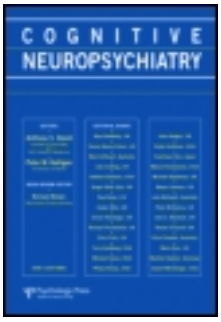

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COMT influences on prefrontal and striatal blood oxygenation level-dependent responses during working memory among individuals with schizophrenia, their siblings, and healthy controls

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COMT influences on prefrontal and striatal blood oxygenation level-dependent responses during working memory among individuals with schizophrenia, their siblings, and healthy controls

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Introduction. Recent theories have suggested that corticostriatal interactions may play an important part in mediating working memory demands and may impact clinical symptomology of schizophrenia. These effects are thought to occur through changes in dopamine signalling from the midbrain and via feedback from the frontal cortex. The catechol-O-methyltransferase (COMT) Val158Met polymorphism may prove useful for studying these effects in vivo.
Methods. In this study, patients with schizophrenia, their well siblings, and healthy controls were genotyped and scanned using functional magnetic resonance imaging (fMRI) while they performed a working memory task.
Results. We found that patients and their siblings, but not controls, who were Val homozygotes displayed greater activity of the DLPFC, striatum, and the cerebellum during the task than respective Met carriers. We also found a relationship between striatal activity and negative symptoms for the Val homozygote group.
Conclusions. Our findings support and extend previous studies of COMT effects on cognition and neural activity, and suggest that changes in dopamine availability may differentially impact corticostriatal functioning of individuals at risk for

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schizophrenia from those who are not. We also found some evidence supporting the proposed role of striatal dopamine signalling and clinical symptoms associated with anhedonia and apathy.

Keywords: Catechol-O-methyltransferase; Dopamine; Schizophrenia; Striatum; Working memory.

INTRODUCTION

Working memory (WM) is a critical part of complex goal-directed cognitive control and is a hallmark deficit of serious mental illnesses like schizophrenia;, however, the neural architecture that supports working memory processing is not fully understood. Previous imaging studies have identified involvement of the prefrontal cortex (PFC) and parietal cortex during WM processing (Braver et al., 1997; Cohen et al., 1997; Collette et al., 2007; Jonides et al., 1997; Smith & Jonides, 1997; Smith, Jonides, & Koeppe, 1996). A growing body of literature examining subcortical involvement during WM processing has demonstrated basal ganglia (BG) engagement during various WM tasks (Chang, Crottaz-Herbette, & Menod, 2007; Heyder, Suchan, & Daum, 2004; Lewis, Dove, Robbins, Barker, & Owen, 2004; McNab & Klingberg, 2008; Ravizza & Ciranni, 2002; Voytek & Knight, 2010), which is consistent with known anatomical connections between the BG and the cortex, including the PFC, (Middleton & Strick, 1994). Further, there is some support for the idea that the nigrostriatal dopamine system and its targets in the striatum (the caudate nucleus and putamen) also play a role in schizophrenia aetiology (Graybiel, 1997; Heyder et al., 2004; Kegeles et al., 2010).

Previous cellular and computational studies have indicated that dopamine (DA) availability likely plays a critical role in WM processing (Braver & Cohen, 2000; Cohen, Braver, & Brown, 2002; Cohen, Braver, & O'Reilly, 1996; Durstewitz, Seamans, & Sejnowski, 2000; Frank, Loughry, & O'Reilly, 2001; Goldman-Rakic, 1995a, b, 1996). Catechol-O-methyltransferase (COMT) is an important enzyme involved in the extraneuronal breakdown of DA and has been studied as a candidate risk gene for psychosis (see, for a review, Craddock, Owen, & O'Donovan, 2006). A functional polymorphism within the COMT gene, at the codon 108/158 (Val158Met), has also been used to explore the interaction of dopamine availability, neural activity, and cognition (Dickinson & Elvevag, 2009; Savitz, Solms, & Ramesar, 2006). This polymorphism results in a substitution of methionine (Met), for valine (Val) resulting in an approximate four-fold increase of enzymatic activity for individuals homozygotic for the Val allele compared to Met homozygotes (Lotta et al., 1995), which may result in increased metabolism of DA. Given its putative effect on DA availability in the frontal cortex, a number of

studies have examined the effects of this polymorphism on the cognitive test performance of healthy controls, patients with schizophrenia, and their well siblings across a variety of cognitive domains and have shown the Val allele confers poorer performance on these tasks (Barnett, Jones, Robbins, & Muller, 2007; Barnett, Scoriels, & Munafò, 2008; Goldberg et al., 2003, MacDonald et al., 2007; Malhotra et al., 2002; Rosa et al., 2004). However, the specific pattern of this effect has varied (see Prata et al., 2009; Rosa et al., 2004). The association between COMT Val158Met, working memory, and schizophrenia is supported by a recent study examining the influence of a number of COMT polymorphisms on WM, IQ, and executive functioning performance of patients with bipolar disorder, schizophrenia, and a healthy control group (Wirgene et al., 2010). They found that only Val158Met significantly affected WM, such that the Val allele was associated with poorer performance, and was so only within the schizophrenia group. However, evidence supporting the association between COMT and schizophrenia is modest (Fan et al., 2005) and the impact that COMT has on cognitive behavioural performance may be small and is inconsistently found (Barnett et al., 2008).

Imaging studies of COMT have consistently shown Val homozygotes demonstrate increased PFC activity during working memory tasks as compared to Met carriers, thought to represent inefficient cortical processing (Bertolino et al., 2004; de Frias et al., 2009; Egan et al., 2001; Ho, Wassink, O'Leary, Sheffield, & Andreasen, 2005; Mattay et al., 2003; Meyer-Lindenberg et al., 2006). One way that COMT may impact frontal and subcortical neural processing is via downstream effects. Studies have shown that COMT is minimally expressed in DA neurons and regions that project to the striatum it is expressed in striatal and cortical regions that receive DA input, supporting the idea that COMT influences DA signalling via feedback from nondopaminergic neurons (Akil et al., 2003; Kastner et al., 1994). Evidence for this downstream effect is provided by Meyer-Lindenberg et al. (2005), who used positron emission tomography (PET) and fMRI to study the effects of COMT on WM performance in a healthy population. Although they did not find a direct effect of COMT on midbrain or striatal DA synthesis they did, consistent with Akil et al. (2003), find strong correlations between prefrontal activity and midbrain DA synthesis during an *n*-back WM task that differed between COMT genotypes. They suggest that their task, given that it was a WM task, was not optimal for detecting COMT effects in the BG. Other studies have demonstrated that COMT impacts activity in regions of the BG during response inhibition (Congdon, Constable, Lesch, & Canli, 2009), reward processing (Krugel, Biele, Mohr, Li, & Heekeren, 2009; Schmack et al., 2008; Yacubian et al., 2007), as well as WM (Caldú et al., 2007; Chang et al., 2007; de Frias et al., 2009; Lewis et al., 2001; McNab & Klingberg, 2008). Thus, whereas imaging studies of COMT

