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The Effects of The COMT val$^{108/158}$met Polymorphism on BOLD Activation During Working Memory, Planning, and Response Inhibition: A Role for The Posterior Cingulate Cortex?

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Catechol-O-methyl transferase (COMT) val$^{108/158}$met polymorphism impacts on cortical dopamine levels and may influence functional magnetic resonance (fMRI) measures of task-related neuronal activity. Here, we investigate whether COMT genotype influences cortical activations, particularly prefrontal activations, by interrogating its effect across three tasks that have been associated with the dopaminergic system in a large cohort of healthy volunteers. A total of 50 participants (13 met/met, 23 val/met, and 14 val/val) successfully completed N-Back, Go-NoGo, and Tower of London fMRI tasks. Image analysis was performed using statistical parametric mapping. No significant relationships between COMT genotype groups and frontal lobe activations were observed for any contrast of the three tasks studied. However, the val/val group produced significantly greater deactivation of the right posterior cingulate cortex in two tasks: the Go-NoGo (NoGo *vs* Go deactivation contrast) and N-Back (2-back *vs* rest deactivation contrast). For the N-Back task, the modulated deactivation cluster was functionally connected to the precuneus, left middle occipital lobe, and cerebellum. These results do not support findings of prefrontal cortical modulation of activity with COMT genotype, but instead suggest that COMT val/val genotype can modulate the activity of the posterior cingulate and may indicate the potential network effects of COMT genotype on the default mode network.

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INTRODUCTION

Catechol-O-methyl transferase (COMT) is a major catabolic regulator of synaptic catecholamine neurotransmitters. COMT is particularly important for the regulation of cortical dopamine levels, especially in the prefrontal cortex, due to the relative lack of dopamine transporters in these areas (Tunbridge *et al*, 2006). A missense COMT val$^{108/158}$met polymorphism functionally impacts on the rate of COMT catabolism resulting in more efficient cortical dopamine catabolism and lower synaptic dopamine levels in val/val homozygotes (Chen *et al*, 2004). Converging evidence from experimental animal and human studies implicates COMT allelic variations in tuning cortical dopamine levels and consequent function (Mier *et al*, 2009; Tan *et al*, 2009; Tunbridge *et al*, 2004). In humans, a number of studies have examined the influence of the COMT polymorphism on functional MRI activations (see Table 1 for summary), employing different tasks and analytical strategies. Initial studies clearly showed a correlation between val allele genotype and frontal activations (Mier *et al*, 2009), with val/val homozygotes showing the greatest activation (eg, see Egan *et al* (2001)). Increased activation among val carriers is interpreted as being indicative of inefficient prefrontal function secondary to lower prefrontal synaptic dopamine levels, potentially due to reductions in cortical noise (Winterer *et al*, 2006). However, as is clear from Table 1, subsequent results have been more mixed; 10 studies indicate that the val/val group has increased frontal activations, whereas 7 studies show the opposite effect. One interpretation of these results is that decreased activations within the frontal cortex can be

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Table 1 Independent Effect of COMT Genotype on Frontal fMRI Activations in Normal Volunteers

Study	Task	No. of vols	COMT effect	Statistical threshold
Egan *et al* (2001)	N-Back	16 and 11	VV>MM: DLPFC	$p<0.005$ uncorr
Smolka *et al* (2005)	Unpleasant visual emotional	35	MM>VV: VLPFC and middle frontal gyrus	$p<0.05$ FDR corr
Blasi *et al* (2005)	Variable attentional control	23	VV>MM: ACC	$p<0.05$ FWE corr
Schott *et al* (2006)	Word recall	49	VV>MM: right PFC	$p<0.005$ uncorr
Bertolino *et al* (2006a)	N-Back	62	Met allele no. negatively correlated with response in bilateral mid frontal lobe	$p<0.05$ FWE corr in a 10 mm ROI frontal sphere
Winterer *et al* (2006)	Oddball task	44	MM>VC: bilateral medial frontal lobe	$p<0.05$ cluster corrected for ROI defined in whole group
Bertolino *et al* (2006b)	Recognition memory task	27	Met allele load: negative correlation for VLPFC	$p<0.005$ uncorr and FWE corr in a 10 mm frontal ROI
Drabant *et al* (2006)	Emotional facial recognition	101	MM>VV: right VLPFC	$p<0.05$ corr for a 35 voxel frontal ROI
Tan *et al* (2007)	N-Back	29	VV>MM: DLPFC and VLPFC ROI's	$p<0.001$ uncorr for a 10 mm frontal ROI
Caldu *et al* (2007)	N-Back	75	No effect	$p<0.005$ uncorr
Tan *et al* (2007)	Numerical working memory	24	VV>MM: right DLPFC	$p<0.05$ corr for a functionally defined frontal ROI
Ettinger *et al* (2008)	Saccades	36	MM>VC: DLPFC and VLPFC during antisaccades	$p<0.05$ corr for a 10 mm frontal ROI
Bishop *et al* (2008)	Verbal and spatial problem solving	22	VV>MM: right DLPFC	$p<0.05$ corr for DLPFC volume
Prata *et al* (2008)	Verbal fluency	48	MM>VV: ACC	$p<0.001$ uncorr
Congdon *et al* (2009)	Response inhibition	43	MM/VM>VV: in all 4 ROI's during both Go and StopInhibit blocks	$p<0.05$ corr for right IFC, right STN, right SMA, right GP ROI's
Sambataro *et al* (2009)	N-Back	75	VV>MM: DLPFC MM>VV:VLPFC	$p<0.05$ corr for 20 mm DLPFC and VLPFC ROI's
Rasch *et al* (2010)	Emotional pictures	56	MM>VM/VV: medial PFC	$p<0.001$ uncorr
De Frias *et al* (2010)	N-Back	22	VV>MM: Superior DLPFC sustained activity during maintenance	$p<0.05$ corr for 3 ROIs
Camara *et al* (2010)	Reward gambling task	36	VV>MM: ACC gain > loss	$p<0.01$ corr for cluster

