 2005). The differences between the two structures seem to be partially related to the time course of the molecular events that occur between the two structures that follows stress. This cascade begins in the amygdala, which exerts molecular and chemical downstream effects on the hippocampus. Cells in the amygdala participate in the earliest reaction to environmental stressors, often initiating the HPA cascade. They are quickly activated by stress and express immediate-early genes (Honkaniemi et al., 1992), whereas the hippocampus begins to play its role later in the cascade, negatively feeding back on the HPA axis to inhibit its activity (Herman and Cullinan, 1997). CRH-induced seizures produce the earliest discharges in the amygdala (Baram et al., 1992), which propagate later to the hippocampus (Haas et al., 1990). Therefore, stress-induced changes in the amygdala may have downstream effects on the HPA axis that over time can change the structure and function of later stages in the axis (Brunson et al., 2001b), like the hippocampus. These timing differences may contribute to the reasons why hippocampal alterations tend to developmentally follow amygdala alterations from stress (Admon et al., 2009).

Observation of these effects may depend on when they are measured. The effects of early-life adversity have been observed in the amygdala during development (Sabatini et al., 2007; Mehta et al., 2009; Tottenham et al., 2009b). Although there is evidence for molecular-level stress-induced changes in the immature hippocampus (e.g., Hatalski et al., 2000; Chen et al., 2006), stress-induced changes in the hippocampus are often difficult to observe during the juvenile period, but may be apparent later in life. Maternal deprivation stress in non-human primates failed to show hippocampal differences as measured by structural MRI in the age-equivalent of childhood (Spinelli et al., 2009). Rodent studies that have manipulated timing of stress and time of measurement have found that hippocampal alterations may be developmentally delayed and appear later in life; neonatal maternal deprivation did not result in a drop in hippocampal synaptophysin (a synapse related protein) until postnatal day 60 (adulthood; Andersen and Teicher 2004). Behaviors that depend on the hippocampus follow a similar trajectory; early-life insults often result in "acquired" cognitive decline, only appearing in older animals, not younger (Brunson et al., 2005). During early-adulthood, the hippocampally-mediated cognitive decline associated with maternal deprivation was unobservable and were not apparent until animals were middle-aged. These behavioral impairments are accompanied by decreased hippocampal LTP, dendritic atrophy, and mossy fiber expansion – hippocampal phenotypes typically associated with stress that occurs in adulthood (McEwen, 1999). It is noteworthy that, unlike with the mature hippocampus, which shows recovery after the termination of a stressor, stress administered early in life has long-term effects on the hippocampus.

Contributing to the differences between the two regions during development may be the differential emergence of HPA-related peptides following stress. Stress hormones and CRH mRNA production in amygdala have a developmentally early onset, appearing as early as postnatal day 2 in the rat (Avishai-Eliner et al., 1996; Vazquez et al., 2006), an earlier onset than in the hippocampus (Fenoglio et al., 2004). This discrepant time course between the amygdala and hippocampus maybe related to the modulatory role that the amygdala has over the hippocampus (Packard and Teather, 1998). These temporal discrepancies appear early in life, where stressful events produce elevations in CRH that occur first in the amygdala and are observed only afterwards in the hippocampus (reviewed in Baram and Hatalski, 1998). Environmental manipulations that result in changes in stress-related gene expression (CRH/GR receptors) are apparent in the amygdala at an age when they are not present in the hippocampus (during the early postnatal period). Neonatal separation stress resulted in an increase in CRF containing neurons in the amygdala, but not the hippocampus in juvenile rodents (Becker et al., 2007). Taken together these data illustrate the interaction between developmental timing and timing of stress exposure (Fenoglio et al., 2004) and suggest that the amygdala response to stressors is a temporal prerequisite for hippocampal changes following stress (Kim et al., 2001). In support of this postulate, a rare prospective study with new recruits to the Israeli Defense Forces showed that while amygdala and hippocampal activity closely correlated with subjective reported stress, hippocampal vulnerability to stress depended on how hyperactive the initial amygdala response was (Admon et al., 2009).

If future studies continue to show that the effects of stress occur in the amygdala developmentally earlier than the hippocampus, it might shed some light on the timing effects often observed in human neuroimaging studies. Evidence from the human literature supporting this temporal hypothesis is mounting. The effects of adversity on these two regions vary by when the stressor was administrated and by age at which testing occurred. For example, larger amygdala volumes are observed during childhood following neonatal early adverse caregiving, although no hippocampal differences were observed (Tottenham et al., 2009b). Age at test is critical since developmental studies that include adolescent subjects often do not find amygdala differences following trauma exposure (Carrion et al., 2001; De Bellis et al., 2001, 1999b). These amygdala effects are typically unobservable, or have not been measured, in adults who experienced childhood stress (Bremner et al., 1997; Cohen et al., 2006), where it has even been reported that childhood stress may actually result in smaller amygdala volumes in adulthood (Driessen et al., 2000). This pattern suggests that initial stress-induced hypertrophy and hyperactivity of amygdala neurons eventually leads to neuronal atrophy or cell death by adulthood (Teicher et al., 2003), a pattern identified in other populations (e.g., autism spectrum disorders) who demonstrate amygdala hypertrophy in childhood followed by smaller volumes in adulthood (Schumann et al., 2004). Effects of trauma do not appear in amygdala volumetric measures in adulthood, while such effects are observable in the hippocampus (reviewed in Lupien et al., 2007; Bremner et al., 2008), leaving open the possibility that hippocampal effects may not emerge until adulthood, possibly as a result of stress-induced changes in the amygdala.

It is also possible that the hippocampus (perhaps due unique developmental timelines) is less vulnerable to stress effects early in life than the amygdala (at least as measured by volume). This may be related to the developmental timing of the structural development of these two structures. As noted earlier, there are data consistent with the hypothesis that amygdala development slightly precedes hippocampal development, and this developmental differential may have consequences for each structure's vulnerability. Alternatively, hippocampal effects may be masked during development. Perhaps measuring changes in volume caused by stress are difficult in the context of developmental changes (Giedd et al., 1996) as measured by MRI. The assertion that the developing hippocampus is entirely invulnerable to environmental stressors is weakened by molecular studies in rodents that show that juvenile stress is followed by altered expression of molecules involved in neural development and synaptic plasticity (i.e., neural cell adhesion molecules), effects that extend into adulthood (Tsoory et al., 2008) (Isgor et al., 2004). Perhaps volumetric studies may not be sensitive enough to show stress-induced hippocampal changes in childhood, although the effects in the amygdala are robust enough to be observed by this measure.

Technological advances are improving our ability to better address these questions in humans. Using diffusion tensor imaging, DTI, studies have replicated and extended findings from volumetric studies of adversity. In one study young adults exposed to verbal abuse as children had significant differences in the white matter tracts associating medial temporal lobe structures to the prefrontal cortex (cingulum bundle) and the fornix fibers of the hippocampus (Choi et al., 2009). DTI reveals the structural connectivity of neural structures and may aid researchers in better describing how associations between structures, such as the ones hypothesized here between the amygdala and hippocampus support developmental patterns in the effects of stress exposure. Other methods for exploring associations between structures such as resting state fMRI, or resting state functional connectivity have not yet been employed in the service of understanding the effect of adversity on neural development. But these techniques have been used to great effect in understanding development generally (Fair and Dosenbach, 2007; Fair and Cohen, 2008) and in defining functionally connected regions of interest in individual subjects (Cohen et al., 2008) a technique which would be well suited to the investigation of individual differences associated with stress. In addition, resting state connectivity and DTI could be used in a range of ages down to children as young as 2 years who have been successfully studied while asleep (Redcay and Courchesne, 2008). Thus, through a combination of connectivity and volumetric studies, it would be possible to, in children as young as 2 and extending through adulthood, examine the structural and functional networks that underlie the embedding of adversity, and directly test the hypothesis, proposed here, that amygdala changes lead and produce hippocampal effects of adversity.

The techniques proposed here have in common with volumetric studies that they can be performed in the same way with children, adolescents, and adults. Notably absent are functional neuroimaging techniques which require the use of a task. In examining the developmental effects of adversity the use of functional neuroimaging presents potential challenges. Examining functional differences across studies is difficult because subtle differences in experimental design disallow certain findings. In addition, across ages the same task may be more salient and/or more difficult. For example a young child may not understand a task which includes a great deal of peer feedback about behavior where as an adolescent will both understand this task and peer feed back will be a very salient emotional stimulus. Including information like this in the interpretation of results can be difficult. These caveats aside, neural activation is most closely linked to behavior and obtaining functional neuroimaging data as a function of early adversity would have the potential to expose important differences and similarities structure-function relationships.

Transitions between developmental stages have not been studied extensively with regard to early adversity, and longitudinal work that includes a close examination of transitions into childhood and adolescence is necessary to delineate the effects of early adversity. Indeed, there is some behavioral evidence that the effects of early adverse caregiving may exacerbate once children age into adolescence. In a longitudinal study of children adopted from Romanian orphanages, Rutter and colleagues have shown that unlike other domains of development (e.g., cognitive development, height, head circumference) which show massive catch-up following removal from orphanage care, there was a significant increase in emotional difficulties caused by institutional care once children transitioned into adolescence (Colvert et al. 2008). These findings are consistent with the notion that the transition into adolescence potentially marks another period when the environment can exert large effects on the brain (Sisk and Foster, 2004; Schulz et al., 2009). Findings from the rodent suggest that pubertal onset may be an important agent in amplifying or making obvious environmental influences on brain development. For example, stress-induced sex difference in hippocampally-dependent trace learning only emerge after adolescence (Hodes and Shors, 2005), perhaps as a result of the organizing effects of increased pubertal testosterone on the hippocampus (reviewed in Sisk and Zehr, 2005.

Thus far we have considered the timing of stress exposure as a variable that may moderate the effect this variable has on the hippocampus and amygdala. There are various other aspects of stress that may modify its impact on neural structures. Chief among these are the kind and duration of stress. Because stress is chiefly a psychologically defined construct (Gunnar and Quevedo, 2007), one that is subjective in nature, determining which stressors (e.g., abuse vs. neglect) are "the worst" is difficult since there are multiple stressors that can present challenges to the individual's homeostasis. However, certain aspects of stress, for example, controllability and predictability may be particularly effective at influencing HPA activity (Davis and Levine, 1982). Presumably the stressors described throughout this manuscript are unpredictable and uncontrollable from the standpoint of the infant or child experiencing them, thus increasing the potential impact of early stressors. Stress duration should additionally be considered as a factor, since chronic stressors may affect neural development differently, often detrimentally, than single instance traumatic events (reviewed in Brunson et al., 2003). Again, the literature reviewed throughout this manuscript provides examples of chronically stressful environments. Nonetheless, dose-response relationships between stress duration and brain development have been reported (Brunson et al., 2001a; Mehta et al., 2009; Tottenham et al., 2009b). A final variable that should be considered when examining the effect of adversity on neural development is the temporal context in which neural development is tested. For example we argue here that amygdala development and its modification by stress precedes that of the hippocampus. However many developmental studies of traumatic stressors (for example, studies of child abuse) may occur when children are still under a great deal of stress. Duration since stressor needs to be considered, since the amygdala and hippocampus may have different recovery rates (Vyas et al., 2004; Yang et al., 2007). For example, when measured almost a decade after removal from orphanages, post-institutionalized children show amygdala hypertrophy and hyperactivity, but do not show differences in hippocampal structure (Mehta et al. 2009; Tottenham et al., 2009b). However, differences in duration since stressor cannot account for all of the data since many studies (in older individuals) show decreased hippocampus years after stress termination (Andersen et al., 2008), suggesting again that developmental timing of exposure will influence the outcome measure. Nonetheless, more control over duration since stressor is warranted in order to better understand the effects of adversity. To summarize, we suggest that (1) early life is a period of increased vulnerability although the effects of stress may be difficult to detect for years (as seems to be the case with the hippocampus) (2) stress-induced changes in amygdala (initial increases in activity and growth) are apparent earlier in life and more robustly than the hippocampus (decreases in growth), and (3) later in life, when hippocampal changes are finally apparent, the initial amygdala volume increases may ultimately change to volumetric decreases (although it may remain hyperactive).

CONCLUSIONS

Adverse experiences shape brain development, and these changes are often global in nature. However in this manuscript, we have focused on the effects on amygdala and hippocampal development, two regions that are particularly vulnerable to stress in adult populations. We argue that a developmental approach is necessary in understanding how environmental conditions such as trauma can impact outcome in these structures. While this review is limited in its scope, the restriction was purposeful in that it allowed for greater discussion of how timing of exposure and age at measurement can differentially influence these neural phenotypes. The hypothesis driving the manuscript was that developmental timing of adverse experiences and the age at testing would influence structure and function of the amygdala and hippocampus. The literature discussed in this review suggest that the amygdala and hippocampus are highly vulnerable to the effects of adverse early environments, although these effects may be more evident in the amygdala early in life, whereas the hippocampus effects may be more subtle early in life and may be more readily observable in humans studies later in life.

We included data from animal models of stress to address issues of timing and biological mechanism that are difficult to address with most human studies. Two of these issues in the human literature include methodological differences between studies and the methods of identifying the timing of stressful experiences. First, studies differ on how/whether they control for total brain volume when examining the effects of stress on regional morphometry. This difference will have a significant impact on volume findings since children with a history of trauma are more likely to have a smaller total brain volume relative to control samples (Casey et al., 2009; De Bellis et al., 1999b). In addition, recognizing changes in the volume of subcortical structures over time may be difficult because cortical volume decreases rapidly across development. Controlling for total cortical volume across development while measuring *changes* in structure volume may inadvertently increase observed structure development.