and WM in schizophrenia have tended to focus on involvement of the cortex, given evidence suggesting an indirect influence of COMT on midbrain DA synthesis via its expression in cortical and subcortical regions of the brain and known anatomical connections between the midbrain, the BG, and the cortex, we felt it would be informative to further investigate the effect of COMT on BG activation of patients with schizophrenia during WM processing.

Despite the challenges of examining COMT effects on cognition and clinical pathology, these studies allow for in vivo hypothesis testing of systems-level neurobiological and neurochemical changes and their effect on cognition and clinical symptoms. For example, one possible explanation for performance and neural functioning differences between Val/Met homozygotes is provided by Bilder, Volavka, Lachman, and Grace (2004). They suggest that the relationship may be explained by considering the tonic-phasic DA hypothesis (Grace, 1991), where changes in extrasynaptic DA produced by COMT degradation in the frontal cortex ultimately may lead to changes in the responsiveness of DA neurons in the midbrain to task related information. This same theory suggested that increased postsynaptic tonic DA associated with the Met allele would lead to decreased phasic DA release within the synapse, and therefore cognitive rigidity and negative symptoms, whereas tonic DA degradation, associated with the Val allele, would lead to dysregulated DA signalling subcortically and an increase in positive symptoms. Evidence for this is provided by Goghari and Sponheim (2008), who found that Val homozygotes in the schizophrenia group had higher positive symptoms scores than heterozygotes and Met homozygotes in that group, but Met homozygotes had significantly greater negative symptomatology than the other patient genotype groups. This is also consistent with the proposed role of frontostriatal circuitry in symptoms of apathy, such as disrupted emotional-affective processing, cognitive processing, and volition (see, for a review, Levy & Dubois, 2006).

In the current study, we examined the influence of the Val158Met polymorphism on activation of the PFC and BG in individuals with schizophrenia, their siblings, and healthy volunteers. Because siblings of schizophrenic patients share, on average, 50% of their genes, are medication naïve, and are free of severe symptoms that may confound performance on cognitive tasks, studying siblings may enhance sensitivity for detecting subtle gene effects. All participants underwent a functional magnetic resonance imaging (fMRI) scan while performing a WM task. We hypothesised that Val homozygotes would display greater activation of the PFC, specifically in the DLPFC, during WM performance than Met carriers. We also conducted masked analyses of the PFC and BG to increase the activity signal during our task. We predicted that COMT would impact striatal activity during WM performance via feedback from nondopaminergic regions in the cortex,

such that the Val allele would be associated with increased neural activity. With regard to behavioural performance, we predict that the Met allele will be associated with higher accuracy during the working memory task. We predict that this would be true for all diagnostic groups, but that overall patients will be less accurate than controls and sibling performance would be intermediate to that of patients and controls. However, in terms of behavioural performance, given the findings of Barnett et al. (2008), we did not predict a large effect of COMT on WM performance.

METHODS

Participants

Participants were recruited through the Conte Center for the Neuroscience of Mental Disorders (CCNMD) at Washington University in St. Louis. Participants were 20 individuals with DSM-IV schizophrenia, 15 siblings of individuals with schizophrenia, and 66 healthy controls. Exclusion criteria for controls included a lifetime history of any Axis I psychiatric disorder and having a first-degree relative with a psychotic disorder. Siblings were also excluded for a lifetime history of psychotic disorders. Our controls were required to have no family history of psychosis and no personal history of any Axis I disorder. However, we could not impose such a criterion on the siblings of individuals with schizophrenia, as many have past depression or anxiety and to exclude such individuals would result in an unrepresentative sample. Thus, we also recruited the siblings of controls, and allowed them to have the same personal history of nonpsychotic AXIS I disorders as the siblings of individuals with schizophrenia. The two sets of siblings were recruited with the same methods and inclusion/exclusion criteria, other than the diagnosis of their sibling. We combined the siblings of healthy control participants with the healthy control group to address confounds associated with differential recruitment and screening criteria for controls versus the siblings of patients. Participants in any group were also excluded if they met criteria for substance abuse or dependence within the past 6 months, had a clinically unstable or severe medical disorder, had a medical disorder that would confound the assessment of psychiatric diagnosis or render research participation dangerous, had head trauma with loss of consciousness, or met DSM-IV criteria for mental retardation. Patients were on stable antipsychotic medication doses for at least 2 weeks before participating in the study. Of the 20 participants with schizophrenia, 13 (65%) were taking atypical antipsychotic medication, five (25%) were on a combination of both typical and atypical, and two (10%) weren't taking any antipsychotic medication.

Diagnoses for all participants were determined using the Structured Clinical Interview for DSM-IV (SCID-IV; First, Spitzer, Miriam, &

Williams, 2002). These interviews were conducted by a master's-level research assistant, who had completed SCID-IV training and participated in regular diagnostic training sessions as part of the CCNMD. The SCID-IV interview had access to all data from present and past hospital records and corroborative family sources. In addition, a psychiatrist conducted a semistructured interview, also using DSM-IV criteria and all available records. A consensus meeting between the SCID-IV interview and the expert clinician determined the participant's final diagnosis. We also gave all participants the Vocabulary subtest from the third edition of the Wechsler Adult Intelligence Scale (WAIS-III; Wechsler, 1997) as a measure of crystallised intelligence.

Clinical rating scales

Clinical symptoms were assessed using the Scale for the Assessment of Negative Symptoms (SANS; Andreasen, 1983a), the Scale for the Assessment of Positive Symptoms (SAPS; Andreasen, 1983b), and the Structured Interview for Prodromal Syndromes (SIPS; McGlashan et al., 2000). These assessments were conducted by a psychologist, psychiatrist, or a trained master's-level research assistant. All participants also completed the Chapman Psychosis Proneness Scales (Chapman, Chapman, & Kwapil, 1995), which included the Perceptual Aberration Scale, the Magical Ideation Scale, the Physical Anhedonia Scale, and the Social Anhedonia Scale. Three clinical symptom clusters were used for assessment: Positive, Negative, and Disorganised symptoms. All rating scale scores were z-scored using the mean and standard deviation of the current sample and averaged within symptom clusters.