Abbreviations: ACC, anterior cingulate; corr, corrected; DLPFC, dorsolateral prefrontal cortex; GP, globus pallidus; IFC, inferior frontal cortex; MM, met/met genotype; ROI, region of interest; SMA, supplementary motor area; STN, subthalamic nucleus; uncorr, uncorrected; VC, val carriers; VLPFC, venterolateral prefrontal cortex; VV, val/val genotype.

explained by the use of emotional tasks (Mier *et al*, 2009) such as facial emotion recognition (Drabant *et al*, 2006), although studies using the N-Back (Sambataro *et al*, 2009) and antisaccades (Ettinger *et al*, 2008) tasks have also shown this direction of effect. Another possible interpretation is that the variations across studies represent different tasks, or subprocesses within tasks, which have differential sensitivity to variations in dopamine tone (Tan *et al*, 2007).

In this study, we sought to replicate previous studies using the N-Back task and additionally selected two further tasks, the Tower of London (TOL) and Go-NoGo, which have also been associated with the dopaminergic system. These additional tasks were selected to first interrogate whether the COMT val$^{108/158}$met polymorphism does have selective effects on prefrontal cortical activations within the same cohort of volunteers, and second to determine whether COMT genotype may also impact on activations in other associated cortical regions.

The first task, the N-Back, is known to be sensitive to the effect of COMT genotype on prefrontal activations (Caldu *et al*, 2007; Egan *et al*, 2001; Tan *et al*., 2007). We selected the second task, the Go-NoGo task, to address the relationship between response inhibition networks and COMT genotype. A previous study of the motor response effects during a response inhibition task showed that a COMT val/val group produced reduced activation in the inferior frontal gyrus and basal ganglia during both inhibition and non-inhibition blocks (Congdon *et al*, 2009). The third task, the TOL, was chosen because of extensive evidence linking both brain dopaminergic markers, dopaminergic manipulations, and dopamine release to performance (Lappin *et al*, 2009; Mehta *et al*, 2005; Reeves *et al*, 2005). These imaging studies in healthy volunteers, as well as studies indicating reduced TOL performance in volunteers with disease-related dopamine depletion (Lawrence *et al*, 1998; Owen *et al*, 1996), implicate the *striatal* dopaminergic system. However, we have recently provided preliminary evidence of possible dopamine release in the prefrontal cortex during performance of the TOL task (Egerton *et al*, 2009), although direct evidence for the involvement of prefrontal dopamine systems in accurate planning is lacking.

In this study, we hypothesized that val/val homozygotes would show the greatest prefrontal cortical activity during

the N-Back and TOL tasks, with the opposite effect during the response inhibition Go-NoGo task. We also sought to identify regions that were functionally connected to cortical areas sensitive to COMT genotype and hypothesized that COMT genotype would alter the strength of these functional connections.

MATERIALS AND METHODS

A total of 65 healthy male and female volunteers (28–61 years of age, mean 43.0 years) were initially recruited for the study by means of public advertisement. To avoid ethnicity effects, only white British volunteers, whose parents were also white British, were recruited to the study. Volunteers were free from any physical illness, had no history of psychiatric disorder (as assessed using an abridged non-patient version of the Structured Clinical Interview for DSM-IV disorders (First and Pincus, 2002), and had not previously used psychotropic medications. Ethical approval for the study was obtained from the Hammersmith, Queen Charlotte's, and Chelsea Research Ethics committee, and all participants gave written informed consent.

DNA Collection and Genotyping

DNA was extracted from saliva using Oragene DNA Self-Collection Kits (DNA Genotek, Ottawa, Canada). The genotyping of the Val^{158}Met polymorphism (rs4680) in the COMT gene was performed using the Amplifluor SNP Genotyping System (Myakishev *et al*, 2001) according to the manufacturer's recommendations (Serologicals Corporation, Norcross, GA, USA). The allele-specific products were resolved on an Analyst fluorescence plate reader (LJL Biosystems, Sunnyvale, CA, USA).

MRI Acquisition

Functional MRI scanning was performed using a 3-Tesla Philips Intera scanner (Best, Holland). Functional T2*-weighted images were acquired using gradient-echo echoplanar imaging, with an automated higher order shim procedure (SENSE factor 2; TE 30; TR 3000 ms; flip angle 90°; FOV 280 mm; voxel dimensions $2.2 \times 2.2 \times 2.75\,mm^3$). Images were acquired in 48 contiguous 2.75 mm axial slices per brain volume. Three experimental tasks were performed: an N-Back, a Go-NoGo, and a TOL task. The total number of brain volumes acquired for each of these tasks was 218, 154, and 169. The first five volumes of each scan were discarded to account for T1 equilibration effects. Functional images were acquired during a single run of 7 min 42 s (Go-NoGo), 8 min 27 s (TOL), and two runs of 5 min 27 s (N-Back). A high-resolution T1-weighted TFE structural scan was also acquired for each participant for normalization purposes (TE 4.6 ms; TR 9.7 ms; Flip angle 8°; FOV 240 mm; voxel dimensions $0.94 \times 0.94 \times 1.2\,mm^3$).

Functional Magnetic Resonance (fMRI) Experimental Tasks

All tasks completed during fMRI scanning followed a block design. Tasks were programmed with E-Prime, version 1.1 (Psychology Software Tools), and presented using the Integrated Functional Imaging System (Invivo). All three tasks were completed during the same fMRI session, in a counter-balanced order.