Secondly, the overwhelming majority of studies on stress rely on retrospective data. This approach, while usually a necessary nuisance in human studies of stress, is problematic because researchers must rely on subject reporting, which may be inaccurate (Maughan and Rutter, 1997), particularly with respect to timing of events. Moreover, even if reporting is accurate, the varying times and durations of the stressful events in most studies make it unclear how timing played a role in the observed effects. Human developmental studies are critical in order to understand how stressful experiences specific to infants and children impact neural development. Drawing firm conclusions about the impact of stressful and traumatic events will rely on methodological advances. Longitudinal studies, where observation occurs at multiple time points allows for direct observation of change over time. Ideally, these studies would be prospective in nature, a design aspect that further complicates data collection. These types of studies are difficult and costly, but provide valuable information about developmental trajectories not often provided by cross-sectional design (Sowell et al., 2004; Durston et al., 2006; Gogtay et al., 2006; Giedd et al., 2008).

The main purpose of this manuscript is to highlight the importance of considering developmental timing, and the investigation into the short-term and long-term effects of adversity on neural development is only at its earliest stages. The hypotheses posited here will continue to be shaped by future results, and approaches like those described in this paper will help constrain interpretations of those data. This approach will be informative regarding the neural mechanisms that underlie mental health problems following early adversity and provide insight into the sensitive periods that define the windows of opportunity and vulnerability to environmental events, both adverse as well as ameliorative.

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Effects of socioeconomic status on brain development, and how cognitive neuroscience may contribute to levelling the playing field

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The study of socioeconomic status (SES) and the brain finds itself in a circumstance unusual for Cognitive Neuroscience: large numbers of questions with both practical and scientific importance exist, but they are currently under-researched and ripe for investigation. This review aims to highlight these questions, to outline their potential significance, and to suggest routes by which they might be approached. Although remarkably few neural studies have been carried out so far, there exists a large literature of previous behavioural work. This behavioural research provides an invaluable guide for future neuroimaging work, but also poses an important challenge for it: how can we ensure that the neural data contributes predictive or diagnostic power over and above what can be derived from behaviour alone? We discuss some of the open mechanistic questions which Cognitive Neuroscience may have the power to illuminate, spanning areas including language, numerical cognition, stress, memory, and social influences on learning. These questions have obvious practical and societal significance, but they also bear directly on a set of longstanding questions in basic science: what are the environmental and neural factors which affect the acquisition and retention of declarative and nondeclarative skills? Perhaps the best opportunity for practical and theoretical interests to converge is in the study of interventions. Many interventions aimed at improving the cognitive development of low SES children are currently underway, but almost all are operating without either input from, or study by, the Cognitive Neuroscience community. Given that longitudinal intervention studies are very hard to set up, but can, with proper designs, be ideal tests of causal mechanisms, this area promises exciting opportunities for future research.

Keywords: developmental neuroscience, environment, poverty, early intervention, review

INTRODUCTION

The neural maturation and plasticity which underpin children's cognitive development provide an endless source of important questions for Cognitive Neuroscience. In some children, this development runs into problems. Consider the example of dyslexia, which, depending upon the criteria that are used, is thought to affect between 5 and 10% of all children. Although much remains unknown and there is no definitive cure, a great deal has been discovered about dyslexia's cognitive and neural bases, a large number of different interventions have been developed and rigorously tested, and the behavioural and neural consequences of several such interventions have been longitudinally explored (e.g. Ahissar, 2007; Ramus and Szenkovits, 2008; Shaywitz et al., 2008; Gabrieli, 2009). From the perspective of basic science, such research has helped to illuminate questions about how the brain normally learns and processes language. In terms of practical consequences, it has helped to improve the reading of many children, to destigmatise the difficulties they are experiencing, and to show them that needing some additional reading instruction is not at all the same thing as being unintelligent. Perhaps the only downside of this impressive body of work is that it is harder these days to carry out novel dyslexia research than it used to be.

In contrast, consider exactly the same questions about the cognitive and neural consequences of growing up in a low socioeconomic status (SES) environment. According to the 2007 Census, 17.4% of children in the United States live in poverty (U.S. Bureau of the Census, 2007, Information retrieved from the University of Michigan National Poverty Center, at http://www.npc.umich.edu/poverty/). As is described in detail in the sections below, there have been a substantial number of behavioural studies of the effects on children's development, and also many intervention studies, but comparatively few of these have had the benefit of rigorously controlled randomised designs. There have been a handful of neuroimaging studies: enough to warrant optimism that this is a promising area of investigation, but a tiny number in comparison to the studies of dyslexia or other learning disabilities. Although there are many crucial basic science questions about how differences in the environment shape neural development, the large environmental differences shaping human neural development have scarcely been addressed. While some interventions have appeared promising, there is much uncertainty about how enduring their effects are, and about what the necessary ingredients of a truly successful intervention should be. Far from destigmatising the learning difficulties caused by low SES, the less-than-distant history of academic psychology has contained some rather unsavoury episodes of seeking to attribute these difficulties to genetic inferiority. Perhaps the only upside of the relative scarcity of research on SES is that this area contains a great many interesting and potentially consequential open questions for Cognitive Neuroscience, ripe for investigation.

THE AIMS OF THIS REVIEW, AND HOW IT DIFFERS FROM OTHERS

There have been a number of recent publications reviewing the role of SES in cognitive development. **Table 1** points the reader to several of them, grouped by their area of focus. In the present paper, we seek to highlight two questions which have not yet received much attention: first, we describe the large and open research opportunities, both pure and applied, which currently exist in this subject area. Despite its direct relevance to many questions which are central to Cognitive Neuroscience, remarkably few studies have so far been carried out. Second, we discuss how neuroimaging can work to ensure that it contributes useful information over and above what can be obtained from purely behavioural studies, especially in terms of diagnostic and predictive power.

AN OPPORTUNITY FOR NEUROIMAGING: LOTS OF BEHAVIOURAL DATA, BUT VERY LITTLE KNOWN ABOUT UNDERLYING MECHANISMS

As the list of reviews in **Table 1** shows, there is a remarkable disconnect at present between the large amount of behavioural data which is available and the almost complete absence of corresponding neural data. This presents quite a research opportunity.

However, we wish to argue that simply measuring the "neural correlates" of SES-disparities would be insufficient. The behavioural data is compelling in its own right, and the mere process of adding of brain pictures may not in itself add any explanatory, diagnostic or predictive power, despite its seductive allure (Weisberg et al., 2008).

Fortunately, there are several ways in which neuroimaging may indeed be able to add substantively to our understanding, particularly in the area of intervention research. We now consider some possible approaches.

WAYS IN WHICH NEUROIMAGING CAN CONTRIBUTE OVER AND ABOVE BEHAVIOUR

The ideal circumstance for neuroimaging to make a contribution is when two people seem the same from outside the head, but actually differ inside the head. This information from inside the head is especially valuable if it contributes diagnostic or predictive power, over and above what purely behavioural measures can provide.

Examples of this are studies in which neuroimaging data helps to predict the degree to which subjects benefit from a subsequent intervention. This model has clear potential relevance to intervention studies in low-SES populations. For example, in studies of depression, activation in frontal and limbic regions has been found to be predictive of patient response to antidepressant drugs (Mayberg et al., 1997; Perlis et al., 2003; Langenecker et al., 2007) and to Cognitive Behavioural Therapy (Siegle et al., 2006). Similar results have been found in predicting gains from movement therapy in stroke patients (Dong et al., 2006). In a study of foreign-language

Table 1 | Summary of recent reviews on the relations between SES and cognitive development, from diverse fields.

Domain	Type of data	Focus	References
Cognitive Neuroscience	Neural and behav.	Brain development	Hackman and Farah (2009), Lipina and Colombo (2009)
Cognitive Psychology	Behav. only	Home environment	Bradley and Corwyn (2002)
		Interaction with environment	Conger and Donnellan (2007)
		Societal context	Huston and Bentley (2009)
		Environmental stressors	Evans (2006)
		Cost-effectiveness of early intervention	Reynolds and Temple (2008)
		Books on early childhood intervention	Shonkoff and Meisels (2000), Feldman (2004), Nisbett (2009)
		Books on language in home environment	Heath (1983), Hart and Risley (1995)
Economics	Behav. only	Inequality and child development	Heckman (2006), Borghans et al. (2008)
	Neural and behav.	Inequality, plasticity and development	Knudsen et al. (2006)
NICHD longitudinal study	Behav. only	Effects of reduced time for maternal care	Brooks-Gunn et al. (2002)
		Effects of different types of child care	NICHD Early Child Care Research Network (2006)
		Long term effects of early child care	Belsky et al. (2007)
		Parental support for children's autonomy	NICHD Early Child Care Research Network (2008)
Intervention and follow-up	Behav. only	Perry Preschool Program	Weikart (1998), Belfield et al. (2006), Muennig et al. (2009)
		Abecedarian Program	Ramey and Ramey (1998a), Campbell et al. (2001)
		Chicago Longitudinal Study	Reynolds and Temple (1998), Reynolds et al. (2001, 2007)
Clinical	Neural and behav.	Interventions from pediatric perspective	Herrod (2007), Bonnier (2008), Bertrand et al. (2008)
	Behav. only	Public health and developing countries	Grantham-McGregor et al. (2007), Beddington et al. (2008)

learning in normal adults, Golestani et al. (2007) found that a structural measure, namely white-matter density in left Heschl's gyrus, predicted subjects' abilities to learn non-native speech sounds. In dyslexia research, a number of studies have shown that neural measures can predict subsequent reading ability, using ERPs in infants (Guttorm et al., 2005) and preschool children (Maurer et al., 2009), and using functional and structural MRI (Hoeft et al., 2007). This last study, by Hoeft et al., is especially notable, as it used cross-validation techniques to ensure that the extra predictive power due to the neural measures was genuinely present, as opposed to potentially being an artifact of over-fitting.

There are other ways for neuroimaging to contribute beyond behavioural studies, other than by making prospective longitudinal predictions. Another potentially valuable service is retrospective: probing the neural changes which underlie intervention-induced behavioural changes. For example, dyslexics often manage to improve their reading via a variety of ad hoc compensatory strategies. Although their reading performance appears outwardly to have gained, it is often supported by different neural systems than those found in normal readers, and is less fluent as a result (Shaywitz et al., 2003). After remediation programs, dyslexics' brain activation tends to becomes more similar to that of normal readers, suggesting that the training has succeeded in acting upon the brain's canonical reading circuits, rather than simply producing another compensatory work-around (Shaywitz et al., 2003; Temple et al., 2003). Similar questions could be investigated after interventions in low-SES children. However, we are not aware of any such study which has been carried out to-date, illustrating yet again the many open opportunities for Cognitive Neuroscience in this area.

It is not only in intervention studies that neuroimaging has the opportunity to carry information over and above what is available from behaviour. In a study of 5-year-old children, Raizada et al. (2008) investigated the relations between SES, fMRI activity and a battery of standardised test scores, and found that SES was strongly correlated with the degree of hemispheric specialisation in Broca's area, as measured by left-minus-right fMRI activation during a rhyming task. However, that fact in itself does not suffice to show that the neural measure is conveying additional information. Both SES and Broca's asymmetry would be expected to correlate with the children's language-test scores, as indeed was found to be the case, and so the correlation between SES and Broca's could potentially be merely a trivial consequence of both measures' correlation with the language scores. However, this concern was ruled out by using partial correlation: the SES-Broca's link remained significant even after the effects of the language scores were removed. This does not imply that SES was influencing Broca's via some kind of non-linguistic pathway. A more likely explanation is that the fMRI is a more sensitive measure of the development of Broca's than any of the behavioural tests are; each behavioural score is a compound function of perception, cognition, attention and motor control, whereas fMRI can probe Broca's more directly. Thus, neuroimaging may be able to provide us with a means to tease apart neural representational competence from behaviourally measured performance.

It should be noted that neural correlates of, and possible predictors for, SES-related impacts on behaviour are unlikely to be isolated to particular localised brain areas. Standard fMRI analysis takes a massively univariate approach, in which signals from the brain are analysed one voxel at a time. However, it is likely that multiple parts of the brain may act together in concert, especially in relation to a multifaceted phenomenon such as SES. Recently developed multivariate pattern-based methods of fMRI analysis are able to capture such multivoxel effects; for a review of the application of these methods to developmental cognitive neuroscience, see Bray et al. (2009) in this volume. These pattern-based fMRI analyses have an additional advantage: by measuring the similarity of the distributed patterns of neural activation that are elicited by different task conditions, they can study the structure of people's neural representations (Kriegeskorte et al., 2008). This allows fMRI to distinguish between neural representations which are well-structured for performing a given task and representations which are poorly structured (Raizada et al., 2010), thereby providing another route to teasing apart neural representational competence and behaviourally measured performance. This distinction may have particular relevance to the puzzle discussed in the Section "The Problem of Fade-out, and the Puzzle of Longer Term Gains", in which intervention-induced changes seem to "fade-out" in the short term, but then to produce significant improvements in people's later lives: it could be that while behavioural performance fades out, an underlying increase in neural representational competence may persist, lying latent for several years but then re-manifesting itself in improved behaviour later in life.