Working memory task

All participants were scanned while performing a verbal and nonverbal two-back working memory task. In this task, participants saw a sequence of stimuli presented in the centre of a computer screen and were told to push one button (target) any time they saw a stimulus that was the same as the stimulus that they saw two trials back and to push a nontarget button otherwise. The stimuli for each task were presented in four blocks of trials, with each block containing 16 trials. Within each 16 trials, one-third were targets and two-thirds were nontargets. Stimuli for the verbal tasks were concrete visually presented words, 3–10 letters in length, presented in 48-point Geneva font. Stimuli for the nonverbal tasks were nonnameable faces. These are the same stimuli used in a number of prior studies (Braver et al.,

2001; Kelley et al., 1998). Participants performed the task in a run lasting 255 s (six runs total). Each run included four task blocks and three fixation blocks interleaved in alternating order with the task blocks. Task blocks lasted 40 s and fixation blocks lasted 25 s. Each of the 16 items in a task block was presented for 2 s followed by a 500 ms interstimulus interval. During fixation blocks, a cross-hair appeared continuously, and participants were told to fixate. Stimuli were presented electronically using the E-prime 2.0 software (Schneider, Eschman, & Zuccolotto, 2002) on a Windows laptop, with each trial onset triggered directly by a pulse from the scanner. The task was projected to participants with a Sharp LCD projector (Mahwah, NJ) onto a screen positioned at the head end of the bore. Participants viewed the screen through a mirror attached to the top of the magnetic resonance (MR) head coil. A fibre-optic keypress interfaced with the E-prime button box was used to record the participant's behavioural performance.

Image collection and preparation

Scanning was performed on the 1.5T Siemens VISION system at the Research Imaging Center of the Mallinckrodt Institute of Radiology at the Washington University Medical School. Functional images were collected using an asymmetric spin-echo echo-planar sequence sensitive to blood oxygenation level-dependent (BOLD) contrast (T2) (TR = 2500 ms, TE = 50 ms, FOV = 24 cm, flip = 90°). During each functional run, 102 sets of oblique axial images were acquired parallel to the anterior–posterior commissure plane (3.75°—3.75 mm in plane resolution). Nineteen 7 mm thick slices were acquired in each image. Structural images were acquired using a coronal MP-RAGE 3D T1-weighted sequence (TR = 9.7 ms, TE = 4 ms, flip = 10°; voxel size = 1°–1°—1.2 mm). These structural images were used for between subject registration and anatomic localisation. Preprocessing of the fMRI data included: (1) compensation for slice-dependent time shifts; (2) elimination of odd/even slice intensity differences due to interpolated acquisition; (3) realignment of data acquired in each subject within and across runs to compensate for rigid body motion (Ojemann et al., 1997); (4) intensity normalisation to a whole-brain mode value of 1000; and (5) spatial smoothing with an 8 mm FWHM Gaussian kernel. Functional data were transformed into stereotaxic atlas space (Talairach & Tournoux, 1988) by computing a sequence of affine transforms and resampled to 3 mm cubic voxels. Methods for movement correction and cross-subject registration are analogous to the linear methods used in AIR (Woods, Grafton, Holmes, Cherry, & Mazziotta, 1998).

Genotyping

The procedure used to genotype the participants was similar to that outlined in Li et al. (Li et al., 2005). Using a procedure similar to that outlined by Willis-Owen et al. (2005) and the Sequenom™ system, polymerase chain reactions (PCRs) were carried out in 10-μL reaction volumes using specific primers. Each reaction included 2 μL DNA template (2 ng/μL), 0.5 μL primers
(1 μm), 0.04 μL Titanium Taq (Clontech) (BD Biosciences, San Jose, California), 1 μL Titanium Taq buffer (BD Biosciences, San Jose, California), 1 μL deoxyribonucleotide triphosphates (dNTPs) (2 mmol/L), 0.4 μL magnesium chloride ($MgCl^2$) (25 mmol/L), and 5.06 μL Milli-Q H^2O. The reaction was carried out as follows: 95°C for 1 min (1 cycle), 95°C for 30 s, 60°C for 30 s, 68°C for 1 min (45 cycles), and 68°C for 3 min (1 cycle). These products were then subject to a shrimp alkaline phosphatase (SAP) digest for removal of nonincorporated dNTPs and a final extension reaction via use of another specific primer. Extension products were cleaned and spotted onto 384 SpectroCHIPs, which were read on a mass spectrometer. Genotyping revealed 17 Met/Met participants, 47 Met/Val participants, and 37 Val/Val participants among the three groups of participants. Of the 17 Met homozygotes, three were patients and two were siblings. Given the small sample sizes for these genotype groups, we combined Met homozygotes and Val/Met heterozygotes into a single group (Met carriers) for the purposes of all subsequent analyses.

Data analysis

The Statistical Package for the Social Sciences version 16 (SPSS Inc., Chicago, IL) was used for statistical analyses of demographic, neuropsychiatric, and behavioural variables. Analysis of fMRI data was performed using in-house software (FIDL analysis package; "fMRI Analysis Methods", n.d.). For each participant, we estimated the magnitude of two-back task-related activation as compared to fixation in each voxel with a general linear model (GLM) using a box-car function convolved with a canonical haemodynamic response, with separate estimates for each stimulus type (e.g., WM-words, WM-faces). However, because we found no effects of stimulus type interactions behaviourally and stimulus type effects were not a focus of the current study, stimulus type effects were not considered further. As such, our primary dependent measure was the average of word and face estimates from each individual subjects fixed-effect GLM. These magnitude estimates were then entered into an ANOVA that treated subjects as a random factor with diagnosis (patients, siblings, and controls) and genotype

