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COMT genotype and resting brain perfusion in children

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ABSTRACT

Levels of extra-synaptic dopamine in the brain vary as a function of polymorphisms at the val158met locus of the catechol-O-methyltransferase (*COMT*) gene. In vivo studies of this polymorphism in the human brain have typically measured patterns of neural activation during dopamine-mediated tasks in adults. This study is the first to investigate the effects of COMT on brain physiology during rest and in children. We used flow-sensitive arterial spin-labeling (ASL) magnetic resonance imaging to examine brain blood flow (CBF) in 42 children. Compared with val-allele carriers, met-allele homozygotes exhibited greater CBF in mesolimbic, mesocortical, and nigrostriatal dopamine (DA) pathways. Higher CBF in DA-rich brain structures reflects COMT-related baseline differences that (1) underlie the selective behavioral advantages associated with each genotype; (2) affect interpretations of previously reported genotype differences in BOLD signal changes; and (3) serve as a foundation for future studies on the effects of COMT on brain development.

Introduction

Catechol-O-methyltransferase (*COMT*) is a gene that encodes a key enzyme in the metabolism of dopamine (DA). A single nucleotide polymorphism (SNP; G→A transition at codon 158) leading to a valine (val) to methionine (met) substitution in a coding region of COMT has been found to be associated with a greater than two-fold decrease in COMT enzyme activity and DA catabolism (Chen et al., 2004; Lachman et al., 1996; Lotta et al., 1995). Consequently, the met allele of this polymorphism confers reduced enzymatic activity and subsequently increased DA availability (Chen et al., 2004; Tenhunen et al., 1994; Tunbridge et al., 2004). Increased synaptic DA availability that characterizes the COMT met allele has been associated with better performance on multiple executive functioning tasks (Barnett et al., 2007; Bruder et al., 2005; Caldu et al., 2007; Diaz-Asper et al., 2008; Egan et al., 2001; Goldberg et al., 2003; Rosa et al., 2004; Tan et al., 2007), but these results have not been replicated consistently (for detailed review see Barnett et al., 2008).

Functional MRI studies have found that the poorer performance on cognitive tasks by adults with a val allele is accompanied by greater BOLD signal change (Bertolino et al., 2004; Bishop et al., 2008; Blasi et al., 2005; Egan et al., 2001; Mattay et al., 2003). These findings have been cited in support of a model postulating that val-allele carriers are characterized by reduced neural efficiency (Heinz and Smolka, 2006). This model, however, has not considered the possible influence of basal brain blood flow on the reported BOLD signal changes. Given DA has a direct vasoactive effect on cerebral circulation (Krimer et al., 1998; Ract et al., 2001; Tuor et al., 1986), in the present study we raise the possibility that the reduced BOLD response reported in met-allele homozygotes in previous studies is due in part to val–met differences in baseline perfusion.

Behaviorally, it had previously been demonstrated that COMT affects executive functioning in healthy children (Barnett et al., 2007; Diamond et al., 2004; Gosso et al., 2008; Wahlstrom et al., 2007; Zhang et al., 2007); until recently, however, the effects of this gene on basic brain biology in children, had not yet been examined. Mechelli et al. (2009) examined the impact of the functional val158met polymorphism in the *COMT* gene on brain function and structure in 10–12 year old children using a combined fMRI and voxel-based morphometry approach. These investigators identified a positive association between the met allele and left hippocampal head volume, and between the met allele and BOLD response to fearful faces in the right parahippocampal gyrus, but they did not obtain the significant frontal effects previously found in adults. This is the first human MRI study showing that COMT influences brain development and highlights the need for further investigation of the effect of COMT in the neural systems of children.

The present study was designed to measure basal brain perfusion as a function of the COMT polymorphism in a sample of healthy children. Understanding the underlying vascular response and blood flow state is necessary for interpreting differences in BOLD signal that have been attributed to the *COMT* gene. Furthermore, the results of perfusion analyses will provide new information about the structural and functional integrity of the brain that has the potential to inform models and theories of the physiological impact and, ultimately, the developmental trajectory of the effects of this gene. For example,

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genetic differences in resting perfusion can be compared to results obtained in disease states or pathologies, and may yield critical information about age-dependent processes associated with COMT. This study is the first to compare patterns of brain function in carriers of different COMT alleles during rest, without the demands imposed by performing a task. The findings of this study, therefore, will provide a foundation for future fMRI investigations of the COMT SNP in children.