WHAT ARE THE BEHAVIOURAL AND NEURAL DIFFERENCES ASSOCIATED WITH LOW SES?

As mentioned above, there is a substantial disconnect between the amount of behavioural and neural data available on SES disparities. However, there is also a large disconnect between general diagnostics of cognitive ability and academic achievement and assessments of specific cognitive processes. For example, previous research has found that children from low SES backgrounds perform below children from higher SES backgrounds on tests of intelligence and academic achievement (Duncan et al., 1994; Bradley and Corwyn, 2002). Children from low SES backgrounds are also more likely to fail courses, be placed in special education, and drop out of high school compared to high SES children (McLoyd, 1998).

Although intelligence tests and academic achievement reflect cognitive ability, they are not particularly informative about brain regions associated with specific cognitive processes (i.e. neurocognitive systems). Accordingly, specific neuropsychological assessments have been employed in recent investigations to decompose cognitive function. In particular, Farah and colleagues used these techniques to derive several relatively independent neurocognitive systems (Noble et al., 2005a, 2007; Farah et al., 2006; Hackman and Farah, 2009). These systems are anatomically and functionally defined by neuropsychological studies with brain-damaged patients and activation studies using neuroimaging techniques with healthy subjects.

Table 2 summarises some of the main SES-related findings in these neurocognitive systems. Collectively, these studies present substantial evidence that the playing field is indeed unlevel. In the following section, we consider a range of interventions which have attempted to level it, and how these interventions create an exciting and also pressing opportunity for Cognitive Neuroscience.

Table 2 | Some of the main experimental findings on SES and cognitive development. From the very small number of neural studies, together with the large number of behavioural findings, it can be seen that there are great opportunities for new research.

Domain	Type of data	Task	Subjects	Finding	References
Language	Behav. only	Lang. in home	Children	SES corrs. with richness of language environment	Heath (1983), Hart and Risley (1995)
	Behav. only	Lang. in home	4–5yo children	SES corrs. with maternal and child syntax	Huttenlocher et al. (2002)
	Behav. only	Lang. in home	2yo children	SES corrs. with maternal and child vocabulary	Hoff (2003)
	Behav. only	Standardised tests	5yo children	SES corrs. with vocab., phon.awareness, grammar	Noble et al. (2005b)
	Struct. MRI	Phon. awareness	11yo children	SES does not corr. with planum temporale asym.	Eckert et al. (2001)
	fMRI	Reading ability	Dyslexic adults	Compensated readers were at higher SES schools	Shaywitz et al. (2003)
	fMRI	Phon. awareness	6–9yo children	Higher SES have less typical brain-behav. relation	Noble et al. (2006)
	fMRI	Rhyming task	5yo children	SES corrs. with left-minus-right Broca's activation	Raizada et al. (2008)
Math	Behav. only	Number tasks	3–5yo children	Low-SES children worse in verbal math problems	Jordan et al. (1994)
	Behav. only	Number intervention	4–5yo children	Low-SES math improved by Number Worlds	Griffin et al. (1994)
	Behav. only	Number intervention	3–4yo children	Low-SES math improved by Pre-K Math	Starkey et al. (2004)
	Behav. only	Number intervention	4–5yo children	Low-SES math improved by linear board game	Siegler and Ramani (2008)
	Behav. only	Home env., math	3–10yo children	SES is a predictor of math attainment at age 10	Melhuish et al. (2008)
	Behav. only	General math	Young children	General reviews of SES and math	Jordan and Levine (2009)
Attention, Exec.func.	Behav, cortisol	Cogn. tasks, cortisol	6–16yo children	Age-dependent cortisol and attention SES diffs	Lupien et al. (2001)
	Behav. only	Attent. Network Test	6yo children	Low-SES children had reduced attentional control	Mezzacappa (2004)
	Behav. only	A-not-B task (exec.)	6–14mo infants	Low-SES infants made more errors	Lipina et al. (2005)
	Behav. only	Working mem./Exec.	5yo children	Low-SES had reduced working mem. and exec.func	Noble et al. (2005a)
	Behav. only	Working mem./Exec.	10–13yo children	Low-SES had reduced working mem. and exec.func	Farah et al. (2006)
	Behav. only	Working mem./Exec.	6–7yo children	Low-SES had reduced working mem. and exec.func	Noble et al. (2007)
	ERP, behav.	Auditory attention	11–14yo children	Nd ERP in high but not low SES, no behav. diff	D'Angiulli et al. (2008)
	ERP, behav.	Visual attention	7–12yo children	Low SES: reduced visual and novelty (N2) ERPs	Kishiyama et al. (2009)
	ERP, behav.	Auditory attention	3–8yo children	Low SES: less ERP suppression of unattended	Stevens et al. (2009)
Memory	Behav. only	Memory	Adults	General review of SES and memory	Herrmann and Guadagno (1997)
	Behav. only	Incidental learning	6–13yo children	Low SES had reduced incidental learning	Farah et al. (2006), Noble et al. (2007)
	ERP, behav.	Recency/recognition	Adults	Elderly low-SES worse on recency task	Czernochowski et al. (2008)
Stress	Behav, cortisol	Cortisol, surveys	6–10yo children	Low SES: higher cortisol, maternal depression	Lupien et al. (2000)
	Behav, cortisol	Cogn. tasks, cortisol	6–16yo children	Age-dependent cortisol and attention SES diffs	Lupien et al. (2001)
	Behav, physiol.	Cortisol, blood press.	13yo children	Poverty corrs. with impaired stress reactivity	Evans and Kim (2007)
	Behav, physiol.	Working mem., stress	Young adults	Poverty corrs. with poorer working mem.	Evans and Schamberg (2009)
	Struct. MRI	Parental care, MRI	Young adults	Parental nurturance corrs. with hippocampal vol.	Rao et al. (2010)

INTERVENTIONS: TOWARDS LEVELLING THE PLAYING FIELD

Given the large body of behavioural data described above, along with the very small but growing body of neural data, there is little doubt that children's development takes place on an unlevel playing field: lower SES children experience environments which are more stressful and less cognitively enriching than those of higher SES children. This immediately raises the question: what can be done to improve the conditions for these children's development?

As was remarked upon in the introduction to this paper, Cognitive Neuroscience finds itself in an unusual and potentially very fruitful position here. There are many intervention programs already ongoing, and more still about to start up, but almost all are operating without either input from, or study by, the Cognitive Neuroscience community. There is clearly an opportunity here to start addressing questions which are important not only from a societal point of view but also in terms of basic science. What kinds of interventions produce enduring neural changes? What kinds of intervention-induced neural changes are most predictive of longer term post-intervention behavioural gains?

EXAMPLES OF SUCCESSFUL INTERVENTIONS, TESTED WITH PROPER CONTROLS

Although many interventions have been attempted, there have been relatively few which meet the strict scientific criteria of a rigorous randomised trial. The confidence with which claims of effectiveness can be made is therefore not as high as it could be. However, there have been a number of studies which do meet these standards, and of those some have provided invaluable longitudinal data about the interventions' enduring effects from childhood into adulthood. In the following section, we wish to highlight the neuroscientific questions and opportunities which some of these interventions suggest, to discuss what the successes and shortcomings of such interventions might tell us about underlying neural mechanisms, and to emphasise the untapped potential for new neuroscientific studies which such interventions offer.

The two best examples of randomised interventions with long-term longitudinal follow-up data are the Abecedarian Program (Ramey and Ramey, 1998b; Campbell et al., 2001, 2008; Barnett and Masse, 2007), and the Perry Preschool Program (Weikart, 1998; Belfield et al., 2006; Muennig et al., 2009). A third source of longitudinal data on the effects of intensive intervention is the Child–Parent Center (CPC) Program, whose follow-up branch is called the Chicago Longitudinal Study (Reynolds and Temple, 1998; Reynolds et al., 2001, 2007). That intervention treated all of its enrolled children and hence did not have a randomised design, but its follow-up studies have used a quasi-experimental cohort design by selecting randomised and matched control groups. All three programs concentrated on low-SES, predominantly ethnic minority children. In the present discussion, we will focus on the two fully randomised studies, namely the Perry Preschool and Abecedarian programs.

The Perry program enrolled 64 children at ages 3 and 4, and consisted of intensive daily sessions lasting 2.5 h each, and also a weekly 90-min home visit to build parental involvement. This lasted for 30 weeks each year, for 4 years. Longitudinal follow-up is ongoing, with the most recent paper describing the participants 37 years later (Muennig et al., 2009). The Abecedarian program was larger, with 111 children, and was even more intensive, involving full-day care from 7.30 am to 5.30 pm, 5 days per week, 50 weeks per year, with free transportation provided. The children started at an average age of 4.4 months, and remained in the program until age 8. Longitudinal follow-up continued until age 21.

The interventions described above all seek to address a broad range of cognitive skills simultaneously. There also exist several low-SES-targeted interventions which are more tailored towards addressing specific skills, some of which have been mentioned in the discussion of different neurocognitive systems above. Examples include the math interventions listed in **Table 2** (Griffin et al., 1994; Starkey et al., 2004; Ramani and Siegler, 2008; Siegler and Ramani, 2008), and the "Tools of the Mind" intervention aimed at improving executive function in pre-school children, which is discussed further in the Section "Metacognitive Skills: Self-control, Perseverance, and Long-term Benefits" (Diamond et al., 2007), and, also in the domain of executive function, an ongoing training study aimed at improving children's fluid reasoning ability (Mackey and Bunge, 2009). These more skill-specific studies have not yet been followed up with long-term longitudinal data, so the crucial question of their enduring impact is as yet unaddressed. The longitudinal follow-up data that we do possess shows a puzzling pattern, which Cognitive Neuroscience may be very well positioned to help explain. In the following section, we consider that puzzle and the possible opportunity which it brings with it.

THE PROBLEM OF FADE-OUT, AND THE PUZZLE OF LONGER TERM GAINS

The Abecedarian and Perry Preschool programs described above were clearly major undertakings. One would expect such strenuous efforts to be almost guaranteed to produce beneficial effects in the participating children, at least in the short term. That was indeed found to be the case, as the references cited above describe. However, they both exhibited a puzzling effect, which is almost tailor-made for a Cognitive Neuroscience investigation: after the children left the program, the benefits in IQ and other test scores appeared to "fade out". Remarkably, though, longitudinal follow-up decades later revealed that the participants did much better than comparable non-enrolled children on several important life-measures, including the proportion who graduated high-school, who studied in a 4-year college, or who owned their own home. A concise summary of these results can be found in Knudsen et al. (2006). Chapter 7 of Nisbett (2009) also provides an excellent discussion of these and related findings.

This is an encouraging long-term result, but also a puzzling one. If intervention-induced gains fade out relatively quickly, then there might seem to be even less chance that any gains would be observed decades later, in adult life. It might appear that the effects of such interventions on cognition might be a little like the weight-loss achieved by a crash diet: once the intervention is ended, the benefits tend to "fade out." The Head Start program in particular has been criticised on these grounds, with a possible cause of the fade-out being the poor quality schools that children are fed into after their participation in Head Start ends (Currie and Thomas, 2000; Fryer and Levitt, 2004). However, such claims are controversial, and it has been argued that much of the apparent fade-out is an artifact of attrition and poor design in follow-up studies (Barnett and Hustedt, 2005).

Although the data for Head Start may be unclear, the data illustrating short-term fade-out in the Perry Preschool Program is unequivocal, and can be clearly seen in Fig. 1 of Knudsen et al. (2006): in their first 2 years of participation in the Perry program, children's average IQs increased by 10–15 points compared to a control group, but by the age of 10, 2 years after the children had left the program, these gains had completely faded out, with no difference between the intervention and control groups. Despite that, the data showing real longer-term gains is equally clear: it is especially striking to compare the fade-out at age 10 shown in Fig. 1 with the major improvements in life circumstances at age 27 which are summarised in Fig. 2A of the same paper. Similar results emerged for the Abecedarian Program, also summarised in the same figures. Clearly this is a puzzle in need of explanation. In the following section, we suggest this constitutes a particularly promising opportunity for Cognitive Neuroscience to make a contribution.

AN OPPORTUNITY FOR COGNITIVE NEUROSCIENCE: WHAT ARE THE NEURAL CHANGES THAT ENDURE WHILE BEHAVIOURAL CHANGES ARE FADING OUT?

As was argued in the Section "Ways in Which Neuroimaging Can Contribute Over and Above Behaviour", the ideal circumstance for neuroimaging to make a contribution is when two people seem the same from outside the head, but actually differ inside the head, especially if these internal neural differences can provide predictive power, over and above what purely behavioural measures can provide.

This is precisely the case in the situation described here of short-term fade-out followed by longer-term gains. Looking only at the behavioural measure of IQ scores, a 10-year-old child who had previously participated in the Perry Preschool Program would have seemed no different from a 10-year-old in a non-intervention control group. However, something about those children must have been different, as the Perry participants went on to be much more likely graduate from high school, to own a home, and to stay off welfare.