N-Back task. This task of sustained attention with a working memory component was derived from the original version by Gevins and Cutillo (1993). A dot was presented in one of four spatial locations, arranged horizontally across the screen. Each location corresponded to a particular button on a response pad. Participants were required to press the button that corresponded to where the dot was currently appearing (0-back), where the dot had appeared one trial previously (1-back), or where the dot had appeared two trials previously (2-back). Dots were presented for 500 ms followed by a blank screen for 1500 ms, with 12 dots being presented in a block. Three '0-back' blocks, three '1-back' blocks, and three '2-back' blocks were presented in a pseudorandom order, interspersed with two blocks of a low-level rest condition (eyes open and fixation on a cross with no responses). Each block lasted for 24 s. Instructions were presented for three seconds before the start of each block. This version of the N-Back task with high demands on continual updating of information stored has been proposed as more sensitive to COMT val$^{108/158}$met polymorphism than versions, which only require indication of correct responses (Goldman *et al*, 2009).

Go-NoGo task. Participants viewed letters (F, H, K, P, S, and V) presented on the screen and responded to each letter with a button press, except for the letter 'V'. Letters were presented for 500 ms followed by a blank screen for 1500 ms, with 18 letters being presented in a block. During the Go condition, all letters required a response (no 'V's were presented). During the NoGo condition, the letter 'V' was presented on eight trials, in a pseudorandom order. Five 'Go' blocks and five 'NoGo' blocks were presented in a pseudorandom order interspersed with two blocks of a rest condition (fixation). Each block lasted for 36 s.

TOL task. This planning task, derived from the one used by Owen *et al* (1996), required participants to determine the minimum number of moves that it would take to re-arrange three colored balls from a start position to a target position. Balls were positioned within three pockets, and each pocket could hold a maximum of one, two, or three balls, respectively. Participants were presented with a display containing an upper 'target' section that indicated the final position of the colored balls, and a lower 'start' section that indicated the start position of the colored balls. They were then asked to indicate the minimum number of moves required to rearrange the balls via a single-finger keypad response. During the 'Count' block participants were requested to count the number of balls in both the start and target pockets and again indicate this via a single-finger keypad response. Four 'Easy Plan' blocks, four 'Difficult Plan' blocks, and four 'Count' blocks were presented in a pseudorandom order, interspersed with three blocks of a rest condition (fixation). Each block lasted for 30 s, however, trials within each block were self-paced. Instructions were presented for 3 s before commencement of the blocks.

fMRI Analysis

Quality of the functional images was assessed before analysis using tsdiffana (http://imaging.mrc-cbu.cam.ac.uk/imaging/DataDiagnostics). Functional image analysis was performed using Statistical Parametric Mapping (SPM5; Wellcome Department of Neurology, UCL) with Matlab version 7.1. Pre-processing steps before statistical analysis included motion correction and spatial normalization to a standard template in MNI space (using the T1 SPM template and resulting in voxels of dimension $2 \times 2 \times 2\,mm^3$). Normalized images were smoothed with an 8 mm full-width half-maximum isotropic Gaussian kernel. Task conditions (Go-NoGo: rest, Go, NoGo; N-Back: rest, 0-back, 1-back, 2-back, instructions; TOL: rest, count, easy plan, difficult plan, instructions) were modelled with appropriate regressors convolved with the standard hemodynamic response function. To minimize the influence of signal changes due to head movement, the six realignment parameters were included in the design matrix. A temporal high-pass filter (cutoff 256 s) was applied, and temporal autocorrelation was modelled as an AR(1) process. Individual participant images were analyzed at the first level to produce estimates for the contrasts of interest. These images were then entered into a second level random effects analyses using one-sample *t*-tests (thresholded at $p<0.001$ uncorrected) to produce the main effects of tasks (activation and deactivations). A $p<0.001$ uncorrected threshold was used as a conservative approach that produces a larger task activation mask for the subsequent genetic analysis compared with a corrected threshold.

To investigate the effect of COMT genotype on fMRI response, a full factorial model within SPM was used with genotype group as a single factor with three levels (met/met, val/met, and val/val; Prata *et al*, 2008). This model accommodates the assumption of genetic co-dominance, similar to a regression approach, but also allows for other patterns of response to be detected. Explicit masks, derived from the analyses of the main effects of the tasks were used to constrain analyses to only those voxels that were activated or deactivated by the task of interest. An additional analysis, restricted to activated voxels of the frontal lobe in line with the *a priori* hypotheses, was performed by multiplying explicit task masks with a mask comprised of the WFU PickAtlas (Wake Forest University, NC) frontal lobe region of interest (ROI), comprised of 70 324 voxels, using image algebra within SPM5. This additional procedure was performed to minimize potential type II errors. Regions showing significant association with COMT genotype (seed regions) were further explored using a psychophysiological interaction (PPI) connectivity analysis (Friston *et al*, 1997). This analysis shows brain regions where the correlation with the seed regions changes in different contexts; ie, context-dependent functional coupling.

MNI coordinates produced from SPM were converted to Talairach coordinates using a nonlinear transformation algorithm (mni2tal; Brett *et al*, 2002). Localization of the peak voxel in each cluster was identified using the Talairach Client (http://www.talairach.org). All coordinates reported here and within the Supplementary Information are MNI coordinates and all results are overlaid on a single-subject image T1-weighted image provided with SPM when graphically presented. Results are reported using a voxel-wise statistical significance threshold of $p<0.05$ FWE corrected for whole-brain multiple comparisons, although for completeness we also report, but do not interpret, results using a statistical threshold of $p<0.001$ (uncorrected for whole-brain multiple comparisons).

Statistical Analysis

All statistical comparisons, with the exception of the SPM analysis, were performed using SPSS 16.0 (SPSS, Chicago, Illinois). Genetic deviation from Hardy–Weinberg equilibrium was determined using χ^2 test. Demographic influences on COMT genotype were assessed either using a one-way ANOVA for continuous data or using χ^2 test for discontinuous data. Behavioral data were assessed using a multivariate general linear model.