We applied a spin-labeling method to examine resting perfusion effects across *COMT*-gene groups in a sample of healthy children. We predicted that met-allele homozygotes would have greater perfusion than would val-allele carriers in major DA pathways of the brain, including the *mesolimbic* (ventral tegmental area (VTA) to nucleus accumbens (NAcc) to medial PFC), *mesocortical* (VTA to PFC), and *nigrostriatal* (substantia nigra to putamen and caudate) pathways. Because genotype effects on behavior have been found to exhibit both dose-dependent linear (Bertolino et al., 2004; Bishop et al., 2008; Blasi et al., 2005; Egan et al., 2001) and inverted U-shaped (Mattay et al., 2003; Meyer-Lindenberg et al., 2005) patterns, we conducted tests of linear, quadratic, and linear + quadratic perfusion effects.

Materials and methods

Participants

Participants were recruited through postings on Craigslist (a community-based, free advertisement website) and community-based parent email lists. Children were screened to meet the following criteria: 9 to 16 years old, right-handed, no past or current psychopathology, fluent in English, and no moderate or severe learning disorder. Parents and their children gave informed consent and assent, respectively, as approved by the Stanford Institutional Review Board. Children participated in a behavioral assessment and orientation session prior to scan scheduling, and subsequently participated in an fMRI scan session. A total of 44 participants were scanned (mean age = 12 years; range 9 to 16 years; Table 1). Two participants were eliminated from analysis: one because a high-resolution structural image was not acquired, and the other because the scan was stopped before perfusion was completed. Thus, 42 children are included in the reported analyses (28 females). Children were paid for their participation.

MRI and perfusion imaging procedures

Structural and perfusion images were obtained on a GE 3.0 T whole body scanner (General Electric Medical Systems Signa, Waukesha, WI). Participants were positioned in a single channel T/R head coil and stabilized by padded 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.

Cerebral blood flow was measured using water protons as endogenous tracers based on the flow-sensitive alternating inversion recovery (FAIR) technique (Kim, 1995; Kim and Tsekos, 1997). In this pulsed arterial spin-labeling technique, flow is assayed by subtracting non-selective inversion (control) images from slice-selective inversion (tagged) images that are collected in an alternating time series. During the delay time after magnetization inversion using an adiabatic 180 degree pulse, magnetized blood spins move into the imaging slice and exchange with tissue water. The signal difference between the tag and control images (the FAIR-CBF image) is directly related to blood flow.

Participants were instructed to lie still with their eyes closed while they were scanned using FAIR for 4 min. Constraints of the FAIR method and optimal slice thickness (≥5 mm) required acquisition of a partial brain volume. To include mesolimbic, mesocortical, and nigrostriatal dopaminergic pathways of the brain, the straight-axial acquisition covered the medial temporal lobe, cerebellum, and prefrontal cortex (Supplementary Fig. 2). One acquisition consisted of 10 axial slices, 5 mm thick (no gap), TR = 3 s, TE = 8 ms, TI = 1200 ms, flip angle = 90°, in-plane resolution 3.43 × 3.43 mm^2, and 80 time frames. The readout utilized a spiral *k*-space trajectory instead of the usual EPI method, allowing a short TE and short readout duration that minimized geometric distortion and signal loss from T2* decay (Glover and Lai, 1998). An axial T1-weighted 3D FSPGR sequence was collected for anatomical reference (140 slices, 1 mm thickness, TI = 500 ms, flip angle 11°, FOV = 25 cm, 256 × 256).

DNA acquisition and processing

Children's saliva was collected for genetic analysis using the Oragene Kit (DNA Genotek, Inc. Ottawa, Ontario, Canada), an all-in-one system for the collection, preservation, transportation, and purification of DNA from saliva. This is a minimally invasive procedure. DNA extracted by this method is of high quality and allows for genotyping with a high success rate (Rylander-Rudqvist et al., 2006). The COMT polymorphism was assayed by the Lachman method (Lachman et al., 1996). DNA was amplified with the primers 5-CTC ATC ACC ATC GAG ATC AA-3 and 5-CCA GGT CTG ACA ACG GGT CA-3. The resulting 109 bp fragment was digested with NlaIII. Digested bands were visualized with ethidium bromide staining after gel electrophoresis (4% agarose).