(Val homozygotes and Met carriers) as between-subject factors and these ANOVAs were used in voxel-wise whole-brain and region of interest (ROI) analyses. Given the prior work showing that COMT genotype modulates functional brain activity in both DLPFC and the BG (Schmack et al., 2008; Yacubian et al., 2007), our primary analyses were focused on a priori regions of interest. We generated masks of voxels within the DLPFC (as defined by Rajkowska & Goldman-Rakic, 1995) and BG (as defined by Wang et al., 2008), and conducted voxel-by-voxel analyses restricted to these a priori regions of interest. We used significance and cluster-size algorithms described in McAvoy, Ollinger, and Buckner (2001) and Ollinger, Corbetta, and Shulman (2001) to protect against false positive rates. Our statistical threshold for this ROI-based analysis took into account the reduced number of voxels (a cluster-size threshold of 10 contiguous voxels and a per voxel z-value of 0.005) compared to a whole-brain analysis. A second, more conservative, whole-brain analysis was conducted, using a more stringent threshold of 21 contiguous voxels and a per voxel alpha of .0001, corresponding to a corrected whole-brain false positive rate of $\sim$.05. This correction factor was determined by Monte Carlo simulations to provide a whole-brain false-positive rate; an approach equivalent to that employed by the Alphasim program in the AFNI software package. The voxel-wise ROI correction factor approach we used is equivalent to the "small volume correction" procedure in the SPM software package.

RESULTS

Demographic data and genotype frequencies for each group are shown in Table 1. The observed distribution of genotypes was consistent with Hardy-Weinberg equilibrium expectations, $\chi^2(2) = 0.1$, $p = .75$. The proportion of males and females differed significantly between the patient and sibling groups, $\chi^2(1) = 8.44$, $p < .01$, and between the patient and control groups, $\chi^2(1) = 16.2$, $p < .001$. No gender differences were observed between the siblings and control groups. We also observed significant differences between our diagnostic groups with regard to IQ, $F(2, 98) = 6.41$, $p < .001$. We entered gender and IQ a covariates and reran our analyses. Our results were unchanged. The three diagnostic groups differed in years of education, $F(2, 98) = 3.87$, $p = .02$, with both siblings and controls having more personal education than patients. However, the groups did not differ in parental socioeconomic status, $F(2, 98) = 0.26$, $p > .50$, or age, $F(2, 98) = 0.64$, $p > .50$. Given the similarity of the groups on parental socioeconomic status we did not attempt to control for education in further analyses, as cognitive disturbances

TABLE 1
Demographic data and genotype sample for all diagnostic groups

	Mean			*Standard deviation*		
	Patients	*Siblings*	*Controls*	*Patients*	*Siblings*	*Controls*
n	20	15	66			
Age (in years)	21.7	21.5	20.8	3.3	2.9	3.5
Sex (% male)	95	53	44			
Parent's education (years)	14.8	14.8	14.5	2.4	2.2	1.9
Education (years)	11.5	13.3	13.1	2.0	2.9	2.6
Handedness (% right)	80	93	81			
Valine homozygotes	10	6	21			
Methionine carriers	10	9	45			

The proportion of males and females differed significantly between the patient and sibling groups, $\chi^2(1) = 8.44$, $p < .01$, and between the patient and control groups, $\chi^2(1) = 16.2$, $p < .001$. No gender differences were observed between the siblings and control groups. No differences in our results were observed when gender was entered as a covariate. The three diagnostic groups differed in years of education, $F(2, 98) = 3.87$, $p = .02$, with both siblings and controls having more personal education than patients. However, the groups did not differ in parental socioeconomic status, $F(2, 98) = 0.26$, $p > .50$, or age, $F(2, 98) = 0.64$, $p > .50$.

associated with risk for schizophrenia may impair educational achievement (Meehl, 1969; Resnick, 1992).

TABLE 2
In-scanner verbal, nonverbal, and combined working memory performance

	Patients		*Siblings*		*Controls*	
In scanner data	*Val*	*Met carrier*	*Val*	*Met carrier*	*Val*	*Met carrier*
Word accuracy	0.88 (0.13)	0.89 (0.07)	0.89 (0.13)	0.93 (0.11)	0.97 (0.04)	0.96 (0.07)
Face accuracy	0.83 (0.13)	0.83 (0.16)	0.802 (0.17)	0.94 (0.05)	0.95 (0.11)	0.94 (0.09)
Combined accuracy	0.85 (0.13)	0.86 (0.11)	0.85 (0.09)	0.94 (0.08)	0.96 (0.03)	0.95 (0.06)
Positive symptoms	1.05 (1.21)	1.67 (0.81)	−0.29 (0.46)	−0.38 (0.22)	−0.39 (0.32)	−0.33 (0.42)
Negative symptoms	1.31 (0.82)	1.07 (0.69)	0.37 (0.74)	−0.31 (0.42)	−0.41 (0.45)	−0.44 (0.21)
Disorganised symptoms	0.77 (0.91)	0.79 (0.86)	0.15 (0.51)	−0.33 (0.16)	−0.35 (0.21)	−0.4 (0.2)

Percentage correct with standard deviations.

Behavioural data

Group differences in WM performance (see Table 2) were examined with a repeated measures ANOVA with accuracy as the dependent variable, diagnosis (patients, siblings, and controls) and genotype (Val homozygotes and Met carriers) as between-group factors, and stimulus type (word or face) as a within-participants factor. The ANOVA revealed a main effect of stimulus type, $F(1, 99) = 15.8$, $p < .01$. In general, participants were more accurate during the word condition than they were during the face condition. We did not observe a diagnosis by stimulus type effect, $F(2,98) = 1.4$, $p > .05$, a genotype by stimulus type interaction, $F(1, 99) = 1.8$, $p > .05$, nor did we find a three-way stimulus by diagnosis by genotype interaction, $F(2, 98) = 2.6$, $p > .05$. We did find an omnibus effect of diagnosis, $F(2, 98) = 15.1$, $p < .01$. Overall, patients were the least accurate, followed by siblings. Controls had the highest accuracy within our sample. We did not observe a main effect of genotype, $F(1, 99) = 2.6$, $p > .05$. A trend towards an effect of diagnosis by genotype, $F(2, 98) = 2.9$, $p = .06$, was observed, such that patient and sibling Vals performed worse than control Vals, patient Met carriers performed worse than control and sibling Met carriers, and sibling Vals performed worse than sibling Met carriers. However, the performance of patient and control genotype groups were nearly identical to one another. We did test for normality and found that our data was skewed. We then performed an arcsine transform on our data and reran our analyses but our results were unchanged. We again found significant main effects of stimulus type, $F(1, 99) = 23.3$, $p < .01$, and diagnosis, $F(2, 98) = 18.9$, $p < .01$, but did not observe significant interactions between any of our variables.