RESULTS

Participant Characteristics

A total of 56 volunteers were included in the final analyses. Nine volunteers were excluded for the following reasons: scanner failure ($n=3$), genotyping failure ($n=4$), disclosure of dyslexia after scanning ($n=1$), and brain structural abnormalities ($n=1$). In addition, because of poor functional image quality, a further six volunteers were excluded from the N-Back task, seven volunteers from the Go-NoGo task, and nine volunteers from the TOL task. Therefore, the final number of volunteers included in the analyses was $n=50$, $n=49$, and $n=47$ for the N-Back, Go-NoGo, and TOL tasks, respectively.

COMT genotype did not deviate from Hardy–Weinberg equilibrium for any of the three volunteer task groups (N-Back: $\chi^2=0.3$, df 1, $p<0.6$; Go-NoGo: $\chi^2=1$, df 1, $p<0.4$; TOL: $\chi^2=1$, df 1, $p<0.4$). There were no significant differences in age, predicted IQ (one-way ANOVAs, all $p>0.2$) or handedness (χ^2, all $p>0.8$) between the COMT genotype groups. Gender was included as a covariate in the analyses of all three tasks as there were significantly more females in the val/val and val/met genotype groups compared with the met/met group for both the Go-NoGo and TOL tasks ($\chi^2=6.27$, df 2, $p=0.04$ and $\chi^2=9.30$, df 2, $p=0.01$, respectively), and at trend level significance for the N-Back task ($\chi^2=5.08$, df 2, $p=0.08$). Participant characteristics are provided in Table 2.

Behavioral Data

For the behavioral results please see Table 3. None of the performance measures differed between the COMT genotype groups.

Functional Imaging Data

Main effect of the tasks. All three tasks produced robust activation of distributed networks, including frontal cortical regions. Areas of activation for the N-Back task included the middle and inferior frontal gyri, and inferior parietal cortex. Areas of activation for the Go-NoGo task included the

Table 2 Participant Characteristics

	Met/Met	Val/Met	Val/Val
N-Back (n)	13	23	14
Age, years	37.2 (3.4)	35.5 (2.5)	35.6 (3.1)
Gender (M/F)	9:4	7:16	6:8
Handedness (R/L)	12:1	22:1	13:1
Predicted IQ	120.1 (0.9)	119.0 (1.1)	117.1 (1.0)
Go-NoGo (n)	14	21	14
Age, years	40.2 (3.2)	35.4 (2.3)	35.6 (3.1)
Gender (M/F)	10:4	6:15	6:8
Handedness (R/L)	13:1	20:1	13:1
Predicted IQ	118.8 (1.4)	118.8 (1.1)	117.1 (1.0)
TOL (n)	12	23	12
Age, years	37.9 (3.7)	34.8 (2.4)	35.4 (3.6)
Gender (M/F)	9:3	5:18	5:7
Handedness (R/L)	11:1	22:1	11:1
Predicted IQ	120.3 (1.0)	119.6 (1.0)	117.4 (1.2)

Values are mean (SEM).

Table 3 Behavioral Data

	Met/Met	Val/Met	Val/Val	*p*-value
N-Back				
0-Back errors	5.94 (1.17)	7.62 (0.85)	5.77 (1.23)	0.36
0-Back RT	1323 (54)	1352 (69)	1271 (84)	0.74
1-Back errors	9.50 (2.03)	9.88 (1.60)	8.69 (1.63)	0.90
1-Back RT	1049 (87)	1130 (109)	1273 (153)	0.50
2-Back errors	20.8 (2.99)	19.1 (2.40)	17.6 (3.51)	0.78
2-Back RT	1088 (110)	1106 (105)	1238 (183)	0.72
Go-NoGo				
Go omission errors	1.69 (0.31)	1.73 (0.42)	2.00 (0.35)	0.86
Go correct RT	488 (34)	447 (19)	436 (31)	0.40
NoGo omission errors	1.25 (0.28)	1.42 (0.33)	1.21 (0.32)	0.88
NoGo correct RT	474 (25)	458 (19)	442 (21)	0.65
NoGo commission errors	1.87 (0.52)	2.65 (0.52)	3.79 (0.89)	0.16
TOL				
Control errors[a]	2.53 (0.56)	4.22 (0.52)	4.84 (0.52)	0.085
Control RT	1707 (114)	1650 (88)	1663 (86)	0.95
'Easy' errors[a]	6.73 (0.66)	6.59 (0.44)	6.69 (0.86)	0.99
'Easy' RT	6385 (419)	5711 (484)	6286 (390)	0.43
'Difficult' errors[a]	7.40 (0.74)	7.04 (0.57)	7.77 (0.74)	0.35
'Difficult' RT	11816 (1034)	11094 (672)	13356 (1498)	0.26

Abbreviation: RT, reaction time in msec
Values are mean (SEM), all comparisons one-way ANOVAs.
[a]Analyses corrected for number of trials attempted; this did not differ between COMT genotype groups.

superior, middle, and inferior frontal gyri, and the posterior cortex. Similarly, the TOL task produced activations in these areas, and in addition activated the medial frontal gyrus and the precuneus (for a summary of the main activation coordinates for the three tasks see Supplementary Table 1; activation maps for the main contrast of interest for the N-Back, Go-NoGo, and TOL tasks are contained within Supplementary Figures 1–4). Age had no significant effect on either the activations or deactivations produced by the main contrasts of interest (all p values >0.05 FWE corrected).

Influence of COMT genotype on fMRI data.