Image analysis

For each slice and for the brain volume, magnitude and FAIR-CBF images were reconstructed for each participant. Magnitude images correspond to the average for the time series, while FAIR-CBF images correspond to average perfusion across the scan. Using SPM2 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm), the FAIR brain volumes (magnitude and brain blood flow (CBF) images) were co-registered to the participant's anatomical image. Co-registered magnitude and CBF mages were normalized to the Montreal Neurological Institute (MNI) template, using the participant-specific transformation parameters created by fitting the T1 SPGR image to the single reference standard SPM T1 template. A smoothing kernel was not used because spatial smoothing already occurs during normalization.

Global perfusion

We extracted the mean global perfusion value across the inferior brain and cerebellum for all participants. Participants' normalized FAIR-CBF images were averaged together, then segmented and truncated, to make a grey matter mask image in which mean global perfusion was calculated. The normality of the distribution of global perfusion within each genotype group was tested using Kolmogorov–Smirnov (K–S). The effects of genotype on global perfusion were tested with analyses of variance (ANOVAs).

Table 1
Participant demographics and summary statistics.

	Met/Met	Met/Val	Val/Val	Statistic
N	6	23	13	$\chi^2_{(2)} = 0.63$, $p = 0.73$
Gender (F:M)	3:3	13:10	12:1	$\chi^2_{(2)} = 5.66$, $p = 0.06$
Age (year)	11.5 (1.9)	12.1 (2.2)	12.5 (1.9)	$F_{2,39} = 0.57$, $p = 0.57$
Global perfusion	29.47 (8.97)	27.85 (9.58)	27.23 (7.49)	$F_{2,39} = 0.13$, $p = 0.88$

Data expressed as mean (S.D.). Perfusion given in ml/100 g/min.

Regional perfusion

We used a three-step preparation to classify both linear and quadratic effects across the genetic groups. Assessing both linear and quadratic brain perfusion effects allowed us to examine both a dose-dependent genetic model in which the effect of allele load is additive, as well as the 'inverted-U' curve that has been found to characterize behavioral and neural responses of individuals with different copies of the *COMT* gene (Mattay et al., 2003; Meyer-Lindenberg et al., 2005). Portions of this classification scheme were previously implemented by Kirschen et al. (2005) in their study of load effects on working memory.

Step 1

We used non-parametric tests to construct a binary map of the voxels for which there was a significant effect of COMT. Participant's spatially normalized CBF maps were analyzed in a random-effects analysis implemented using the SnPM3 toolbox (Statistical non-Parametric Mapping, http://www.sph.umich.edu/ni-stat/SnPM/) (Holmes et al., 1996; Nichols and Holmes, 2002). The benefits of using non-parametric permutation tests are that minimal assumptions are made about the distribution of CBF values, and it is more powerful than parametric approaches when the groups have uneven sample sizes, some of which are quite small. The SnPM3 one-way (by genotype group) ANOVA was implemented using 10,000 iterations.

Previous research has found dynamic cortical perfusion across the age range of our participants (Chiron et al., 1992). Consequently, even though the three genotype groups did not differ in age (Table 1), we specified age as a covariate in the SnPM3 ANOVA. The resulting SnPM3 F-contrast image was then thresholded at $p<0.05$, cluster minimum 5 voxels, and saved as a binary brain mask for subsequent analysis.

Step 2

For the voxels that exhibited a significant omnibus effect of COMT, we further specified the pattern of results by computing linear and quadratic trends for each voxel within the F-mask. We conducted separate one-way (by genotype group) ANOVAs for each voxel in SPM2 and specified orthogonal contrasts of [−1 0 1] for the linear trend and [−1 2 −1] for the quadratic trend. Results were thresholded by using Monte Carlo simulations to establish the minimum cluster size of voxels ($p<0.005$) in the F-contrast map that exceeded a cluster-wise corrected p-value of 0.05 (Ward, 2000). This resulted in a cluster size of $k=3$, Because we selected this contrast map based on significant omnibus results, however, we increased the minimum cluster size to $k=5$ to further protect against Type 1 error inflation. Next, we classified each voxel within each image as exhibiting that trend (=1) or not (=0). Thus, supra-threshold voxels were characterized as (a) exhibiting only significant linear effects; (b) exhibiting only significant quadratic effects; or (c) exhibiting both linear and quadratic effects.