Imaging

A priori regions of interest. The ROI analysis did not reveal any significant main effects of diagnosis that did not also show higher order interactions (Table 3). A region in the left putamen demonstrated a significant main effect of genotype, with Val homozygotes showing higher activation than Met carriers, $F(1, 95) = 12.32$, $p < .01$. We also observed a significant diagnosis by genotype interactions in the putamen, $F(2, 95) = 6.6$, $p < .01$, caudate, $F(2, 95) = 6.99$, $p < .01$, and two middle frontal gyrus (MFG) regions, BA 46, $F(2, 95) = 6.36$, $p < .01$, and BA 9, $F(2, 95) = 7.93$, $p < .01$, respectively (Figure 1). Interestingly, in all four of these regions Val homozygotes in the patient and sibling groups showed higher activation than Met carriers (Figure 2). Further, for the regions that displayed a diagnosis by genotype interaction, patient and sibling Val homozygotes significantly differed from control Val homozygotes (Figure 2), whereas patient and

TABLE 3
Regions from the a priori analysis showing interaction effects of genotype, and diagnosis

Region	*BA*[1]	*X*	*Y*	*Z*	*F-value*	*Pattern*
Region of interest						
COMT						
Putamen		−26	−15	3	12.32	Val > Met
DX × COMT						
Putamen		−25	−7	1	6.60	Val > Met*
Caudate		9	7	13	6.99	Val > Met*
Middle frontal gyrus	46	27	44	26	6.36	Val > Met*
Middle frontal gyrus	9	−20	46	36	7.93	Val > Met*
Whole-brain analysis						
COMT						
Cerebellum		−43	−65	−14	27.77	Val > Met
Putamen		−29	−17	5	14.87	Val > Met
DX × COMT						
Lingual gyrus	18	0	−97	−10	15.60	Val > Met**
Putamen		−29	4	0	9.15	Val > Met*

[1]Brodmann's Area. *Pattern observed for patients and siblings only. **Pattern observed for patients only.

sibling Met carriers only differed from control Met carriers in one region (caudate region 9, 7, 13; see Figure 2).

Whole-brain analysis. We found no regions that showed a main effect of diagnosis that did not also show higher order interactions. A main effect of genotype was observed in the cerebellum (Figure 3), where Val homozygotes showed higher activation than Met carriers. Regions in the lingual gyrus and putamen showed interactions between diagnosis and genotype. In the putamen, as in the ROI analysis, Val homozygote patients and siblings

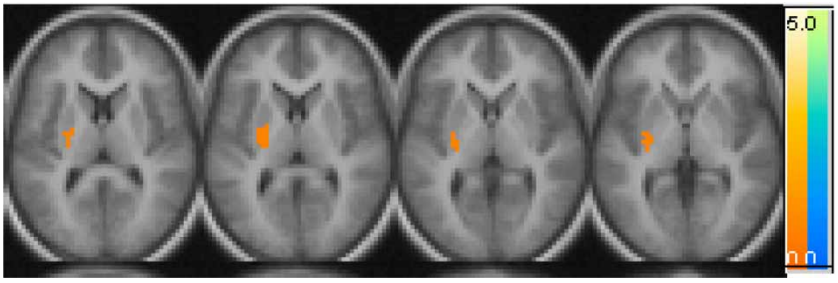

Figure 1. Regions from the ROI analysis that showed COMT effects.

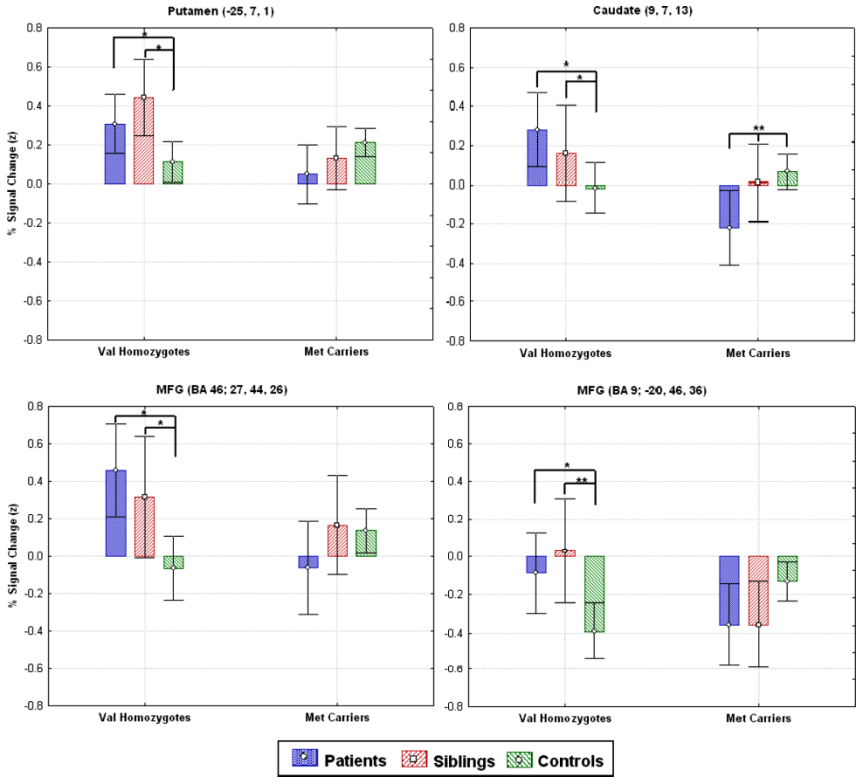

Figure 2. Regions from the ROI analysis that showed a diagnosis by genotype interaction. In the putamen, the caudate, and one of the middle frontal gyrus regions (BA 46), the patient and sibling Val homozygotes showed significantly greater activation than control Val homozygotes (*p*s $<.05$). In contrast, in the caudate, the patient and sibling Met carriers actually showed significantly less activation than control Met carriers (*p*s $<.01$), but did not differ from control Met carriers in the putamen or the middle frontal gyrus regions.

showed higher activation than Met carriers. For the lingual gyrus, only patient Vals showed higher activation than Met carriers. In the putamen, there was a trend for patient and sibling Val homozygotes to show greater activation than controls ($p = .07$), whereas the patient and sibling Met carriers again showed less activation than controls ($p < .05$). A similar pattern was observed in the lingual gyrus.