N-Back task*:* For this task, we performed both parametric and subtraction analyses. For the parametric analysis, there was no effect of COMT genotype on the linear change in activations with task difficultly. For the subtraction analysis, we focussed on the most difficult condition (2-back) due to the clear activation of brain regions consistent with previous studies (Owen *et al*, 2005). The only differences with COMT genotype were for the 2-back *vs* 0-back activation and 2-back *vs* rest deactivation contrasts.

2-Back *vs* 0-back activation contrast: Val/val homozygotes activated a small cluster of voxels within the inferior frontal gyrus to a greater extent than the met/met homozygotes ($x=-28$ $y=28$ $z=-2$, cluster size 5 voxels, $t=3.55$, $p<0.001$ uncorrected). Met/met homozygotes activated regions within the superior parietal lobe ($x=32$ $y=-76$ $z=44$, cluster size 10 voxels, $t=3.59$, $p<0.001$ uncorrected) and the middle frontal gyrus ($x=38$ $y=18$ $z=40$, cluster size 6 voxels, $t=3.55$, $p=0.001$ uncorrected) to a greater extent than the val/val homozygotes. None of these effects survived correction for multiple comparisons across the task network or frontal lobe networks.

2-Back *vs* rest deactivation contrast*:* Analysis of areas that were deactivated during the task demonstrated that val/val homozygotes and val/met heterozygotes exhibited greater activity within the right posterior cingulate cortex than met/met homozygotes ($x=10$ $y=-58$ $z=28$, cluster size 140 voxels, $t=5.15$, $p=0.03$ FWE corrected; see Figure 1).

Go-NoGo task*:* For this task, the effect of inhibition trials *vs* go trials constitutes the main contrast. Additional contrasts against the null (or 'rest') trials were also performed. The only contrast that showed significant effects of COMT genotype was the NoGo *vs* Go deactivation contrast.

NoGo *vs* Go deactivation contrast: Val/val homozygotes, compared with met/met homozygotes, activated a cluster in the right retrosplenial region of the posterior cingulate cortex (BA30) ($x=16$, $y=-60$, $z=14$, $t=4.82$, cluster size 82 voxels, $p<0.05$ FWE corrected; see Figure 2)

TOL task: There was no significant difference between genotype groups for the problem *vs* control blocks or the task *vs* rest contrasts.

Task *vs* Control activation contrast: Val/val homozygotes activated a cluster of 90 voxels in the inferior parietal region ($x=-44$, $y=-50$, $z=42$, $t=3.83$, $p<0.001$ uncorrected) to a greater extent than met/met homozygotes. This effect did not survive correction for multiple comparisons across the task network.

PPI connectivity analysis: For the N-Back task, the posterior cingulate region modulated by COMT genotype on the 2-back *vs* rest deactivation contrast showed

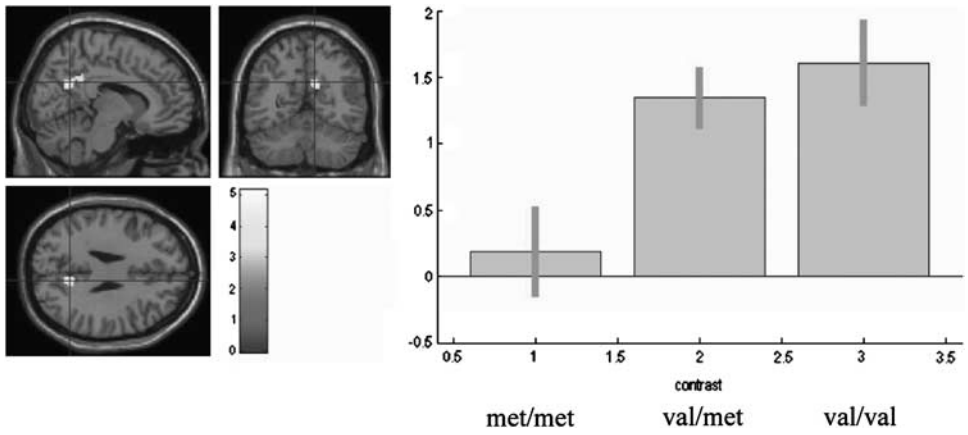

Figure 1 Significant correlation between COMT val/val and val/met genotype and deactivation of the right posterior cingulate during the 2-back task (thresholded at 100 voxels for illustration purposes, left is left). Ordinate on bar chart shows degree of deactivation (rest >2-back).

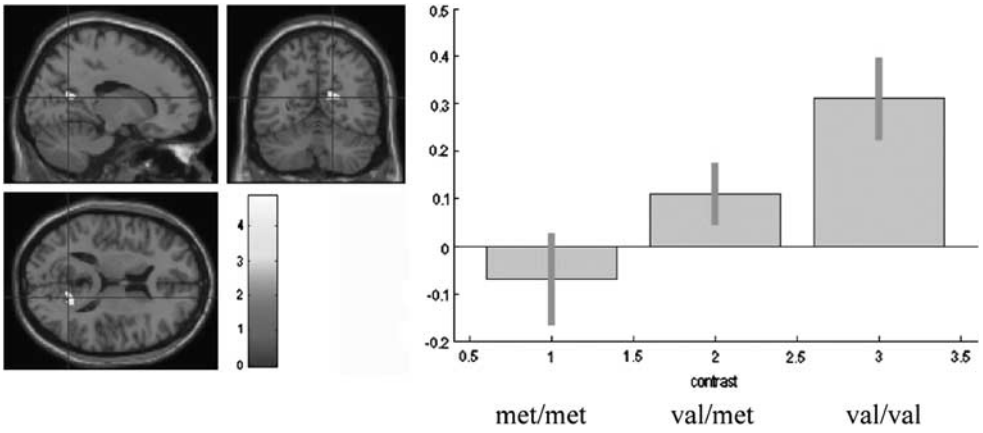

Figure 2 Significant correlation between COMT val/val genotype and right posterior cingulate deactivation during the Go *vs* NoGo contrast (thresholded at 82 voxels for illustration purposes). Ordinate on bar chart shows degree of deactivation (Go >NoGo).