Step 3

Finally, these boolean images were repopulated with the appropriate contrast values as follows: (1) the linear effects image was repopulated with the linear t-test contrast values; (2) the quadratic effects image was repopulated with the quadratic t-test contrast values; and (3) the linear + quadratic effects image was repopulated with the original F-test values generated in Step 1. The resulting maps for these results are summarized in Supplementary Tables 1–3.

Results

Participant characteristics

Genotyping yielded three groups of children: homozygous met ($n=6$); homozygous val ($n=13$); and met/val ($n=23$). Demographic data for the three COMT genotype groups are presented in Table 1. The three groups did not differ significantly with respect to age, $F(2,39)=0.57$, $p>0.5$, or gender, $\chi^2_{(2)}=5.66$, $p>0.05$. Nevertheless, we tested for gender effects in cerebral perfusion, collapsing across the three genotype groups and observed that males and females did not differ significantly in global perfusion, $t(40)=1.26$, $p=0.21$. Finally, the allelic frequencies were in Hardy–Weinberg equilibrium, $\chi^2_{(2)}=0.63$, $p>0.5$.

Global perfusion

As expected, the three genotype groups did not differ with respect to global perfusion, $F(2,41)=0.13$, $p=0.88$. Moreover, the distribution for global perfusion was normal within each group, all K–S<0.23, $ps>0.05$. Supplementary Fig. 1 shows the aligned dot plot for the global perfusion data by group.

Regional differences

Fig. 1 shows brain blood flow (CBF) values in representative slices for each genotype. Supplementary Tables 1–3 detail the regions with perfusion differences among the three genetic groups for the linear, quadratic, and linear + quadratic contrasts at a threshold of $p<0.005$, and $k\geq 5$. Several regions showed a significant linear effect in which perfusion increased with allelic load. For a majority of these regions, perfusion was associated with increased met-allele load, including numerous frontal cortical, temporal cortical, and lateral cerebellar regions that are associated with executive processing and visual attention (i.e., anterior cingulate and fusiform), and regions along the mesolimbic, mesocortical, and nigrostriatal pathways (i.e., midbrain, NAcc, caudate, medial PFC). In contrast, far fewer regions showed increased perfusion with val-allele load, including clusters in the occipital and cerebellar regions, with the most significant and largest cluster in the vermis of the cerebellum.

Quadratic effects were obtained in fewer, more punctate regions of the brain. These are summarized in Supplementary Table 2 as either U-shaped (homozygotes>met/val) or inverted-U-shaped (homozygotes<met/val) quadratic effects. U-shaped quadratic effects were

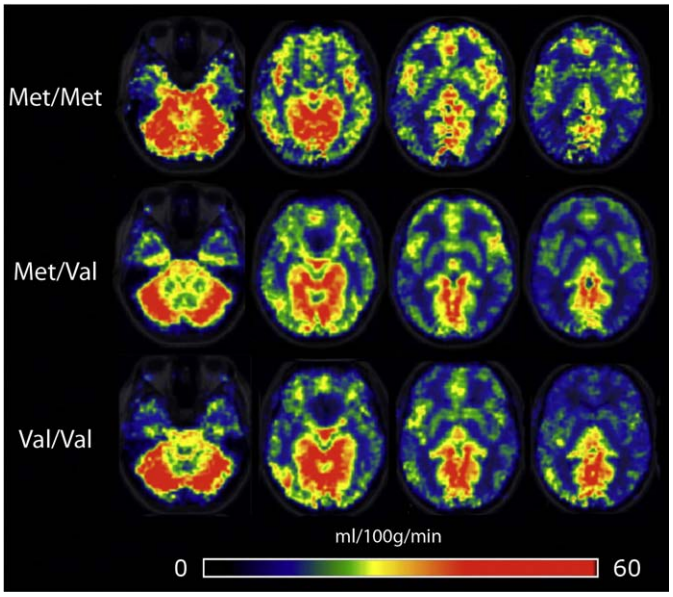

Fig. 1. Quantitative resting perfusion images for three genotype groups. The genotype groups are represented in rows. The columns (left to right) contain select slices at $z=-30$, −12, 0 and 6 mm, respectively. These normalized, non-smoothed group mean perfusion images were derived using SPM's *Imcalc* function. Perfusion images are displayed on anatomical background images attained by averaging participant SPGR images.

observed in superior and medial temporal regions, bilateral thalamus, numerous anterior and posterior cerebellar regions, and right insular cortex. Inverted-U-shaped quadratic effects were observed in the medial frontal gyrus, fusiform cortex, and the pyramis of the cerebellum.