One possibility, which has yet to be explored, is that differences in neural maturation and neural representational capacity may be induced by interventions, but that they may manifest themselves in behaviour only gradually over the course of many years. Two newly built houses may both look good upon completion, but the one with more firmly built foundations and more weather-proof paint will be in much better shape after 10 or 15 years. Neuroimaging could, potentially, be able to reveal how an intervention acts to strengthen the neural foundations upon which a child's later cognitive development depends. Which neural measures, if any, might turn out to have the greatest long-term predictive power is a potentially important empirical question which is currently completely open.

METACOGNITIVE SKILLS: SELF-CONTROL, PERSEVERANCE, AND LONG-TERM BENEFITS

Another possible explanation for the occurrence of longer-term gains after short-term fade-out, and one which is not at all exclusive of the neural hypothesis suggested above, is that the intervention programs may have induced greater powers of self-regulation and self-control in the children, and that these enhanced executive skills may have manifested themselves in greater academic attainment much later in life. In kindergarten, improved self-control may have only a weak effect on how much a child learns, but in high-school, when self-directed study and homework start to become important, the effect could be substantial.

In an important series of studies, Duckworth and colleagues have shown that such self-discipline and perseverance, which they capture using the term "grit", is more predictive of academic performance than are more conventional measures such as IQ (Duckworth and Seligman, 2005; Duckworth et al., 2007; Duckworth and Quinn, 2009).

Such powers of self-regulation may be trainable from a young age. Working with low-SES preschool children, Diamond et al. (2007) have recently produced exciting evidence that self-control and executive function can be increased, using an intervention called "Tools of the Mind" which is based on Vygotsky's principles of executive function and development (Bodrova and Leong, 1996). The children in the study showed improved accuracy on tests that measure core aspects of executive function. In a follow-up study, Barnett et al. (2008) replicated these effects on executive function, but found only small improvements in language development. It will be important to carry out long-term follow-up of such studies, to see whether academic gains may start to emerge later in high-school, when self-regulatory skills start to have a more direct impact on academic outcomes via homework, revision for exams and so on.

Given these considerations, it is possible that the longer-term gains exhibited by the Perry Preschool and Abecedarian Program participants may have stemmed from enduring improvements in self-regulation. Such gains may have little effect on measured IQ scores, but may make all the difference in helping children to avoid dropping out of high-school. This possibility also suggests several specific neural and cognitive hypotheses that could be tested, using prefrontal/executive tasks such as stop-signal inhibition and Stroop interference (Stuss and Alexander, 2000).

POSITIVE FEEDBACK LOOPS: HOW A SMALL INTERVENTION MAY EVENTUALLY HAVE LARGE EFFECTS

One particularly appealing aspect of training executive function in young children is that it could potentially trigger a long-term self-reinforcing trend, with improved self-control enabling greater attentiveness and learning, which would in turn help to make a child's educational experiences more rewarding, thereby facilitating yet more intellectual growth. That rather rosy-sounding scenario of course raises the question of whether such positive feedback loops can in fact be induced.

One phrase that has been used to describe such phenomena is "the Matthew effect" (Merton, 1968), based on the following text from the Gospel of St. Matthew: "For unto every one that hath shall be given, and he shall have abundance: but from him that hath not shall be taken away even that which he hath" (XXV:29). In other words, the rich get richer and the poor get poorer. In the domain of cognitive development, Stanovich (1986) argued that learning to read can produce precisely such effects: the better a child can read, the more likely they are to seek out and find new reading material, thereby improving their reading ability still further. Conversely, a child who experiences reading difficulties may become more and more likely to avoid reading, thus dropping further and further behind. The degree to which this phenomenon actually holds true in reading development is a topic of some debate (Shaywitz et al., 1995; Scarborough and Parker, 2003; Morgan et al., 2008). It may well hold true in interventions of the sort considered in the present review: in a meta-analysis of interventions aimed at making children's home environments more conducive for cognitive development, it was found that higher SES households showed more improvements than did lower SES ones (Bakermans-Kranenburg et al., 2005).

In two studies reported in Science, Cohen and colleagues have shown that brief self-affirmation writing assignments aimed at reducing feelings of academic threat in ethnic minority high-school students had the effect of producing significant improvements in grade-point average, which endured over a period of 2 years (Cohen et al., 2006, 2009). They argue that this small intervention can induce large effects precisely due to triggering a positive feedback loop, writing that "because initial psychological states and performance determine later outcomes by providing a baseline and initial trajectory for a recursive process, apparently small but early alterations in trajectory can have long-term effects" (Cohen et al., 2009).

Another source of evidence that seemingly small interventions can have large effects if they induce enduring changes in mindset comes from the work of Dweck and colleagues (Blackwell et al., 2007; Dweck, 2007). They have shown that teaching children a "growth mindset", in which achievement is viewed by the child as deriving from hard work and therefore being under their individual control, as opposed to a "fixed mindset", in which achievement is viewed as being determined by how much innate ability one happens to have, can lead to markedly improved long-term educational outcomes. Specifically, in an intervention study with seventh graders drawn from a range of SES and ethnic backgrounds, Blackwell et al. (2007) found that the math scores of children who had been taught the growth mindset increased with respect to a fixed-mindset group over a period of 1.5 years.

KIPP, THE HARLEM CHILDREN'S ZONE, AND A COMING WAVE OF INTERVENTIONS IN NEED OF NEURAL MEASURES

The Perry Preschool and Abecedarian studies described above show that sustained and intensive interventions can indeed make lasting differences in the lives of low-SES children. However, those studies were of very limited size, raising the question of whether they can be scaled up in order to help larger populations of children. Naturally, the larger a program becomes, the harder it is to preserve sustained and intense high-quality intervention. Some exciting programs attempting to do precisely that are taking place right now.

One such effort is an organisation of charter schools called the "Knowledge is Power Program", or KIPP[1]. Founded in 1994, KIPP currently includes 82 schools across 19 states with around 20,000 students in all. The KIPP students are drawn almost entirely from low-SES neighbourhoods, with more than 80% of the children being eligible for free or subsidised school lunches, and are highly intensive. There is a strong emphasis on self-discipline and commitment to learning, and children receive small monetary reward "paychecks" each week, based on their academic performance and standards of behaviour (Mathews, 2009).

An even more ambitious program, although currently not operating on as large a scale, is the Harlem Children's Zone, or HCZ[2]. This program seeks to create a continuous "pipeline" to promote the cognitive development of low-SES children, starting from birth and continuing through preschool, elementary school and middle school. One of the main aims of having such an unbroken chain of high quality care is to prevent fade-out from having any opportunity to arise. An excellent description of the program and its creation can be found in Tough (2008).

Unlike the much smaller Perry Preschool and Abecedarian programs, there is no randomised control group available against which the KIPP or HCZ schools' performance can be compared, and indeed such an arrangement at so large a scale would probably be impossible. Thus, studies of their effectiveness must be interpreted with caution. With that borne in mind, initial results are promising: KIPP children's scores on California State and national language and math tests were markedly higher than those of children from comparison schools (Woodworth et al., 2008), and Harlem Children's Zone students ranked in the top fifth of all eighth grade classes in the whole of New York City, a comparison group in which most of the schools are from higher SES neighbourhoods than Harlem (Tough, 2008).

In what may by now be a familiar refrain to readers of this review, we wish to point out that this very large scale intervention program has, as far as we are aware, been not yet been accompanied by any Cognitive Neuroscience measures whatsoever, not even at the behavioural level. Clearly there are potential research opportunities here, although the absence of a pre-existing randomised control group means that, in order to be statistically valid, studies may need to use very carefully controlled prospective longitudinal designs.

In 2008, more than 10,000 children were enrolled in the various sections of the Harlem Children's Zone program. However, it is likely that over the coming 2–3 years, a far larger number of children will participate in such programs: the Obama administration has proposed in its 2010 budget plan to set up "Promise Neighborhoods" in urban centres across the country, directly modelled on the Harlem program[3]. As we discuss in the Section "A Pressing Opportunity: Ongoing Intervention Programs Without Any Cognitive Neuroscience Studies", this coming wave of large-scale intervention programs constitutes a pressing opportunity for Cognitive Neuroscience. They could either be a source of invaluable data about the impact of cognitive stimulation on neural development, or they could become yet another example of a major behavioural study passing by, without any knowledge being gained about the underlying neural mechanisms that the intervention is fundamentally acting upon.

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[1] http://www.kipp.org

[2] http://www.hcz.org

[3] http://www.whitehouse.gov/omb/fy2010_key_education

CHALLENGES, OPPORTUNITIES AND FUTURE DIRECTIONS

MINEFIELDS ASSOCIATED WITH INVESTIGATING SES, AND APPROACHES FOR DEFUSING THEM

Any researcher who starts to investigate the relation between cognitive development and SES will quickly find that this is very much a touchy topic. There is often a suspicion that some attempt may be made to put a scientific veneer of respectability onto a claim that people from lower socioeconomic backgrounds are somehow inferior or undeserving. From the current authors' own perspective, which we have no reason to believe is atypical of the field in general, this is absolutely the opposite of what is intended. On the contrary, the hope would in fact be to provide scientific support for educational and social intervention programs which would help to reduce socioeconomic disparities. In order pre-emptively to fend off misunderstandings of this sort, it may be wise to make such intentions as explicit and salient as possible. We hope that the title of this paper reflects that.

Another objection frequently levelled against studies of SES, and not always without some justification, is that scientific studies may take too reductive a view of the complex and multifaceted environments in which children grow up. A variety of factors interact at multiple scales, including parents, schools, social peer groups, and neighbourhood communities, yet despite that SES is often characterised by just a single number. Such a one-dimensional measure is undoubtedly an over-simplification, but such over-simplifications can be useful at least as an initial step, especially for trying to find important trends in large and complex datasets. Fortunately, much more detailed and multidimensional measures of children's environments are available, such as the HOME Inventory of Bradley, Caldwell and colleagues (Bradley et al., 1988, 2001). Recent reviews emphasising the importance of viewing SES in a sociological context can be found in Conger and Donnellan (2007) and Huston and Bentley (2009).

A related potential criticism is the suggestion that Cognitive Neuroscience studies portray being of low SES as some kind of "deficit". Here again, it seems to us that a helpful response is to emphasise that the aim is quite the opposite, namely to help build the foundations for improving children's educational opportunities. There can also be an understandable discomfort about having a bunch of rich, white university professors coming along and trying to tell poorer people how they should raise their children. A promising solution to this problem is exemplified by the Harlem Children's Zone, which is staffed by people who have lived and grown up in the in the local community.

Unfortunately, the suspicion of academic psychology described above has not always been unwarranted. The field has, in the not very distant past, been associated with some rather unsavoury claims that socioeconomic achievement gaps are caused by some alleged genetic inferiority. The best known expression of this view is the book "The Bell Curve" (Herrnstein and Murray, 1994). Several very detailed and thorough dismantlings of that thesis have been published, perhaps most notably the books by Gould (1996) and, more recently, Nisbett (2009). Without attempting to repeat all those arguments here, we wish to highlight two points in particular. First, as Nisbett (2009) argues in detail, studies that appear to measure heritability may in large part be measuring environmental homogeneity. If all children grew up in exactly the same environment, then all characteristics would appear to be 100% hereditary and 0% due to environmental variation: no effects can be accounted for by environmental variation if there is no environmental variation in the first place. In terms of providing environments conducive to children's cognitive development, the households of adoptive parents or of high-SES families may in fact be highly homogeneous, with them all containing educated adults who play with and read to their children. In contrast, the environments in lower SES households may have much greater variability. Based purely on these environmental homogeneity considerations, one would therefore predict that intelligence should appear to be much more heritable in high-SES families than in low-SES families. Indeed, this is precisely what is observed (Turkheimer et al., 2003). A second point worth making, which is not discussed in Nisbett (2009), is that the whole premise of the nature-vs.-nurture debate is rendered highly questionable by recent discoveries in genetics. Although our genes remain the same, the *expression* of those genes, i.e. whether those genes are turned on or off, is hugely influenced by the environment throughout life (Champagne and Mashoodh, 2009). Indeed, the activation and deactivation of genes within the nuclei of neurons is precisely the pathway via which the environment makes long-term changes to our synapses during learning (McClung and Nestler, 2008). Given this dynamic interplay between genes and the environment, with influence running in both directions, old controversies about nature-vs.-nurture may have hinged upon a distinction that is false.

A PRESSING OPPORTUNITY: ONGOING INTERVENTION PROGRAMS WITHOUT ANY COGNITIVE NEUROSCIENCE STUDIES

Longitudinal intervention studies are perhaps the most difficult of all types of experiments to set up and run. They are time-intensive and very costly, and pose major challenges of subject recruitment and retention. This is true enough when the participants are well-off middle class families with their own transportation and the ability to find free time, but is greatly more difficult when they are low SES families struggling to get by. However, longitudinal intervention studies are also the only way to address many of the most important questions about cognitive development.

From this perspective, it would seem like quite a missed opportunity if several large-scale longitudinal interventions serving low SES children were to take place and run their course without any study by, or input from, the Cognitive Neuroscience community. Yet this is precisely what is happening right now, and what will be happening on a broader nationwide scale if a network of Promise Neighbourhoods is set up across the country without any neuroscientific measures being made. There is clearly an opportunity here to start addressing questions which are important not only in from an applied point of view but also in terms of basic science. What kinds of interventions produce enduring neural changes? What kinds of neural changes are most predictive of longer term post-intervention behavioural gains? Which neural processes fade away without constant support, and which can trigger self-sustaining improvements? In scientific and also societal terms, the impact from starting to answer these questions could be substantial.