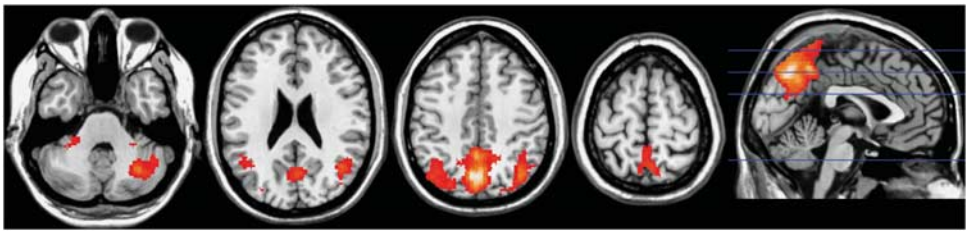

Figure 3 Clusters of fMRI deactivation significantly functionally connected to right posterior cingulate deactivation during the 2-back *vs* rest deactivation contrast ($p<0.05$ FWE corrected, figure shows slices 24, 36, 44, and 64).

increased coupling with proximal posterior regions during rest compared with the task blocks (Figure 3). The four significant clusters of increased connectivity had peak voxels in the precuneus ($x=-2$, $y=-70$, $z=42$; cluster size 6523 voxels, $t=7.04$, $p<0.05$ corrected), the right ($x=32$, $y=-62$, $z=-38$; cluster size 518 voxels, $t=5.9$, $p<0.05$ corrected) and left cerebellum ($x=-30$, $y=-40$, $z=-44$; cluster size 187 voxels, $t=4.4$, $p<0.05$ corrected), and left middle occipital lobe ($x=-48$, $y=-80$, $z=-4$; cluster size 136 voxels, $t=4.04$, $p=0.072$ corrected). There were no statistically significant effects of COMT genotype on the task-dependent posterior cingulate cortex connectivity.

For the NoGo *vs* Go deactivation cluster, there was no differential connectivity with the retrosplenial region of the posterior cingulate cortex for the Go and NoGo blocks of the task and no effect of COMT genotype.

DISCUSSION

The main aim of this study was to determine whether the common functional polymorphism COMT val$^{108/158}$met has

selective effects on prefrontal cortical activation across three tasks that have previously been associated with dopaminergic functioning in both drug modulation and PET ligand studies. The main finding was modulation of the posterior cingulate gyrus across two tasks, with greater deactivation in the val carriers on the N-Back task when compared with 'rest' and during response inhibition trials compared with the Go trials. Interestingly, we did not detect statistically significant associations between prefrontal cortical activation and COMT genotype for any of the three tasks used in the study using a combination of task-appropriate subtraction and parametric analyses.

One explanation for the lack of frontal cortical COMT effects over the three tasks could be that the study lacked sensitivity or power to detect an effect. Indeed, small clusters of activity which correlated with COMT genotype were found in the inferior and middle frontal gyri for the 2-back *vs* 0-back contrast, although neither of these effects survived correction for multiple comparisons, even after restriction of the analyses to the frontal cortex. Nonetheless, poor statistical power cannot be completely discounted, but we contend it is unlikely to explain our results for a number of reasons. First, we selected tasks that are known to be particularly sensitive to COMT polymorphisms (N-Back; Goldman *et al*, 2009) or with known sensitivity to dopaminergic manipulations (TOL; Cheesman *et al*, 2005; Egerton *et al*, 2009; Lappin *et al*, 2009; Owen *et al*, 1992; Reeves *et al*, 2005; Rektorova *et al*, 2008) and response inhibition (Go-NoGo; Hershey *et al*, 2004). Second, for the TOL task we have recently demonstrated significant changes in both striatal and prefrontal dopamine ligand binding indicative of probable dopamine release during execution of the task (Egerton *et al*, 2009; Lappin *et al*, 2009). Finally, the number of volunteers included in our study exceeded the average number of volunteers per study in the summary on Table 1 ($n = 44$). The largest studies to date were either unable to demonstrate an independent effect of COMT genotype on N-Back activation maps (Caldu *et al*, 2007) or described a COMT effect that relied on statistical correction within small-frontal cortical ROIs (Bertolino *et al*, 2006b; Drabant *et al*, 2006; Sambataro *et al*, 2009). However, it should be noted that we used a more conservative region based on the entire prefrontal cortical area as the ROIs used in these published studies were all different.

Given these previous results, the findings from this present study add to the literature questioning the degree to which COMT val$^{108/158}$met polymorphism independently accounts for variations in recruitment of the prefrontal cortex. However, this does not preclude an interaction effect between the COMT val$^{108/158}$met polymorphism and COMT haplotypes (Meyer-Lindenberg *et al*, 2006) or other dopaminergic genetic variants, such as dopamine transporter polymorphisms (Caldu *et al*, 2007), influencing prefrontal fMRI activations. It also does not preclude variations in COMT genotype selectively modulating component processes with the tasks. The use of block fMRI designs, although efficient in detecting BOLD effects, may record multiple processes potentially diluting process-specific effects. For example, in a small group of volunteers, De Frias *et al* (2010) recently suggested that COMT genotype modulated prefrontal cortical activity depending on the temporal dynamics of the BOLD signal, with transient activity changes and sustained activity differences present in the hippocampus and the prefrontal cortex, respectively.