In addition to regions in which only linear or only quadratic effects were observed, there were a small number of regions in which both linear and quadratic effects were present; these included the temporal lobe and select subcortical regions. Plotting the results of these analyses as a function of genotype group indicated that, across these regions, met-allele homozygotes exhibited greater perfusion than did val-allele homozygotes. Unlike the pure linear effects, however, individuals heterozygous for the *COMT* gene (met/val carriers) exhibited perfusion levels in these regions similar to the val-allele homozygotes. Supplementary Table 3 summarizes the peaks of these effects, labeled "met/met>val carriers" because of the observed character of these effects when plotted.

Discussion

This is a foundational study of COMT genotype effects on neurophysiology in children. This study is novel both in examining a wide area of cortical and subcortical brain regions for possible neurobiological effects of COMT, and in the analytic approach taken. The results of this investigation indicate that children with different COMT polymorphisms do not differ in their *global* perfusion values (Supplementary Fig. 1), but do differ in *regional* perfusion (Fig. 2).

As hypothesized, met-allele homozygotes exhibited higher resting perfusion than did val-allele homozygotes in numerous cortical and subcortical brain regions (frontal and temporal cortices, insula, caudate, brainstem, lateral cerebellum), many of which are DA-rich regions of the brain. In contrast, val-allele homozygotes exhibited increasingly greater perfusion than did met-allele homozygotes in very few regions; these included the cerebellar vermis and occipital regions.

The analytic methods in the present study allowed us to characterize CBF values in the met/val carriers relative to other gene groups. By designating all significant voxels as linear, quadratic, or both linear and quadratic, we distinguished regions in which met/val carriers exhibited intermediate perfusion effects from regions in which met/val carriers clustered with one of the homozygous groups. Overall, the predominant effect observed across groups was linear; with the exception of a subset of regions in the temporal and midbrain cortices, met/val heterozygotes exhibited perfusion values that fell between the two groups of homozygotes. This pattern of results suggests that the effects of the COMT val158met phenotype on regional perfusion are largely dose-dependent — exhibiting increasing perfusion with allelic load. Even in those regions in which quadratic effects were observed, the effects were driven by significantly higher perfusion in met homozygotes than in the other two groups (U-shaped). The lack of a linear relation in these regions may be a result of a weaker association between synaptic DA and perfusion, producing a 'threshold' effect that required the presence of two met alleles and significantly higher synaptic DA to influence perfusion.

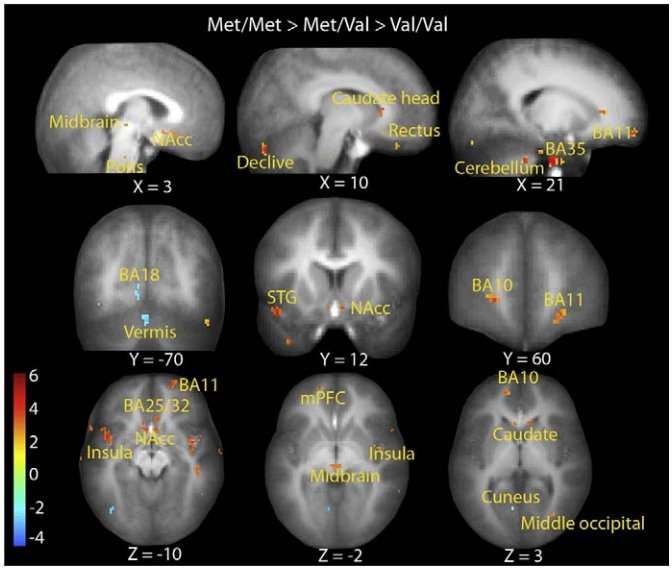

Fig. 2. Main effect of genotype on resting perfusion. Results from the GLM thresholded at $p<0.005$ showed higher resting perfusion with met-allele load (shown in orange) in several critical nodes along dopaminergic pathways. Lower resting perfusion was observed with increasing met-allele load (shown in blue) in the occipital cortex and cerebellar vermis. Group differences are displayed on the average of all children's SPGR images. NAcc, nucleus accumbens; STG, superior temporal gyrus, mPFC, medial prefrontal cortex.