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Neural circuits can bridge systems and cognitive neuroscience

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There has been an emerging focus in neuroscience research on circuit-level interaction between multiple brain regions and behavior. This broad circuit-level approach creates a unique opportunity for convergence and collaboration between studies of humans and animal models of cognition.

Measurement of broad-scale brain networks may be particularly important for understanding changes that occur in brain organization and function during development. Recent studies in humans have gained much leverage from trying to understand circuit-level interactions among brain regions over the course of development. Such studies use connectivity analyses of functional magnetic resonance imaging both during cognitive activity and during rest (fcMRI), and diffusion tensor imaging (DTI) to measure (respectively) the functional and structural connectivity between discrete brain regions (e.g. Rissman et al., 2004; Snook et al., 2005; Mori and Zhang, 2006; Fox and Raichle, 2007). Studies using these approaches have revealed that, over the course of development, functional connectivity increases between distant brain regions (in the rostro-caudal axis) while it decreases between local regions of the frontal, parietal, and cingulate cortex (Fair et al., 2008). Developmental trajectories may be altered in diseased brains (e.g., Church et al., 2009), and functional and structural differences in connectivity may reflect individual differences in cognitive abilities (e.g., Niogi and McCandliss, 2006; Seeley et al., 2007; also see reviews this issue).

These findings emphasize the importance of understanding the development of associative neural circuits. Going forward, a key challenge will be to gain an understanding of what these circuits do during development at multiple levels of analysis, from cellular mechanism to cognitive function. Currently, the cellular and synaptic basis of changes in functional connectivity and DTI imaging remain unclear. Are these changes due to myelination, novel growth, elaboration or pruning of new connections? What happens when development is altered or connections are silenced?

Understanding the mechanistic basis of connectivity changes in humans, and how these changes relate to changes in behavior, is challenging and may benefit greatly from animal models. We propose that an emerging focus on broad-scale neural circuits provides a unique opportunity for collaborative studies that bridge research in mice and humans. New research methods and technology targeting neural circuits in both human and mouse neuroscience labs have great potential for enhancing overlap and collaboration between these two scientific cultures.

Furthermore, many of the cognitive paradigms in humans draw from, or have parallels in, the animal conditioning literature, such as reward prediction, reversal learning, relational memory, rule extraction, and set shifting. This overlap in behavioral paradigms and cognitive domains suggests the promise of integrating a circuit-level understanding of cognitive development across species. To facilitate such collaborations, there is a need for researchers to communicate across technical and cultural boundaries. Communication and education in the research possibilities available to each subfield will also facilitate the opportunity for researchers on both sides to make explicit predictions that can be tested in the most appropriate species, advancing research progress on common questions.

WHAT MOUSE MODELS CAN OFFER DEVELOPMENTAL COGNITIVE NEUROSCIENCE

Multimodal association areas are thought to support cognitive development and learning across mammalian species. Many of the same key cognitive regions of the brain (e.g. parietal and frontal cortex, basal ganglia, amygdala, and the hippocampus) can be found in both humans and mice and these broadly defined regions are connected in comparable circuits. For example, mice have cortical-basal ganglia loops and have parietal-frontal cortex and amygdala-frontal cortex connections. Elaboration and specialization of associative regions has likely occurred with evolution and growth in brain size, but the common genetic and anatomical architecture of the mammalian brain suggests similar rules may govern the development of basic associative brain circuits in mice and men alike.

Mice are particularly advantageous for study because of their relatively short development (puberty begins about 30 days of age, with adulthood at about 60 days), and their long history as a genetically tractable species, where increasingly specific identified populations of neurons can be genetically altered. Studies of the connectivity between association areas can be performed in mice with greater resolution to answer questions about the mechanisms regulating developmental circuit changes.

Thus, common circuit architecture in mice and humans offers the opportunity to perform controlled environmental, genetic, and behavioral experiments during development and adulthood. Such studies have enormous value, despite the obvious gaps in cognitive abilities between the species.

TECHNOLOGIES FOR STUDYING CIRCUITS IN MICE WITH HIGH RESOLUTION

New technologies have recently enhanced the study of neural circuits in mice, with important implications for understanding brain circuits underlying human cognitive development:

IMAGING PLASTICITY AND ACTIVITY WITH CELLULAR AND SYNAPTIC RESOLUTION: 2PLSM

Two photon laser scanning microscopy (2PLSM) through a thin skull or cranial window allows time- lapse imaging of dendrites, spines and axonal and boutons in developing and adult mice (Holtmaat et al., 2009). Chronic preparations allow longitudinal study of developmental or experience-dependent process or the time scales of hours to several months. Imaging studies to date have revealed spine and bouton loss and gain, and reorganization of axonal arbors in the living brain. Similar imaging techniques can also be used to monitor cellular activity using calcium sensitive indicators (Stosiek et al., 2003; Dombeck et al., 2007). These techniques can become particularly powerful as identified cell types or cells with known afferents or efferents can be identified via fluorescent genetic labeling strategies.

MAPPING LONG-RANGE CONNECTIONS BETWEEN SPECIFIC NEURON TYPES: CRACM

Light can also be used to isolate and measure the function of long-range connections between identified neurons. Channel Rhodopsin Assisted Circuit Mapping (CRACM) (Petreanu et al., 2007) uses ion channels "borrowed" from light sensitive bacteria to stimulate activity in channel expressing neurons (Boyden et al., 2005). Genetic and viral tricks allow light sensitive ion channels to be delivered to the membranes of specific cells or regions of interest, loading even long-range axons that traverse large portions of the brain (Petreanu et al., 2007). Expression of the channel in cells of interest enables isolated stimulation of cells or even severed axon terminals of interest (without stimulation of neighboring cells) by easily delivered remote flashes of blue light. *Ex vivo* slice patch clamp recording of cells in a specific location, or cells labeled with genetic tools, enables measurement of long-range afferent synapses made between specific cell types.

MAPPING LOCAL CIRCUIT CONNECTIVITY BETWEEN SPECIFIC NEURON TYPES: LSPS

Local circuit connectivity can also be measured using light. Laser scanning photostimulation (LSPS) uses light to uncage neurotransmitters in a defined region (Katz and Dalva, 1994). Light based uncaging produces focused concentration of transmitter, which can be used to cause action potentials at soma near the uncaging beam focus. With patch clamp recording of a single neuron and controlled scanning of an uncaging beam to distributed points across a brain slice it is possible to map local connections between a patched cell and its neighbors.

2PLSM, CRACM, and LSPS techniques have first been applied to sensory cortices in mice and are now beginning to be applied to motor areas (Zuo et al., 2005; Yu et al., 2008; Xu et al., 2009). An important next step is to apply these technologies to multimodal association areas and the development of circuits that connect them. Studies of association area connectivity in mice should be of great interest to developmental cognitive neuroscience. For example, changes in the "default state network" that occur with development or disease could be modeled by looking at changes in parallel areas in mice.

Some questions that can be answered with mouse experiments:

- Which synapses are generally pruned with development? Which are gained?
- How are the connections between brain regions (long range and short range) altered by the maturation of inhibition and the connectivity of local cortical circuits?
- Which cell types within these regions demonstrate the most radical developmental changes? What modulates these changes? Which genes are unique to these cell types?
- How do genetic differences and/or experience alter developmental circuit changes in a controlled environment?
- How do cellular level changes in circuits correlate with changes in behavior?

HOW CAN CONVERGING STUDIES OF CIRCUIT DEVELOPMENT ALSO ADVANCE OUR UNDERSTANDING OF COGNITIVE DEVELOPMENT?

To maximize the knowledge gained from comparative study of mice and humans it is important to take into account species differences in evolution and behavior and select the most auspicious cognitive comparisons. Many would agree that emotional and motivational behaviors supported by limbic, basal ganglia, and midbrain structures are readily comparable between species. Cortex-based cognition in mice and humans may differ both qualitatively and quantitatively, yet the sophistication of mouse cognition should not be underestimated. Mice are capable of rapid associative learning and reversal of learned associations (within a single training session) on tasks designed to approximate tests for humans with frontal lobe damage (Bissonette et al., 2008). The role of learning, memory and reward in decision making has also been found to be highly nuanced in studies of rodents and may be supported by distinct subcircuits that are parallel to those found in humans (E.g. Dusek and Eichenbaum, 1997; Shohamy and Wagner, 2008; see Eichenbaum and Cohen, 2001; Yin and Knowlton, 2006; Schoenbaum et al., 2009 for reviews).

When making cross species comparisons it should be noted mice tend to learn cognitive tasks using olfactory cues more efficiently, but they can also discriminate tactile, aural, and visual cues. The complexity of human social systems and the lack of pheromone-related circuits in humans may make comparison of social cognition between species more difficult. Although behavior may not align perfectly in mice and men, with careful selection of cognitive measures with regard to ethological caveats we can enhance our understanding of the basic function of relays between associative regions.

Studies of mouse association circuits can also provide a bridge between genetics and behavior. Studies that attempt to link human genetic variation to disease have not been as clear as once hoped (Goldstein, 2009), and this is especially true for psychiatric disease. A search for common biological pathways and cellular and circuit endophenotypes that link rare genetic variants may be more successful (Hirschhorn, 2009). The effect of human genetic variants on neural circuit development and plasticity may be readily tested in mice at both the circuit and behavioral level. Furthermore, imaging in awake mice and repeat longitudinal imaging allows for greater possibility and power in assessing correlations between developmental cognitive changes, cellular level circuit measures, and genetic differences.

Studies of whole brains or multiple brain regions and correlations between them can be performed most efficiently in human studies. These studies, along with genetic data, can then be used to pinpoint circuits and cells for further study with higher resolution in mice.

Studies of the brain are revealing that specialized knowledge is most powerful when shared across networks. Ideally, neuroscientists studying humans and mice can similarly work and move forward together.

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Applications of multivariate pattern classification analyses in developmental neuroimaging of healthy and clinical populations

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Analyses of functional and structural imaging data typically involve testing hypotheses at each voxel in the brain. However, it is often the case that distributed spatial patterns may be a more appropriate metric for discriminating between conditions or groups. Multivariate pattern analysis has been gaining traction in neuroimaging of adult healthy and clinical populations; studies have shown that information present in neuroimaging data can be used to decode intentions and perceptual states, as well as discriminate between healthy and diseased brains. While few studies to date have applied these methods in pediatric populations, in this review we discuss exciting potential applications for studying both healthy, and aberrant, brain development. We include an overview of methods and discussion of challenges and limitations.

Keywords: multivariate pattern classification, development, MRI, fMRI, clinical

INTRODUCTION

The human brain is a distributed processing machine, with even the most basic tasks requiring the cooperation of neurons in multiple brain regions. It has long been recognized that important information about brain function is encoded in distributed patterns of brain activity (Mesulam, 1981; Vaadia et al., 1995; McIntosh et al., 1996; Fox et al., 2005). Nonetheless, univariate analysis methods, which treat each spatial location in the brain independently, have predominated in both functional and structural magnetic resonance imaging (fMRI and sMRI) research (Cox, 1996; Worsley et al., 1996; Ashburner and Friston, 2000; Smith et al., 2004).

In recent years, multivariate pattern analysis (MVPA) approaches to studying the brain have been gaining momentum (e.g., Kay et al., 2008; Mitchell et al., 2008). These methods are designed to identify spatial and/or temporal patterns in the data that differentiate between cognitive tasks or subject groups. The excitement about these methods has been borne out by several studies showing that patterns of brain activity carry information that reliably predicts what the subject is seeing (Eger et al., 2008), attending to (Kamitani and Tong, 2005), or planning to do (Bode and Haynes, 2009). In addition, by quantifying distributed neural representations for specific components of visual images or semantic concepts, it is possible to construct a general visual decoder (Kay et al., 2008), reconstruct a subjects' perceptual experience, even for novel stimuli (Miyawaki et al., 2008), and make quantitative predictions about how the brain will respond to new stimuli (Mitchell et al., 2008).

Neuroimaging studies of the developing brain have found that activity in specific regions differs between children and adults (Casey et al., 1997; Thomas et al., 2001; Bunge et al., 2002). However, as the brain matures, many regions undergo structural changes (Reiss et al., 1996; Gogtay et al., 2004), and cognitive maturation likely occurs as a result of distributed changes in the brain. Indeed, recent studies employing network analysis methods have found that the configuration of brain networks also changes over the course of development (Fair et al., 2009; Supekar et al., 2009), suggesting that studying development in terms of changing distributed patterns of structure or activity could be more sensitive than studying changes in individual regions.

Furthermore, an important challenge in pediatric clinical neuroimaging is identifying patterns of brain activity or structure that reliably predict disease onset (Koutsouleris et al., 2009), or can distinguish treatment responders from non-responders. Multivariate methods have particular relevance for research problems related to prediction of clinical outcomes from neuroimaging data, as they may afford greater sensitivity to widespread changes in the brain (Davatzikos et al., 2008; Koutsouleris et al., 2009; Misra et al., 2009), or differences in local information coding (Kriegeskorte et al., 2006).