Although COMT may not have a *direct* role in modulating prefrontal cortical activation, we did find evidence that COMT genotype effects fMRI activation in the right posterior cingulate. Because of the relative nature of contrasts in fMRI block designs the main contributor to these effects cannot be fully determined. However, the location of the modulatory effects in the posterior cingulate, commonly associated with the default mode network, is intriguing. The posterior cingulate is typically deactivated when focussed attention is harnessed at the expense of broadly monitoring the external environment or self-referential thought (Buckner *et al*, 2008). Increases in cognitive task load produce increased posterior cingulate deactivation (McKiernan *et al*, 2003) and conversely attentional lapses produce less posterior cingulate deactivations in normal volunteers (Weissman *et al*, 2006), although not in patients with schizophrenia (Harrison *et al*, 2007). We found greater posterior cingulate deactivations in the val/val group across two task-specific deactivation contrasts, 2-back *vs* rest and NoGo *vs* Go, although the latter was positioned more inferiorly within the retrosplenial region. The results from these two contrasts show that at the same task load, and to produce a similar level of behavioral task achievement, the val/val group deactivated the posterior cingulate to a significantly greater extent than the met/met group. The implication of this finding is that the greater deactivation in the val/val group may be a consequence of decreased cortical dopamine levels secondary to the functional consequences of the polymorphism (or *vice versa* in relation to the met/met group).

For the N-Back task, the region modulated by COMT genotype also showed functional coupling with the precuneus and posterior cingulate regions. During rest, coupling between the posterior cingulate and these other posterior components of the default mode network was increased compared with the 2-back blocks, indicating the potential network level influences of COMT genotype. This finding is also complemented by the recent demonstration of decreased functional coupling between the posterior cingulate cortex and anterior components of the default mode network in val/val homozygotes compared with met carriers when scanned at rest using fMRI (Liu *et al*, 2010). As working memory performance has been found to positively correlate with the connectivity strength of default mode network components including the posterior cingulate (Hampson *et al*, 2006), we would suggest that further exploration of the effects of COMT genotype on the default mode network is important both at a behavioral and a neurophysiological level.

In summary, we have shown that across three tasks commonly used to examine dopaminergic basis of cognitive function, the COMT val/met polymorphism does not directly impact on frontal cortical fMRI activations. Instead, we found that COMT val/val polymorphism results in increased deactivation of the posterior cingulate during N-Back and Go-NoGo tasks. These results imply that COMT genotype status may indirectly impact on prefrontal function through the modulation of the posterior cingulate possibly via its connections with components of the default mode network. Our results add to the literature in this area

by specifying cognitive subprocesses that warrant further investigation using tasks, which engage the posterior cingulate cortex. Ultimately, we would suggest that the careful separation of cognitive subprocess, a process already begun by the Tan *et al* (2007) study defines the future of further COMT fMRI studies.

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DISCLOSURE

Dr Stokes and Dr Rhodes report no conflict of interest. Professor Grasby has served as an occasional consultant to GlaxoSmithKline, Merck, and Pfizer. Dr Mehta reports research grant support from Pfizer, GlaxoSmithKline, and Evotec Neurosciences.

REFERENCES

Bertolino A, Blasi G, Latorre V, Rubino V, Rampino A, Sinibaldi L *et al* (2006a). Additive effects of genetic variation in dopamine regulating genes on working memory cortical activity in human brain. *J Neurosci* **26**: 3918–3922.

Bertolino A, Rubino V, Sambataro F, Blasi G, Latorre V, Fazio L *et al* (2006b). Prefrontal-hippocampal coupling during memory processing is modulated by COMT val158met genotype. *Biol psychiatry* **60**: 1250–1258.

Bishop SJ, Fossella J, Croucher CJ, Duncan J (2008). COMT val158met genotype affects recruitment of neural mechanisms supporting fluid intelligence. *Cereb Cortex* **18**: 2132–2140.

Blasi G, Mattay VS, Bertolino A, Elvevag B, Callicott JH, Das S *et al* (2005). Effect of catechol-O-methyltransferase val158met genotype on attentional control. *J Neurosci* **25**: 5038–5045.

Brett M, Johnsrude IS, Owen AM (2002). The problem of functional localization in the human brain. *Nat Rev Neurosci* **3**: 243–249.

Buckner RL, Andrews-Hanna JR, Schacter DL (2008). The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci* **1124**: 1–38.

Caldu X, Vendrell P, Bartres-Faz D, Clemente I, Bargallo N, Jurado MA *et al* (2007). Impact of the COMT Val108/158 Met and DAT genotypes on prefrontal function in healthy subjects. *NeuroImage* **37**: 1437–1444.

Camara E, Kramer UM, Cunillera T, Marco-Pallares J, Cucurell D, Nager W *et al* (2010). The effects of COMT (Val108/158Met) and DRD4 (SNP -521) dopamine genotypes on brain activations related to valence and magnitude of rewards. *Cereb Cortex* **20**: 1985–1996.

Cheesman AL, Barker RA, Lewis SJ, Robbins TW, Owen AM, Brooks DJ (2005). Lateralisation of striatal function: evidence from 18F-dopa PET in Parkinson's disease. *J Neurol Neurosurg Psychiatr* **76**: 1204–1210.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S *et al* (2004). Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet* **75**: 807–821.

Congdon E, Constable RT, Lesch KP, Canli T (2009). Influence of SLC6A3 and COMT variation on neural activation during response inhibition. *Biol Psychol* **81**: 144–152.

de Frias CM, Marklund P, Eriksson E, Larsson A, Oman L, Annerbrink K *et al* (2010). Influence of COMT gene polymorphism on fMRI-assessed sustained and transient activity during a working memory task. *J Cogn Neurosci* **22**: 1614–1622.

Drabant EM, Hariri AR, Meyer-Lindenberg A, Munoz KE, Mattay VS, Kolachana BS *et al* (2006). Catechol O-methyltransferase val158met genotype and neural mechanisms related to affective arousal and regulation. *Arch Gen Psychiatry* **63**: 1396–1406.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE *et al* (2001). Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA* **98**: 6917–6922.