In this study we describe a pattern of perfusion data that may serve as the underlying basis of task-related activation. BOLD and CBF contrasts are distinct but related in a number of ways. CBF contrast measured using spin-labeling techniques is more spatially localized than is blood oxygenation contrast measured using BOLD imaging, but the temporal resolution of perfusion imaging is lower than that of BOLD imaging, because the labeled blood must travel from the labeling plane into the imaging plane. Neither method measures the amount of basal oxygen, but both are the accumulation of several factors that include oxygen concentration/quantity and utilize oxygen for their signal properties. As a result, BOLD and CBF increase together in response to task-related activation. Thus, it is reasonable to expect that if CBF is already high at rest, the amount of increase measured above this baseline may be reduced.

Understanding the effect of COMT on CBF may aid in the interpretation of BOLD fMRI studies. Most fMRI studies that have documented task-related differences in BOLD response as a function of COMT have shown decreased BOLD response in met-allele homozygotes (Bertolino et al., 2004; Bishop et al., 2008; Blasi et al., 2005; Caldu et al., 2007; Egan et al., 2001; Kempton et al., 2008; Mattay et al., 2003). However, some studies have shown greater BOLD response in met carriers (Drabant et al., 2006; Smolka et al., 2007; Smolka et al., 2005); indeed, the only study of BOLD fMRI in children showed greater BOLD effects in met carriers (Mechelli et al., 2009). The influence of resting perfusion on BOLD was recently highlighted in a study of Alzheimer's disease: compared to individuals at low risk for this disease, high-risk participants exhibited higher resting baseline perfusion, no difference in absolute perfusion during a cognitive task, and a concomitant reduction in BOLD fMRI amplitude (Fleisher et al., 2008). In light of this pattern of perfusion differences, Fleisher et al. concluded that BOLD activations should be interpreted with caution, as they may not reflect differences in neuronal activation. Here, we reiterate the same caution in interpreting BOLD differences previously associated with COMT. We know that the size and extent of BOLD activations are directly influenced by the magnitude of resting brain perfusion, which essentially forms the baseline over which activation is measured. The differences we found among genetic groups was region-specific, suggesting that mapping perfusion baseline within the same scan session will be useful for future BOLD fMRI studies comparing groups with differing neurotransmitter levels, particularly when those neurotransmitters are known to be vasoactive.

In the present study, we show that met-allele carriers, and particularly met-homozygotes, have higher resting perfusion rates than do val-allele homozygotes in numerous cortical and subcortical brain regions. The COMT genotype has most often been implicated in PFC differences, as it is expected to exert its greatest effect in the PFC. However, the number of non-invasive systems-level human genomic imaging studies is rapidly expanding and these offer new insight into behavioral and brain differences that extend beyond PFC localization. Virtually all COMT fMRI studies have shown group COMT differences

in regions outside of the PFC, including anterior cingulate, parietal, temporal pole, and medial temporal regions (Bertolino et al., 2004; Bishop et al., 2008; Blasi et al., 2005; Drabant et al., 2006; Egan et al., 2001; Kempton et al., 2008; Mattay et al., 2003; Smolka et al., 2007; Smolka et al., 2005). Moreover, structural neuroimaging studies also show genotype differences beyond the PFC (Cerasa et al., 2008; Taylor et al., 2007; Zinkstok et al., 2006). Finally, studies investigating the effect of COMT on affect and affective traits implicate processing differences that extend beyond the PFC (Jabbi et al., 2007; Waugh et al., In press; Wichers et al., 2008). Taken together, our results and these previous findings suggest that the effects of COMT extend beyond the PFC, and further studies of COMT on brain structure, function, and/or development should include these other DA-rich regions in their conceptualizations. Here we report an intriguing dissociation among genotype groups for brain regions with greatest resting perfusion. Previous research has provided evidence that whereas met-allele homozygotes perform better on cognitive control tasks, val-allele homozygotes outperform their met-allele counterparts on tasks involving affect regulation (Zubieta et al., 2003) and report lower levels of anxiety (Enoch et al., 2003; Woo et al., 2004) and hostility (Volavka et al., 2004). Thus, it appears that whereas met-allele carriers have a cognitive advantage, val-allele homozygotes have an affective advantage. We supplemented this formulation by documenting a neurobiological dissociation in which the resting perfusion effects were localized to regions associated with the psychological processes for which each genotype group is advantaged. That is, perfusion was greatest in regions important for cognitive processing in met-allele homozygotes, (e.g., prefrontal, temporal and cerebellar regions), and was greatest in a cerebellar region important for emotion regulation (Levisohn et al., 2000; Schmahmann and Caplan, 2006; Tavano et al., 2007) in val-allele homozygotes.