COMT effects within controls. We observed no differences between Val and Met carriers in these regions for our control group, although numerically Met carrier controls typically displayed greater activation than control Val homozygotes. However, because we had a larger sample of control participants we decided to break the Met carrier group up into

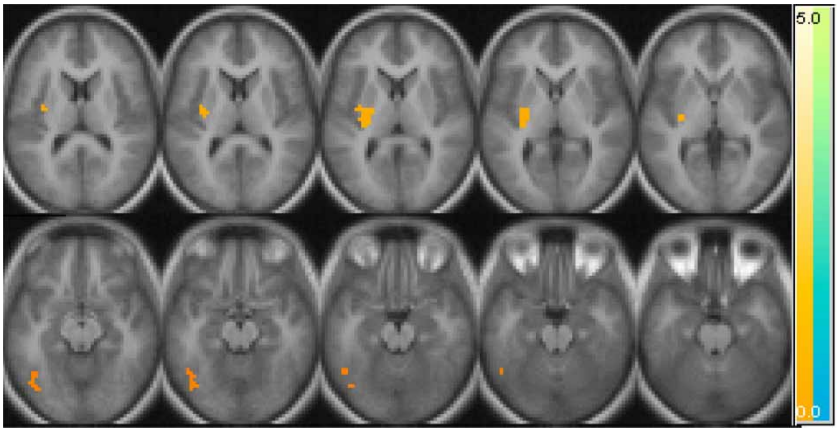

Figure 3. Regions from the whole brain analysis that showed COMT effects.

respective heterozygote and Met homozygotes, to examine differences between control Val homozygotes and Met homozygotes for the regions that showed COMT effects in our main analysis. Once done we did find an effect of COMT in our control group, such that one region in the caudate (9, 7, 13), MFG (−20, 46, 36), and a region in the putamen (−29, 4, 0) control Met homozygotes demonstrated increases in activity during working memory performance when compared to Val homozygotes. We also examined whether combining the control group and their well siblings impacted the pattern of results in our control group. To examine whether this was the case, we removed control siblings from the control group and reran our analyses and found that our results were essentially unchanged. For regions that previously only showed a main effect of COMT, once sibling controls were removed from the control group, they now also showed a COMT by diagnosis interaction. These regions (one region in the caudate and one in the putamen) were similar in location to regions that already showed significant interactions of COMT and diagnosis.

The relationship between behavioural performance, symptoms, and brain activation. Given the differential performance between our subject groups, we performed ANCOVAs with in-scanner behavioural performance as a covariate for regions that showed significant effects of diagnosis. Previous studies have found a relationship between reaction time duration and brain activation (Honey, Bullmore, & Sharma, 2000, 2002), so we included both accuracy and reaction time as covariates. The results indicated that differences in accuracy and a combination of accuracy and reaction time did not appear to be responsible for differences in activation, as all regions continued to show significant effects of diagnosis (all $ps < .05$) when

performance was used as a covariate. Further, Graybiel (1997) suggests that, given previous lesion work, disruption of functioning within the striatum could lead to symptoms like apathy and anhedonia that may be expressed as

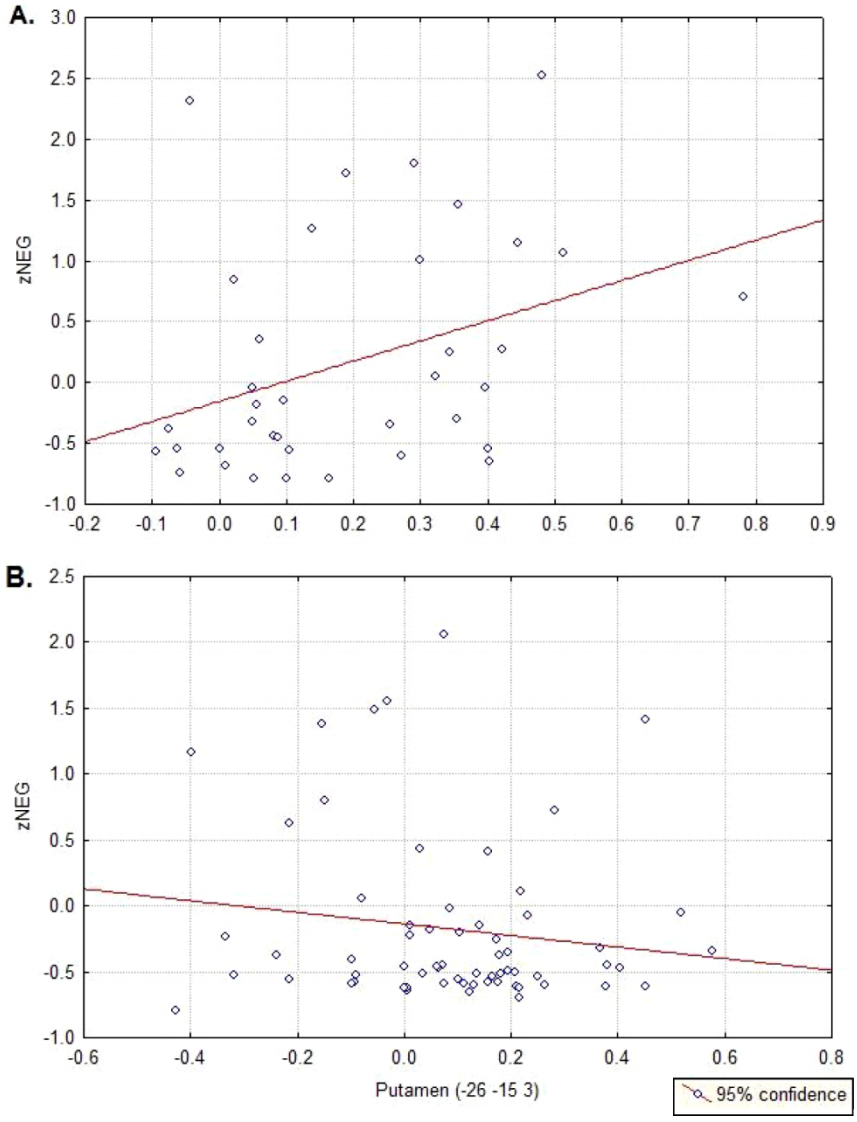

Figure 4. (A) Correlation between *z*-scores of the negative symptom category and the only region that showed a main effect of COMT. The correlation was significant, $r = .36$, $p = .03$, for Val homozygotes in this region, whereas (B) the correlation between positive symptoms and neural activity for this region was not significant for Met carriers.

increased negative symptom expression. To assess whether there was an association between COMT related striatal activity and clinical symptoms, we ran correlation analyses between the left putamen region (−26, −15, 3) that showed a main effect of genotype (Table 3) and the three symptom categories (positive, negative, and disorganised symptom *z*-scores). We found that putamen activity of Val participants only significantly correlated with negative symptoms (Figure 4), $r = .36$, $p = .03$, but not positive or disorganised symptoms. Activity within the left putamen of Met carriers did not significantly correlate negative symptoms (Figure 4), nor did it significantly correlate with positive or disorganised symptoms.