Several excellent reviews have discussed the application of multivariate pattern classification to fMRI data, with a focus on decoding of subjective states (Haynes and Rees, 2006; O'Toole et al., 2007; Pereira et al., 2009). There is also a growing literature on classifying group differences, which is of particular interest for clinical investigations. While few studies to date have applied

MVPA in pediatric neuroimaging (Hoeft et al., 2007; Hoeft et al., 2008; Zhu et al., 2008), there are many promising applications in this field. Here we review relevant literature on studies in adult healthy and clinical populations, including functional, structural, and multimodal studies, and provide an outlook for future studies in pediatric neuroimaging. We will begin with a brief overview and motivation for using MVPA, followed by an introduction to methods and choices to be made when implementing MVPA. Next, we will discuss applications in the literature with an emphasis on those relevant to pediatric neuroimaging in healthy and clinical populations. Finally, we discuss some specific challenges and limitations of MVPA methods.

CONCEPTUAL OVERVIEW AND MOTIVATION

fMRI studies generate on the order of 100,000 time series from voxel locations throughout the brain. These time series, especially from proximal locations, are not independent, and yet mass univariate analysis methods, which treat each voxel independently, are the most widely used in the neuroimaging literature [general linear model (GLM); Friston et al., 1995a; Worsley et al., 2002]. These methods allow the researcher to specifically model the expected neural response over time, and are useful for testing detailed hypotheses about the involvement of specific regions in task performance. However, brain activity, i.e., neuronal firing, is in itself just a means of communicating with other neurons, and it is clear that even the simplest cognitive tasks are not accomplished solely by the neurons contained in individual voxels. This suggests that analysis techniques designed to learn distributed spatial patterns that best distinguish one condition from another (Kriegeskorte et al., 2006; De Martino et al., 2008; Pereira et al., 2009) may be more sensitive than univariate techniques. MVPA can be applied to any multivariate data, including fMRI and sMRI.

MVPA methods are sensitive to spatially covarying patterns of activity, and are therefore intrinsically linked to functional connectivity analyses (McIntosh et al., 1994; McIntosh et al., 1996; Friston et al., 1997; Calhoun et al., 2001; Friston et al., 2003), which seek to uncover functional networks in the brain. As such, depending on the spatial scale on which a particular analysis is performed, one might interpret a pattern of activity as representing a spatially distributed network of brain regions. MVPA techniques are also frequently used at a finer spatial scale to uncover how information is encoded within a particular region (Kamitani and Tong, 2005; Kriegeskorte et al., 2006; Kay et al., 2008). By far, the most popular application in the functional imaging literature has been to study object representation and visual consciousness (Haxby et al., 2001; Carlson et al., 2003; Cox and Savoy, 2003; Hanson et al., 2004; Polyn et al., 2005; De Martino et al., 2008; Eger et al., 2008; Kay et al., 2008).

Demonstrating that fMRI data contains enough information to distinguish between a restricted set of stimulus conditions may seem like merely a validation that fMRI is robust and repeatable (Bandettini, 2009). However, with the right experimental design, MVPA studies can provide detailed insight into information representation and functional organization in the brain. For example, studies which map neural responses to the building blocks of visual objects or semantic concepts (Kay et al., 2008; Mitchell et al., 2008; Miyawaki et al., 2008) lead us to a better understanding of the brain's responses to more complex stimuli. In the clinical domain, mapping gross differences in structure or brain activity may be relatively straightforward, but developing tasks that elucidate how these differences in distributed processing affect behavior will be a greater challenge.

DEVELOPMENTAL AND CLINICAL NEUROIMAGING

Widespread structural changes in the brain occur as a function of development in childhood (Reiss et al., 1996). While many studies have successfully identified local changes in brain activity (Casey et al., 1997; Bunge et al., 2002), a complementary approach is to study how networks of regions change as the brain develops (e.g., Fair et al., 2009; Supekar et al., 2009); see Stevens (2009) for a review. For studying the evolution of distributed activity patterns, MVPA may be more sensitive than univariate methods and could be applied to distinguish individuals at different stages of cognitive development, as they have similarly been applied to distinguish different stages of cognitive decline (Fan et al., 2008a).

In clinical populations, MVPA techniques are of particular interest for studying the development of brain disorders, as studies in adults have shown that these disorders rarely affect single brain structures (e.g., Harrison, 1999; Lee et al., 2007), and may be better understood using an approach designed to capture network behavior (Greicius and Menon, 2004; Celone et al., 2006; Garrity et al., 2007). As many psychiatric diagnoses are based on behavioral symptoms that overlap between disorders and have poorly understood etiology, the promise of MVPA methods for uncovering biomarkers (endophenotypes) is extremely attractive [e.g., in psychosis or Alzheimer's (AZ), Koutsouleris et al., 2009; Misra et al., 2009]. An important challenge in pediatric clinical neuroimaging is identifying patterns of brain activity or structure that reliably precede disease onset (Koutsouleris et al., 2009), or can distinguish treatment responders from non-responders.

METHODS

UNIVARIATE VERSUS MULTIVARIATE

Analysis of both fMRI and sMRI data has traditionally been performed in a univariate sense. That is, the time series of each voxel in the brain is separately modeled and statistically tested for a condition of interest, usually in the framework of the GLM (Friston et al., 1995b; Worsley et al., 2002). Thus, questions regarding how blood oxygen level dependent (BOLD) signal activity relates to a continuous outcome measure (e.g., developmental stage), or differs across populations (e.g., in patients with distinct neurological diseases), are addressed using information from each region of the brain separately.

In contrast, MVPA of neuroimaging data simultaneously considers the BOLD signal measured across a number of spatial locations. Rather than asking to what degree each voxel responds to one experimental condition versus another, MVPA turns the question around, asking instead whether – and which – patterns of brain activity across many voxels are characteristic of the brain during one experimental condition versus another, or of one clinical population versus another. In addition, many MVPA approaches do not require the specification of a hemodynamic model, which may differ considerably across subject populations (Handwerker et al., 2004; Thomason et al., 2005; Handwerker et al., 2007). Combining information from multiple spatial locations yields a descriptive power beyond that of single voxels, potentially allowing for greater sensitivity in differentiating between individuals and conditions.

PROBLEM SPECIFICATION

For neuroimaging studies, MVPA has been employed primarily in two ways. The first aims to infer the mental state of a subject (i.e., what the subject is perceiving, feeling, or thinking) or the stimulus or experimental condition presented at a particular time, based on information from the BOLD fMRI volumes acquired at that time (see Haynes and Rees, 2006 for a review, and "'Brain reading' with fMRI" below for further discussion).

The second major application, which is perhaps more relevant to clinical studies, is to predict the state of an individual given his/her functional or structural imaging data. For example, Zhang et al. (2005) were able to differentiate between the brains of subjects with and without drug addictions; Zhu et al. (2008) applied classification to discriminate between children with attention-deficit/hyperactivity disorder and normal controls. Applying MVPA in such contexts can yield insight into the neural signatures or determinants of various diseases, as well as guide the identification of biomarkers and courses of treatment or intervention. A general pipeline for applying MVPA is shown in **Figure 1**.

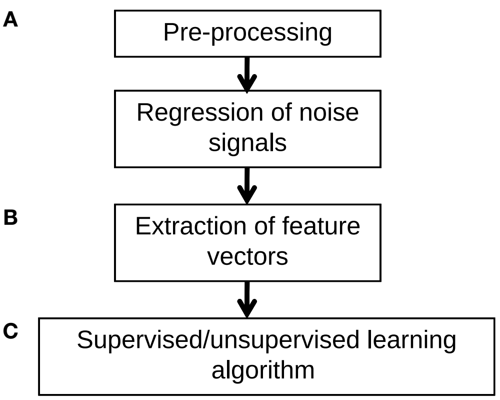

FIGURE 1 | Example workflow for pattern classification analysis. **(A)** Preprocessing of fMRI data can follow that used for conventional GLM analyses, consisting of slice-timing correction, realignment, and optional spatial normalization and smoothing steps. However, while the GLM can reduce potential noise sources by including signals such as motion parameters and physiological noise models as covariates in the GLM design matrix, MVPA does not typically have a framework for modeling confounds. Thus, when applying MVPA to the raw time-series, expected confounds should be removed from the data prior to performing MVPA, such as by obtaining the residuals from a regression over the nuisance variables. In addition, if the features are activation patterns (parameter estimates for a particular model), one may wish to first run a GLM analysis on the fMRI data and extract the relevant contrast estimates. **(B)** Next, the pre-processed data should be transformed into "feature vectors". This involves creating, for each data point (e.g., subject) a vector in which the *i*th entry corresponds to the value of the *i*th feature for that data point. Feature selection may be applied to reduce the number of entries in the feature vectors. **(C)** At this stage, one may choose to run an unsupervised learning algorithm to characterize patterns in the data, or to run a supervised learning algorithm in conjunction with cross-validation. The parameters of the trained model can be examined for further insight, and the model may also be applied to make predictions on additional datasets so as to further explore its generalization ability.

APPROACHES

Supervised learning

Supervised learning is based on determining a mapping between particular attributes, or *features*, of the data (e.g., the BOLD signal activation levels from multiple voxels observed in a subject) and the associated value of a *target label* of interest (e.g., the subject's cognitive or developmental state, or class of psychiatric disorder). A set of data points (the *training set*) is used to estimate (*learn*) the parameters of a model relating the features to the target labels. Once the parameters are learned, the model can be applied to predict the target label of a previously unseen data point. The supervised learning problem is referred to as *classification* when the target labels comprise a set of discrete classes (e.g., different clinical populations), and as *regression* when the target labels assume continuous values (e.g., behavioral performance or the severity of a disorder).

Classification. There are a number of different classification methods, each of which makes a different set of assumptions about the data and posits a particular type of model relating the features to the target labels, as well as a means of learning its parameters. *Linear classifiers* (**Figure 2**) predict the target label of an example based on a linear combination of its features, and are widely used in fMRI studies due to their simplicity, interpretability, and generally good performance.

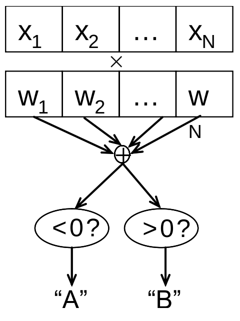

FIGURE 2 | Schematic of linear classification. Each feature (x_i) of the data point ($x_1,\ldots,x_N$) is multiplied by its respective weight (w_i), and the summation of the resulting terms ($\hat{y} = \sum_i x_i \cdot w_i$) is evaluated. The classifier predicts that the data point is in "class A" if $\hat{y} < 0$, and "class B" if $\hat{y} > 0$.

As an example of linear classification, suppose the features ($x_1,\ldots,x_N$) of an fMRI dataset are defined to be the average BOLD signal percent change in each of N regions during a task performed by subjects from two different clinical populations ("A" an "B"). Given the values of ($x_1,\ldots,x_N$) from a subject, the linear classifier might predict that he/she belonged to group "A" if the linear combination $w_0 + w_1x_1 + \ldots + w_Nx_N$ is <0, and "B" if the linear combination is >0 (see **Figure 2**). The weights ($w_0,\ldots,w_N$) are the parameters of the model, which are learned during training. After the training process, the weights can be examined for the specific patterns of brain activity related to each clinical condition. For instance, the relative magnitudes of the individual weights can reveal the relative importance of each of the features in the classification process (a higher magnitude of w_i, compared to w_j, indicates that feature x_i is weighted more heavily in determining the output than is x_j), and the relative signs of the weights w_i and w_j can reveal whether features x_i and x_j are indicative of the same class (which would be the case if the signs of w_i and w_j were the same) or different classes.

Several commonly used linear classifiers in neuroimaging include the linear support vector machine (SVM) (e.g., Kamitani and Tong, 2005; LaConte et al., 2005; Mourao-Miranda et al., 2005), linear discriminant analysis (LDA) (e.g., Haynes and Rees, 2005; O'Toole et al., 2005), and logistic regression (LR) (e.g., Knutson et al., 2007; Yamashita et al., 2008). There is no clearly "correct" choice of classifier for a given problem. Studies comparing different classifiers for fMRI data have shown that SVM outperforms LDA for particular fMRI data sets and feature selection methods (Cox and Savoy, 2003; Mourao-Miranda et al., 2005; Bergstrand et al., 2009). LR and SVM are reported to have comparable performance (Pereira et al., 2009), though SVMs can more efficiently handle high-dimensional feature spaces (Vapnik, 1996). Classifiers that use nonlinear functions of the features (*nonlinear classifiers*) also exist, though they do not always outperform linear classifiers in practice (Cox and Savoy, 2003), and the parameters of a nonlinear model are more difficult to interpret than the weights of a linear classifier.

Regression. For problems in which the target labels assume continuous values, *linear regression* models the target label as a linear combination of the features: the prediction ($\hat{y}$) of the actual target label (y) is given by $\hat{y} = w_0 + w_1 x_1 + \ldots + w_N x_N$. There are a variety of linear regression algorithms, differing from one another primarily according to the criterion for selecting the parameters [weights ($w_0,\ldots,w_N$)]. In standard least-squares regression, the weights are chosen to minimize the squared error between the prediction and the actual target label, summed over each of the M data points in the training set. This function ($J = \sum_{n=1}^{M} (y_n - \hat{y}_n)^2$) is known as the *cost function*.