The finding that higher resting baseline perfusion is correlated with met-allele load provides a new neurobiological foundation for future studies of the effects of the COMT genotype on brain development. Differences in resting brain perfusion have direct implications for research examining BOLD signal in children with different COMT genotypes, and more subtle implications for models of development and individual variation that may be associated with this genotype. Previous work has shown that, compared with adults, children have higher CBF values that decline to adult-level CBF in the adolescent years, with region-specific time trajectories (Chiron et al., 1992). Chiron et al. illustrated that primary sensory cortices (visual, auditory, and sensorimotor) require a shorter time to reach adult levels of perfusion than do associative cortices (lateral frontal, parietal and Broca's area), in which development is protracted (Chiron et al., 1992). The finding that perfusion rates are reliably higher in met-allele homozygotes than in val carriers has potentially important implications for development. It is possible that developmental reductions in CBF are related to brain maturational processes, like synaptic stabilization (Changeux and Danchin, 1976). Greater regional perfusion for the met-allele homozygotes may, therefore, indicate an altered developmental trajectory. It will be important in future studies to collect longitudinal data that will address these fundamental questions about COMT-associated differences in brain function across the life span.

The finding of higher resting baseline perfusion with increasing met-allele load also has implications for understanding other life-span effects. For example, stable baseline perfusion differences should affect neural processes that are associated with aging. The nature of the relation between higher perfusion rates and brain tissue stability and function is not yet known; however, recent studies that have examined the effects of the *COMT* gene on cognition and aging show that the met advantage is maintained or even augmented in late-life (de Frias et al., 2005; Lindenberger et al., 2008; Nagel et al., 2008; Raz et al., 2009) with some evidence for sex differences (O'Hara et al., 2006). Again, the consequences of stable high perfusion rates are not well mapped, and work in this area will have important implications for our understanding of the effects of genes on brain biology, and of the associated behavioral phenotypes.

It is important to note that genotype differences in this study were statistically significant even though there were only six participants in the smallest genotype group (met-allele homozygotes); it is likely that these results would be even stronger in a larger sample. Nevertheless, we reduced the likelihood that outlier effects contributed to our results by limiting our analyses to regions that were significantly different in a non-parametric group analysis. It is also noteworthy that a majority of the effects of COMT on perfusion were found in previously hypothesized regions along DA pathways (i.e., NAcc and prefrontal projection regions).

Haplotype models have further informed our understanding of the effects of the COMT gene (Diaz-Asper et al., 2008). There is little doubt that to fully understand the relation between COMT and neural/behavioral effects, investigators will need to examine haplotype models as well as multiple functional polymorphisms with a single gene and across genes. There is recent evidence, however, to suggest that the simpler val158met SNP model may be the most informative for understanding the effects of COMT on neural processing (Puls et al., 2009). In light of this evidence and to link our examination of brain perfusion to previous studies of BOLD, in the present study we focused on the val158met SNP of the *COMT* gene. Future investigations of the effects of COMT on brain perfusion, however, should investigate the effects of other COMT SNPs as well as possible gene–gene interactions.

This is the first study of COMT-related brain perfusion differences in children, and it is clear that basal perfusion differs as a function of COMT genotype. Future examinations of COMT task-related activations will benefit from taking into account CBF by measuring it directly or, at a minimum, considering their findings in light of these results. The documentation of significant differences in baseline perfusion rates among COMT genotype groups in children suggests that this line of research will be important for understanding COMT brain biology over the life span.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2009.05.076.

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