DISCUSSION

Like previous studies, we found that DLPFC activity was increased in Val homozygotes as compared to Met carriers during a task of WM, even when controlling for performance differences. We replicated previous studies that found involvement of the BG, particularly the striatum, during working memory processing (Chang et al., 2007; Voytek & Knight, 2010). We also replicated previous studies that found Val homozygotes demonstrated greater DLPFC activity as well as striatal activity during WM processing (de Frias et al., 2009; Tan et al., 2007). Further, we were interested in assaying these results across diagnostic groups by assessing the symptoms, behavioural performance, and neural activity of patients with schizophrenia, their first-degree relatives, and healthy control participants. We found that COMT status influenced the pattern of differences across groups, such that patient and sibling Val homozygotes showed greater activation than controls in both striatal and prefrontal regions. In contrast, patient and sibling Met carriers either showed no difference from controls, or less activation. Interestingly, although we did observe COMT effects in our control population, the pattern of effects differed from our patient and sibling group.

COMT genotype and behavioural performance

In-scanner working memory performance of our participants revealed expected differences between the diagnostic groups. However, we did not find a main effect of genotype. Although the pattern of genotype effects suggested that COMT impacted our diagnostic groups differently, we also failed to find a significant genotype by diagnosis interaction. The pattern of performance was similar to what has been observed previously (Goldberg et al., 2003), such that Val homozygotes performed worse than Met carriers, so it may not be surprising that this effect did not reach significance, particularly given the findings of others (Barnett et al., 2008; Stefanis et al.,

2004). In a meta-analysis of the association between the Val158Met COMT polymorphism and cognitive phenotypes like IQ, *n*-back performance, and verbal recall, Barnett et al. (2008) found no robust association between the polymorphism and these phenotypes within schizophrenia patient groups or healthy control groups. However, they did find that the effect size of the association between COMT and *n*-back performance was larger for the patient groups than for nonpatient groups. This effect was not in the predicted direction, such that the Val allele was associated with better performance (Barnett et al., 2008). Other studies seeking to replicate the association between COMT, schizophrenia, and executive functioning have found an association between COMT and executive functioning for controls but not patients (Rosa et al., 2004), or have found an impact of COMT on some cognitive domains for patients but not executive functioning (Bilder et al., 2002). We did not find that COMT significantly impacted behavioural performance during working memory, nor did we find an effect of COMT in either of our diagnostic groups. Taken together, these findings, and ours, suggest that the association between COMT, cognition, and schizophrenia is tenuous at best, which would be consistent with genetic association studies examining the relationship between COMT and schizophrenia (Munafò, Bowes, Clark, & Flint, 2005). However, given the possible confounds of other factors like, but not limited to, measurement error, the best way to assess the subtle effects of polymorphisms like COMT may be with phenotypes that are more refined (Sabb et al., 2009). One approach to doing this is by studying latent variables. Models employing a latent variable approach attempt to control for some of the extraneous variance (e.g., method variance, measurement error, shared variance, etc.) associated with observable variables in an attempt to study the unique variance associated with a particular construct. This method has been successful at furthering our understanding of the unity and diversity of executive functioning (Miyake et al., 2000), as well as revealing genetic and environmental contributions to individual differences in executive functioning (Friedman et al., 2008). Alternatively, examining changes in neural activation may prove more sensitive to the subtle gene effects of COMT on working memory than examining the observable construct alone.

COMT genotype and neural processing

Consistent with our hypothesis, we found that the Val allele was associated with greater activity in the caudate, putamen, and middle frontal gyrus than Met carriers. This further supports neuroanatomic and functional imaging studies that suggest a role for both the PFC and BG in mediating WM demands (Frank et al., 2001; McNab & Klingberg, 2008; Middleton &

Strick, 1994). Specifically, these results provide some evidence suggesting that changes in dopamine availability in the frontal cortex has some influence on striatal functioning, extending the findings of Meyer-Lindenberg et al. (2005). They found a relationship between prefrontal activity subcortical dopamine synthesis in a healthy population during a working memory task, but they did not observe a direct effect of COMT on subcortical activity. Additionally, because this effect was evident in Val homozygotes (with putatively lower levels of available DA), our findings are consistent with the hypotheses that adequate levels of DA in both PFC and striatum are necessary for regulated neural processing during WM tasks. Our findings are also consistent with the proposed relationships between prefrontal DA availability and striatal DA tone (Bilder et al., 2004).

We also found that COMT genotype influenced the ways in which patients and siblings differed from controls in terms of functional brain activation. We found that COMT differentially affected frontostriatal activity, such that patient and sibling Val homozygotes demonstrated greater frontal and striatal activity than controls during a working memory performance. Patients and sibling Val homozygotes not only displayed greater activity than all Met carriers, but they also displayed significantly greater activity than control Val homozygotes. This was true for essentially all regions of our ROI analysis that showed a diagnosis by genotype effect. On the other hand, we observed some evidence for the opposite pattern of effects for our control subjects, such that Met homozygotes demonstrated increased striatal activation during working memory performance and attenuated deactivation in the MFG. The pattern of these findings may be explained by the "inverted U" hypotheses, which suggest that neural activation is most efficient when DA levels are optimal, but increases or decreases in DA can produce neural activity that is inefficient (Mattay et al., 2003). In the case of our control Met homozygotes, increased DA availability may have moved them beyond what would be considered optimal, and thus produced increases in activity the same way suboptimal DA would produce increases in neural activity. Our findings support the proposed association between COMT and schizophrenia, but are distinct from previous studies that found that COMT impacted neural activity associated with working memory processing similarly for patients and controls. However, we are not the first study to demonstrate neural activity differences between patients and controls as a function of COMT. For example, Prata et al. (2009) examined the influence of COMT on verbal fluency and regional brain function and found that patients and controls demonstrated a differential pattern of effects for frontotemporal activity such that the Met allele was associated with greater deactivation for patients, and where patient Val homozygotes demonstrated increased activation control Val homozygotes demonstrated increased deactivation. During recognition memory, Di

Giorgio et al. (2011) also found increased activation of parahippocampal and hippocampal regions to be associated with the Met allele.