In *ridge regression*, the weights are chosen to minimize a slightly different cost function, which contains both the squared error and the amplitude of the weights: $J = \sum_{n=1}^{M} (y_n - \hat{y}_n)^2 + \lambda \sum_{i=0}^{N} w_i^2$, where λ is an additional parameter that controls their relative importance. Including the amplitude term tends to improve the performance of the regression algorithm on data that were not used in the training process, as it reduces the likelihood that the solution (w) *overfits* to the training set (Hastie et al., 2001). Other types of objective functions may produce solutions in which many of the w_i are equal to 0, effectively reducing the number of features used in the model; this will be described further in Dimensionality reduction and feature selection. For clinical studies, regression may be applied toward mapping patterns of brain activity onto continuous outcome measures, such as the progression or severity of a disorder. For example, Hoeft et al. (2007) combined behavioral, functional, and structural neuroimaging data in a regression analysis to predict phonological processing abilities of 8- to 11-year-old poor readers one school-year later.

Partial least squares (PLS) is a regression approach that can identify multivariate patterns within neuroimaging data that covary with experimental condition (McIntosh et al., 1994; McIntosh et al., 1996). Here, the target labels (as well as the features) are multi-dimensional, comprising for instance a variety of clinical outcome measures or fMRI task design parameters. PLS will return a set of spatiotemporal components in the imaging data that explain joint variation between the imaging data and target labels. Clinical applications of PLS have included identifying large-scale brain networks that mediate genetic risk for obsessive–compulsive disorder (Menzies et al., 2007) and predicting the behavioral effects of nicotine use (Giessing et al., 2007).

Unsupervised Learning

Supervised learning requires the user to specify both the features and target label of each example in the training set. In contrast, *unsupervised learning* requires only the features (no labels), and attempts to learn patterns and structure in the data.

Clustering is one form of unsupervised learning, which identifies groups within the data based on the similarity of their features. *K-means clustering* assigns each data point to one of *k* groups, and has been applied to discover relationships among the time series of voxels in fMRI data (Golland et al., 2008; Mezer et al., 2009). *Hierarchical clustering* methods build a succession of clusters: datapoints are first grouped into clusters, and the clusters themselves are merged into groups at a second level according to their similarity, and so forth, building a tree depicting the hierarchical dependence structure across data points (Cordes et al., 2002; Liao et al., 2008). Thus, while k-means clustering is informative of major subdivisions in the data, hierarchical clustering provides a more complete characterization of relationships between data points and may be used to identify more subtle patterns. However, some of the relationships discovered by hierarchical clustering may be driven by attributes specific to the dataset to which it is applied, and may not generalize across other datasets. Thus, one must take care not to "overfit" to a given dataset, and may wish to employ methods such as those used in Talavera (1999).

Independent component analysis (ICA) is another popular form of unsupervised learning. ICA can be applied to decompose a set of fMRI time courses into a set of spatially distinct "networks". ICA has provided insight into the functional organization of the brain (McKeown et al., 1998; Beckmann et al., 2005) and suggested key functional differences across clinical populations (Greicius et al., 2004; Calhoun et al., 2008; Rombouts et al., 2009). In addition to exploring the spatiotemporal structure of brain activity within individuals and groups, the spatial networks and time courses derived using ICA can also be used to derive features within the framework of supervised learning (De Martino et al., 2007; Calhoun et al., 2008). For example, Calhoun et al. (2008) applied ICA to the data of bipolar and schizophrenic patients, extracting two networks from each subject ("default-mode" and "temporal lobe"). The networks were then used as input to a classification algorithm, which demonstrated high accuracy in classifying between the patient groups.

VALIDATION

As described above, an MVPA model can yield useful information about the properties and structure of the dataset it is trained on. However, for MVPA to be applicable for drawing general conclusions relating brain activity patterns and clinical variables, it is important to know how well the model applies to subjects and scans beyond those in the training set. One method for estimating the generalization ability of a supervised learning model is via *cross-validation*. In *hold-out* cross-validation, a subset of the data (*training set*) is used for learning the model parameters, and the remainder (*test set*) is used for validation. In validation, the trained model is applied to predict the target label of each data point, and the prediction accuracy across the test set is summarized using metrics such as the percentage of correctly classified data points (for classification) or mean-squared error (for regression). A cross-validation method that makes more efficient use of the data is known as *k-fold cross-validation*. Here, the N data points comprising the entire dataset are randomly partitioned into k subsets; k iterations are then performed, whereupon one of the subsets is designated as the test set, and the remaining $N–k$ data points are designated as the training set. The prediction accuracy is finally summarized as the average of the error over each of the k iterations. When the number of available data points is small, as is often the case with fMRI subject populations, one might choose to set $k = 1$ (*leave-one-out cross-validation*), though the computational expense will increase since $(N - 1)$ iterations of training are then required. Permutation testing can be used to determine whether the classification accuracy is statistically significant (significantly better than chance) (e.g., see Golland and Fischl, 2003; Pereira et al., 2009).

In addition to accuracy (the fraction of correctly classified data points in the test set), the performance of a classifier can be quantified using *sensitivity* [TP/(TP + TN)] and *specificity* [TN/(TN + FP)], where TP is the number of true positives, TN is the number of true negatives, and FP is the number of false positives.

DIMENSIONALITY REDUCTION AND FEATURE SELECTION

Suppose, as in the example in the "Unsupervised learning" section, that one wishes to apply a classifier to differentiate between two clinical populations on the basis of BOLD signal activity across many voxels during a task. How many voxels should be used as features? If every voxel in the brain is used, computation time may increase prohibitively. In addition, when the number of features greatly exceeds the number of data points in the training set, the performance of classification and regression algorithms will suffer and the model will become less interpretable (Hastie et al., 2001).

It is usually the case that only a subset of voxels will actually provide useful information for classifying between populations. Reducing the set of all possible features to those of likely importance is a problem known as *feature selection* (**Figure 1B**). Feature selection can be performed manually – for example, we may know based on previous studies or complementary functional and structural analyses that only a few particular anatomic regions are hypothesized to differ between the two clinical populations, and can therefore choose to include only voxels contained within those structures. There are also methods for automatic feature selection. Examples include (1) recursive feature elimination, which is based on iteratively eliminating features having smallest SVM weights (De Martino et al., 2008); and (2) "sparse" methods, which are classification and regression algorithms that implicitly select a subset of all input features to use in the model (Grosenick et al., 2008; Yamashita et al., 2008; Carroll et al., 2009). Automatic feature selection methods are also useful in revealing which aspects of the data are critical for a given classification problem.

Another approach for reducing the dimensionality of a dataset is to apply principal component analysis either in lieu of, or in conjunction with, feature selection methods. A data point in a high-dimensional feature space (e.g., the set of all voxels in a subject's brain) is reduced to a smaller number of features, resulting from the projection of that data point onto a set of axes (principal components) that express the greatest directions of variability in the dataset.

APPLICATIONS

"BRAIN READING" WITH FMRI

Typical functional imaging studies compare brain activity during different experimental conditions to discover what brain regions are activated by particular tasks. In contrast, the classic application of MVPA to functional imaging data is for so-called "brain reading", that is, using patterns of brain activity to perform a reverse inference and decide what subjects are looking at or thinking about (Cox and Savoy, 2003).

This technique has grown in popularity, and has successfully been applied to a variety of paradigms, including discriminating between object categories (Haxby et al., 2001; Carlson et al., 2003; Hanson et al., 2004), visually presented and attended stimuli (Kamitani and Tong, 2005), remembered stimuli (Polyn et al., 2005), intention to engage in a task (Haynes et al., 2007), and deception (Davatzikos et al., 2005).

As the field progresses, experimenters are designing tasks for which classifiers can shed new light on how information is represented in the brain. In the domain of object/concept representation, three recent studies have shown that by training classifiers to represent meaningful aspects of the stimuli (e.g., visual properties or semantic content), one can decode from a large set of novel images (Kay et al., 2008), accurately reconstruct novel stimuli that subjects have never seen (Miyawaki et al., 2008), and make quantitative predictions about what responses to novel stimuli should look like (Mitchell et al., 2008).

By interrogating the patterns of activity that classifiers rely on to make inferences, we can gain information about which voxels in the brain are the most informative. Also, by applying classifiers trained in one domain to stimuli in a different domain, we can test hypotheses regarding the functional overlap of neural circuitry. For example, in a recent study Knops et al. (2009) trained a classifier to discriminate between left and right saccades, based on posterior parietal activity during a spatial attention task. In order to test the hypothesis that mental arithmetic uses circuitry involved in spatial coding, the same classifier was used to decode addition or subtraction operations. Interestingly the classifier identified significantly more addition operations as rightward saccades, compared to subtraction, indicating that mental arithmetic engages spatial coding circuitry in the parietal cortex.

Several groups have applied MVPA to detect neural representations related to behavioral variability, taking advantage of the fact that MVPA can be sensitive to information coded by different sub-populations within a specific region. Li et al. (2009) demonstrated that as subjects learn to categorize identical stimuli using different rules, patterns of neural activity in specific brain regions reflect the categorical decision, rather than the stimulus features. Raizada et al. (2009) have also shown that in individuals with differential ability to discriminate/ra/and/la/phonemes (e.g., native English and Japanese speakers), the amount to which a subject distinguishes between two stimuli behaviorally is related to the statistical separability of activity patterns in auditory cortex. This approach may be useful for understanding behavioral differences in a wide range of paradigms, including understanding how behavioral impairments in children relate to differential stimulus coding in the brain.

Decoding subjective experience can also contribute to our understanding of functional differences in brain disorders. Yoon et al. (2008) demonstrated that multivariate techniques may be more sensitive to differences between patients and controls by qualitatively comparing the performance of a neural-network classifier to a GLM-based analysis. They trained a classifier to distinguish between categories of visually presented objects during a 1-back memory task. Classification was significantly more accurate in controls compared to patients; however, the GLM did not show any significant differences between the groups. Note that in this study the classifier did not explicitly distinguish between patients and controls, but rather showed that spatial response patterns were less consistent in the patient group relative to controls.

CLASSIFYING BRAIN DISORDERS

Applying pattern classification to study clinical disorders is attractive for several reasons. One reason is that many brain disorders affect networks of brain regions, and therefore analysis techniques designed to extract distributed spatial patterns may be more sensitive than more traditional mass univariate techniques. Following similar reasoning, MVPA techniques applied to longitudinal data may be useful for extracting patterns of activity or structural abnormalities that are predictive of abnormal cognitive development. Finally, as many psychiatric disorders are diagnosed based on behavioral criteria, MVPA techniques may be useful for identifying endophenotypes associated with disease, and ultimately could be used for biologically based diagnoses.

Functional imaging

Clinical populations often show deficits on specific cognitive tasks, which may provide clues about the underlying etiology of the disease. For example, schizophrenia patients often show a significant difference in the amplitude of the event-related potential related to target detection (P300). Functional tasks, such as auditory target detection, may serve as good starting points for examining how distributed patterns of brain activity are different in patient populations. Studies using univariate analyses of this task have shown that schizophrenia patients show hypoactivition in several cortical and subcortical regions during target detection, relative to healthy controls (Kiehl et al., 2005). Multivariate techniques have extended this work by identifying networks of regions that vary together, suggesting abnormal auditory and executive function networks in schizophrenia (Kim et al., 2009).

MVPA techniques are proving useful for studying the neuropathology of AZ disease. Celone et al. (2006) performed ICA on memory related fMRI activity in AZ and mild cognitive impairment (MCI) patients, as well as age-matched healthy controls. They found distributed networks involved in memory activity, including hippocampal activation accompanied by parietal deactivation, and moreover found a nonlinear trajectory in fMRI network activation across the continuum of impairment.

As in the above examples, functional data used for pattern classification may come from tasks in which different subject populations show known behavioral differences. Another approach is to use fMRI collected while subjects are at rest, not performing any explicit task. Resting state BOLD signal fluctuations are thought to reflect internalized thought processes, and have been shown to exhibit consistent patterns across individuals (Damoiseaux et al., 2006). Multivariate techniques have been applied to resting state data to identify regions whose activity varies together over time. Many studies have examined differences in resting state data between patient populations and controls. Differences in the default mode have been identified in AZ (Greicius et al., 2004; Wang et al., 2006), depression (Greicius et al., 2007), schizophrenia (Garrity et al., 2007), and ADHD (Zhu et al., 2005).

In pattern classification analyses, one can also combine data from more than one source. Calhoun et al. (2008) trained a classifier to discriminate between schizophrenic and bipolar patients using both task-related and resting-state (default mode) activity. Bipolar and schizophrenic patients share overlapping symptoms, motivating the need for reliable brain-based classifiers for assisting in diagnosis. Given that the subjects identified as bipolar or schizophrenic in the study by Calhoun et al. (2008) were triaged based on behavioral criteria, it would be interesting to see how unsupervised approaches would group the subjects, and whether the resulting patterns would resemble those resulting from the supervised classification of behaviorally labeled patient data. Unsupervised methods are designed to uncover structure present in the data without any user-provided labels, and in the future could be used to inform diagnostic criteria for disease (Hrdlicka et al., 2005).

Structural imaging

Brain structure has been found to reflect life experience (Bengtsson et al., 2005), correlate with personality traits (Cohen et al., 2009), and relate to psychiatric diagnosis (Lawrie and Abukmeil, 1998). Multivariate techniques are also relevant for analyses of structural data, as they may be more sensitive to distributed patterns of structural change.