The differential effects of COMT we observed between our patient groups could reflect differences in the influence of other genes on cognition and brain function in controls versus individuals with schizophrenia. Given that the risk of developing schizophrenia may be influenced by multiple risk genes of small effect, or result from an interaction of such genes and environmental stressors, one might expect that patients with schizophrenia and their relatives would possess other genetic alleles that can exert a negative influence on DA function. In this sense, the additive effects of risk genes or epigenetic effects on patient and siblings COMT genotypes may confer cognitive impairment or neural disruption differently than it does for control groups. This is consistent with the idea of a final common pathway proposed by Howes and Kapur (2009). Evidence for differential dopamine signalling between patients with schizophrenia and control subjects is provided by Meyer-Lindenberg et al. (2002), who found that dopaminergic neurotransmission in the striatum differed between patients and controls during a task of executive functioning, and that these changes in dopamine transmission observed in patients was tied to prefrontal activity. COMT, then, is only one small player in a complex series of factors that would contribute to the symptoms of schizophrenia. The degree to which this particular allele contributes to schizophrenia risk is unclear. There is some evidence, provided by Wirgenes et al. (2010), to suggest that out of all of the polymorphisms within the COMT gene only the Val158Met SNP demonstrated a significant association with working memory, and only within the schizophrenia group. Other studies suggest that the Val158Met polymorphism itself is not disease-causing but is in strong linkage disequilibrium with another variant (Handoko, Nyholt, Hayward, Nertney, Hannah et al., 2005), or that COMT exerts its influence on schizophrenia risk at the level of a multi-SNP haplotype (Li et al., 2000; Shifman et al., 2002). Whereas some large genome-wide association studies (GWAS) have had some difficulty replicating the relevance of some SNPs (Need et al., 2009), a large scale meta-analysis of 1179 gene studies found that four genes (DRD1, DTNBP1, MTHFR, and TPH1) were most strongly linked to disease susceptibility for schizophrenia (Allen et al., 2008). An alternative to GWAS is hypothesis-driven candidate gene studies, which have been useful for creating meaningful connections between genes and specific biochemical pathways that may be associated with a particular clinical disorder. However, a comparison of GWAS results and the candidate gene literature for schizophrenia found that the candidate gene literature did not yield markers with predictive power for schizophrenia (Collins et al., 2012), suggesting a poor overlap between genome-wide studies and candidate gene studies. One interpretation of this finding is that candidate gene studies are failing to reveal genes that are

relevant to clinical pathology; however, this issue remains complicated. One possible way forward is provided by Potkin et al. (2009), who used differences in brain activation as a phenotype, and then sought SNPs that influence this phenotype instead of going from the candidate gene to the phenotype. Although it is not clear that GWAS or candidate gene studies are yielding information about genes that play a large role in psychiatric disease pathology, they have yielded information about genes and functional polymorphisms that impact relevant neurobiological pathways implicated in cognition and symptom expression, which is useful for studying the mechanisms in vivo.

Clinical symptoms

The potential relationship between COMT variation and symptom expression not clear. Recent theories about the role of DA in schizophrenia suggest that decreased available DA in the PFC is associated with negative symptoms, whereas dysregulated DA subcortically could lead to positive symptoms (Weinberger et al., 2001). According to this framework, the Val allele would be associated with negative symptoms and, due to corticostriatal interactions, also an increase in positive symptoms. Alternatively, Bilder et al. (2004) suggested that increased postsynaptic tonic DA associated with the Met allele would lead to decreased phasic DA release within the synapse, and therefore cognitive rigidity and negative symptoms. We found that activity in a region of the putamen that demonstrated an effect of COMT significantly correlated with negative symptoms of Val homozygotes but not Met carriers. Given the proposed role of corticostriatal loops and dopamine in the expression of apathy (for a review, see Levy & Dubois, 2006), this finding may make some sense. Although examining COMT effects on symptoms expression was not a focus of this study, this finding is intriguing in that it gives some credence to the notion that a putative disruption of DA signalling and associated changes in activity in the BG may impact clinical symptom expression. Further work is needed to explore the influence subcortical structures, like the BG, have on clinical symptom expression.

Limitations

The majority of the individuals with schizophrenia were treated with antipsychotic medication, and most of these individuals were treated with atypical drugs. Medication status could have interacted with the influences of COMT genotype in our study, but COMT effects have previously been observed in unmedicated patients (Woodward, Jayathilake, & Meltzer, 2007). Furthermore, many of the same genotype effects on cognition and neural

activation of the unmedicated siblings of patients with schizophrenia were observed previously (Egan et al., 2001; Goldberg et al., 2003; Rosa et al., 2004), and were also observed in our unmedicated sibling sample. Another limitation was sample sizes. Given the subtle nature of gene effects from a single allelic variation, larger sample sizes would enhance power to detect differences caused by such variations. However, despite our small samples, we did detect patterns consistent with previous studies of COMT effects on cognition and neural activation. In addition, our WM contrast compared a two-back WM condition to fixation, a contrast that may encapsulate processes that immediately relevant to WM, such as encoding, response selection, and motor execution. Of particular concern, given our focus on the BG, is motor execution. To some degree this may account for the activity we observed in the striatum during WM, but we would not expect striatal activity associated with the motor activity of buttonpresses to vary as a function of genotype or diagnostic category. However, additional work will be necessary to address whether our findings are specific to WM as opposed to more general to a range of processing mechanisms, both of which are important to understand.

CONCLUSIONS

Our findings support and extend previous studies of COMT genotype variability on neural activity during WM performance. We found further evidence to support an important role for COMT genotype variability in the direct regulation of PFC function, and on the indirect regulation of striatal function. Further, we found that COMT allelic status influenced the pattern of group differences in functional brain activation, with patient and sibling Val homozygotes showing evidence for increased activation in PFC and striatal regions compared to controls. These results add to the growing literature suggest that both cognitive performance and functional brain activation during WM is influenced by genetic variation that controls DA function, and that such effects are important for understanding behaviour and brain function among individuals with schizophrenia.

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