Multivariate methods have identified supra-regional fronto-temporal abnormalities in schizophrenia (Wright et al., 1999), in addition to global changes. MVPA has also shown high accuracy at diagnosing AZ disease, and in fact the spatial patterns were robust enough that a classifier trained on one scanner could accurately diagnose based on scans collected at another center (Kloppel et al., 2008).

MVPA techniques may be a useful for predicting disease susceptibility and progression. Studies using pattern classification on AZ patients and MCI patients have found that some MCI patients show greater structural similarities to the AZ patients, while others were more similar to healthy controls (Fan et al., 2008a; Misra et al., 2009). Interestingly, similarities to the AZ group patterns were predictive of subsequent cognitive decline (Misra et al., 2009), indicating that structural brain scans could play a role in treatment decisions.

The onset of psychosis is often preceded by what is referred to as an at risk mental state (ARMS), during which patients show the first symptoms of disorder. However, as symptoms are heterogeneous and not 100% predictive of future psychosis, the discovery of biological markers for disease onset would greatly help with early intervention. To this end, Koutsouleris et al. (2009) trained a classifier to recognize structural scans belonging to healthy controls compared to ARMS patients. They then followed up with these patients 4 years later, and trained a second classifier on the original scan data, labeling the scans based on whether the patient did or did not transition to disease. They were able to distinguish with 82% accuracy those who would transition to disease based on abnormal structural patterns present before the onset of psychosis. This study could have important implications for deciding treatment course in individuals who present with ARMS, and suggests that other disorders may also benefit from similar research into functional or structural patterns in individuals at risk. In general, the ability to correlate patterns with future outcomes is very interesting, and can be facilitated by the assembly of longitudinal databases [e.g., ADNI[1] and the NIH Study of Normal Brain Development[2]].

Unsupervised learning techniques may also be a powerful method for defining subtypes within heterogeneous spectrum disorders such as autism. For example, Hrdlicka et al. (2005) used a clustering algorithm on MRIs from 64 subjects with autism. In this study, experienced neuroradiologists manually traced brain structures on the MRI scans, such as the corpus callosum, amygdala, hippocampus and caudate. Surface areas for these structures, along with a measure of gray matter thickness, were entered into a hierarchical clustering algorithm. On the basis of this fairly course spatial information, they identified several clusters within the subjects that correlated with factors such as age of pregnancy and scores on the Childhood Autism Rating Scale (CARS) autism diagnostic tool. While all individuals in the study were diagnosed as "autistic" based on the CARS, individual symptoms and brain structure were nonetheless heterogeneous. This study provides hope that with finer spatial measures, and a larger sample size, some biologically based subtypes of autism may be identified.

Multimodal

MVPA does not require a model of the expected response, making it easy to combine data across modalities. For instance, Fan et al. (2008b) combined functional PET data and anatomical MRI data from patients with MCI and healthy aging, demonstrating that very high classification rates can be achieved by combining information from different sources.

There is growing interest in using biomarkers from brain imaging to identify disease-relevant genes or patterns of genes. One method for interrogating genetic effects on functional brain activity is to group subjects based on a specific genetic polymorphism of interest. Tura et al. (2008) examined schizophrenic patients with different genotypes for the dopamine receptor DRD1. They found that thought behavior did not differ between the groups, multivariate patterns of activity on a working memory task significantly differentiated between the genotypes.

Another method involves detecting covarying patterns across modalities. Liu et al. (2009) used parallel ICA to uncover covarying patterns of activity in fMRI and genetic data. The authors identified a genetic component – an array of single nucleotide polymorphisms – that significantly correlated with a pattern of brain activity in schizophrenics. Hardoon et al. (2009) applied a conceptually similar method, kernel correlation analysis, to show genetic influence on gray matter structure in healthy controls. This type of study may help the search for disease relevant genes, but results will need to be validated in larger populations.

PEDIATRIC POPULATIONS

While most neuroimaging MVPA studies have involved young and older adults, MVPA techniques have several potential applications in pediatric developmental and clinical studies. Here we discuss possibilities and challenges for studies in pediatric populations.

Healthy development

An interesting application of MVPA for developmental studies is identifying functional and structural patterns that are predictive of development of cognitive skills, such as reading and mathematics. Hoeft et al. (2007) used multiple regression to show that sMRI, fMRI and behavioral measures taken at the beginning of the school year could be combined to predict reading ability at the end of the school year, in 8- to 11-year-old poor readers, with greater accuracy than brain or behavioral data alone. Information from this type of study could be useful to inform educational practice (Meltzoff et al., 2009).

There are many challenges specific to neuroimaging in pediatric populations (Berl et al., 2006; Kotsoni et al., 2006; O'Shaughnessy et al., 2008). Children, compared to adults, are likely to exhibit more variability in both brain structure and activity. Individuals mature at different rates, and therefore a sample of children in a given age range will likely be more heterogeneous than a sample of healthy adults.

One model of brain development suggests that regional activations are more diffuse in childhood and become more focal with development (Durston et al., 2006). However, there is increasing evidence suggesting that functional maturation may proceed differently in different brain regions, and a model of focalization with development is likely not sufficient to explain the full range of developmental changes (Blakemore and Choudhury, 2006; Brown et al., 2006; Durston and Casey, 2006; Durston et al., 2006). In fact, some evidence suggests that brain networks come to rely less on spatial proximity and more on functional importance, becoming less "local" and more "distributed" with development (Fair et al., 2009). MVPA is agnostic with regard to models of development, and may be more sensitive to distributed patterns that differentiate adults and children.

Developmental disorders

A major focus in the study of developmental brain disorders is the early identification of individuals at risk (Koutsouleris et al., 2009). Indeed, one of the NIMH strategic objectives is to "Chart mental illness trajectories to determine when, where, and how to intervene"[3]. Longitudinal studies, in which outcomes can be related to patterns of activation or structure, are one very promising

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[1] http://www.loni.ucla.edu/ADNI/

[2] http://www.bic.mni.mcgill.ca/nihpd/info/

[3] http://nimh.nih.gov/about/strategic-planning-reports/index.shtml

application of this technique. As mentioned above, this technique is already beginning to be applied for predicting transition to disease in adult populations (Fan et al., 2008a; Koutsouleris et al., 2009).

While there are few studies using this technique in pediatric populations to date, Hoeft et al. (2008) showed that fine structural features of the brain can be used to distinguish healthy children from patients with genetic disorders such as fragile X syndrome (FXS) with greater than 90% accuracy, even in children aged 1–3 years (**Figure 3**). It is well known that individuals with FXS have enlarged caudates relative to the general population (Reiss et al., 1995), suggesting that classifier performance could hinge on caudate volume alone. However, even when the caudate was removed from the training data, the classifier was still able to significantly distinguish FXS from both typically developing and developmentally delayed controls with an accuracy of ~89%, implying that information about abnormal brain structure in this disorder is distributed in voxels throughout the brain.

FXS is a genetically defined clinical group with a well characterized influence on brain development, which is strong enough to be uncovered using voxelwise analyses (Reiss et al., 1995; Lee et al., 2007; Hoeft et al., 2008). However, MVPA methods may be most fruitful for studying patient groups with more subtle distributed pathology (Zhu et al., 2008). In particular, we foresee interesting applications in (1) predicting the onset of brain disorders based on structural or functional patterns, (2) predicting individual response to treatment, and (3) identification of disease-relevant endophenotypes/biomarkers.

CHALLENGES AND LIMITATIONS FOR MVPA IN PEDIATRIC POPULATIONS

The challenges of neuroimaging in pediatric populations are well known (Berl et al., 2006; Kotsoni et al., 2006; O'Shaughnessy et al., 2008). In this section we will discuss some of the challenges in applying MVPA to pediatric populations.

MOTION

Subject motion makes brain imaging studies in pediatric clinical populations very difficult. Preprocessing can be used to mitigate this problem; however since motion affects the signal globally, task-correlated motion may have a strong impact on pattern classification techniques that draw power from integrating over many voxels. Therefore, special care should be taken to ensure that patterns are representative of neural, rather than motion-induced, changes in BOLD signal.

REGISTRATION WITH STANDARD TEMPLATES

Standard templates used to align MR images into a common space are built using adult brains (Talairach and Tournoux, 1988). It has been found that the normalization procedure can cause significant distortions in brains of children 6 and under (Muzik et al., 2000), while for older children this is less of an issue (Muzik et al., 2000; Burgund et al., 2002; Wilke et al., 2002; Kang et al., 2003). However, if similar distortions are applied to one group of subjects in a classifier, it may affect MVPA results. It is therefore worth taking precautions to ensure that classifiers are not relying on image distortion.

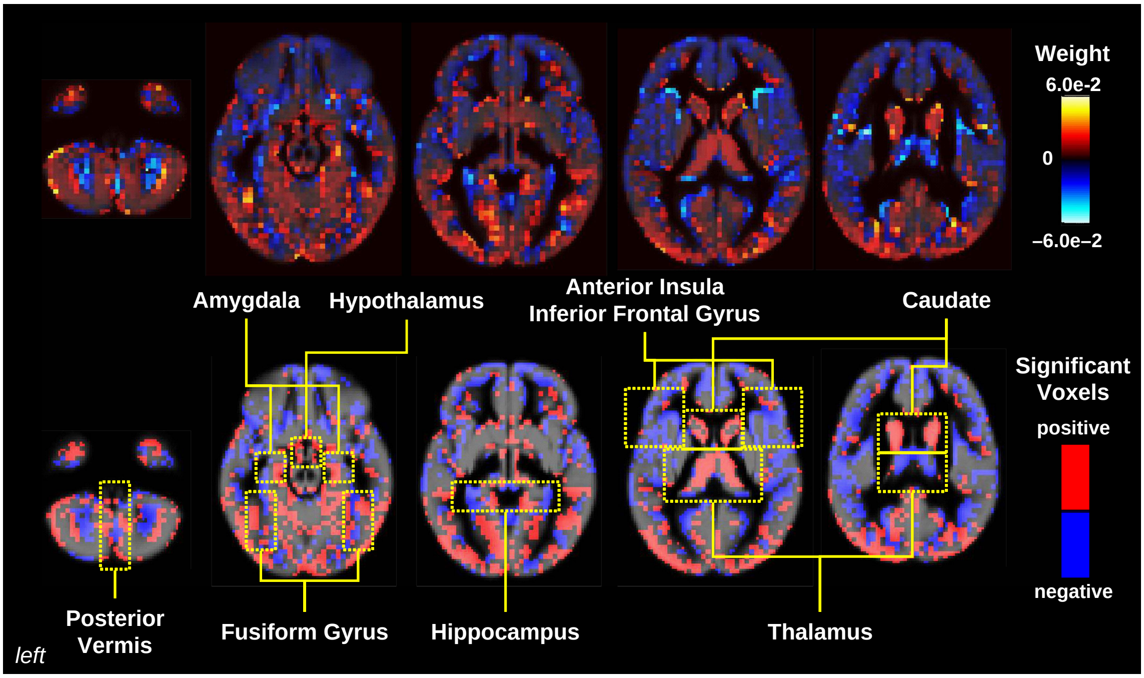

FIGURE 3 | Pattern classification results for FXS versus TD/DD; classification rates above 90% were achieved in this analysis. Whole-brain representation of pattern classification results from FXS versus TD or DD using all gray matter voxels. Axial brain images of weight vectors from leave-one-out support vector machine analysis for all voxels (top) and spatial patterns of the most significant voxels when thresholded at $P = 0.05$ (according to 2000 permutations) (bottom) are shown. Reprinted with permission from Hoeft et al. (2008). Copyright © 2008 American Medical Association. All rights reserved.

OTHER LIMITATIONS

With the relatively small sample sizes that are the norm in neuroimaging studies, overfitting is a concern, and results must be interpreted with caution. Before these methods can be deployed in a clinical setting, studies with larger cohorts must be undertaken (Koutsouleris et al., 2009).

In addition, MVPA methods are not ideal for testing hypotheses regarding the involvement of a specific brain region in a given task. The results of an MVPA analysis can tell you only the relative amount of information given by a particular voxel. Moreover, patterns of weights are likely to be more difficult to interpret than a significant result at a specified region of interest.

CONCLUSION

MVPA is useful for answering questions about functional and structural organization in the brain, and can have greater sensitivity and descriptive power than mass univariate methods. While MVPA techniques have yet to be widely adopted in pediatric neuroimaging, we expect to see applications in several domains in coming years. These include both predicting normal cognitive development and detecting patterns related to brain disorders that may be used for diagnosis or treatment.

While MVPA methods are attractive in a variety of situations, they are likely not appropriate to answer any and all questions about brain function. In particular, because MVPA does not explicitly model the shape of the neural response, univariate GLM models may be more appropriate for testing whether fMRI time courses correlate with specific signals of interest, (e.g., prediction error signals in reward learning or other model derived phasic signals O'Doherty et al., 2003), or for testing for responses within a particular region that vary in a graded manner with task parameters.

It is also worth noting that pattern classification is a research field in and of itself, and methods are continually being improved. Therefore, not every tool should be expected to provide great results "straight out of the box", and a good understanding of analysis methods is always important for interpreting the results.

MVPA is a promising tool for neuroimaging of brain development, with potential to yield novel insights into both healthy brain development and the pathology of developmental brain disorders. While these methods are still relatively new, the rapid acceleration of applications in both structural and functional neuroimaging indicates that MVPA is poised to become a standard analysis tool that can complement GLM-based analyses (e.g., Dux et al., 2009).

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