Egerton A, Mehta MA, Montgomery AJ, Lappin JM, Howes OD, Reeves SJ *et al* (2009). The dopaminergic basis of human behaviors: a review of molecular imaging studies. *Neurosci Biobehav Rev* **33**: 1109–1132.

Ettinger U, Kumari V, Collier DA, Powell J, Luzi S, Michel TM *et al* (2008). Catechol-O-Methyltransferase (COMT) Val(158)met genotype is associated with BOLD response as a function of task characteristic. *Neuropsychopharmacology* **33**: 3046–3057.

First MB, Pincus HA (2002). The DSM-IV Text revision: rationale and potential impact on clinical practice. *Psychiatr Serv* **53**: 288–292.

Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ (1997). Psychophysiological and modulatory interactions in neuroimaging. *NeuroImage* **6**: 218–229.

Gevins A, Cutillo B (1993). Spatiotemporal dynamics of component processes in human working memory. *Electroencephalogr Clin Neurophysiol* **87**: 128–143.

Goldman D, Weinberger DR, Malhotra AK, Goldberg TE (2009). The role of COMT Val158Met in cognition. *Biol psychiatry* **65**: e1–e2; author reply e3-e4.

Hampson M, Driesen NR, Skudlarski P, Gore JC, Constable RT (2006). Brain connectivity related to working memory performance. *J Neurosci* **26**: 13338–13343.

Harrison BJ, Yucel M, Pujol J, Pantelis C (2007). Task-induced deactivation of midline cortical regions in schizophrenia assessed with fMRI. *Schizophr Res* **91**: 82–86.

Hershey T, Black KJ, Hartlein J, Braver TS, Barch DM, Carl JL *et al* (2004). Dopaminergic modulation of response inhibition: an fMRI study. *Brain Res Cogn Brain Res* **20**: 438–448.

Lappin JM, Reeves SJ, Mehta MA, Egerton A, Coulson M, Grasby PM (2009). Dopamine release in the human striatum: motor and cognitive tasks revisited. *J Cereb Blood Flow Metab* **29**: 554–564.

Lawrence AD, Weeks RA, Brooks DJ, Andrews TC, Watkins LH, Harding AE *et al* (1998). The relationship between striatal dopamine receptor binding and cognitive performance in Huntington's disease. *Brain* **121**(Part 7): 1343–1355.

Liu B, Song M, Li J, Liu Y, Li K, Yu C *et al* (2010). Prefrontal-related functional connectivities within the default network are modulated by COMT val158met in healthy young adults. *J Neurosci* **30**: 64–69.

McKiernan KA, Kaufman JN, Kucera-Thompson J, Binder JR (2003). A parametric manipulation of factors affecting task-induced deactivation in functional neuroimaging. *J Cogn Neurosci* **15**: 394–408.

Mehta MA, Gumaste D, Montgomery AJ, McTavish SF, Grasby PM (2005). The effects of acute tyrosine and phenylalanine depletion on spatial working memory and planning in healthy volunteers are predicted by changes in striatal dopamine levels. *Psychopharmacology (Berl)* **180**: 654–663.

Meyer-Lindenberg A, Nichols T, Callicott JH, Ding J, Kolachana B, Buckholtz J *et al* (2006). Impact of complex genetic variation in COMT on human brain function. *Mol psychiatry* **11**: 867–877, 797.

Mier D, Kirsch P, Meyer-Lindenberg A (2009). Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Mol psychiatry* **15**: 918–927.

Myakishev MV, Khripin Y, Hu S, Hamer DH (2001). High-throughput SNP genotyping by allele-specific PCR with universal energy-transfer-labeled primers. *Genome Res* **11**: 163–169.

Owen AM, Doyon J, Petrides M, Evans AC (1996). Planning and spatial working memory: a positron emission tomography study in humans. *Eur J Neurosci* **8**: 353–364.

Owen AM, James M, Leigh PN, Summers BA, Marsden CD, Quinn NP *et al* (1992). Fronto-striatal cognitive deficits at different stages of Parkinson's disease. *Brain* **115**(Part 6): 1727–1751.

Owen AM, McMillan KM, Laird AR, Bullmore E (2005). N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. *Hum Brain Mapp* **25**: 46–59.

Prata DP, Mechelli A, Fu CH, Picchioni M, Kane F, Kalidindi S *et al* (2008). Opposite effects of catechol-O-methyltransferase Val158Met on cortical function in healthy subjects and patients with schizophrenia. *Biol psychiatry* **65**: 473–480.

Rasch B, Spalek K, Buholzer S, Luechinger R, Boesiger P, de Quervain DJ *et al* (2010). Aversive stimuli lead to differential amygdala activation and connectivity patterns depending on catechol-O-methyltransferase Val158Met genotype. *NeuroImage* **52**: 1712–1719.

Reeves SJ, Grasby PM, Howard RJ, Bantick RA, Asselin MC, Mehta MA (2005). A positron emission tomography (PET) investigation of the role of striatal dopamine (D2) receptor availability in spatial cognition. *NeuroImage* **28**: 216–226.

Rektorova I, Srovnalova H, Kubikova R, Prasek J (2008). Striatal dopamine transporter imaging correlates with depressive symptoms and tower of London task performance in Parkinson's disease. *Mov Disord* **23**: 1580–1587.

Rubia K, Russell T, Bullmore ET, Soni W, Brammer MJ, Simmons A *et al* (2001). An fMRI study of reduced left prefrontal activation in schizophrenia during normal inhibitory function. *Schizophr Res* **52**: 47–55.

Sambataro F, Reed JD, Murty VP, Das S, Tan HY, Callicott JH *et al* (2009). Catechol-O-methyltransferase valine(158)methionine polymorphism modulates brain networks underlying working memory across adulthood. *Biol psychiatry* **66**: 540–548.

Schott BH, Seidenbecher CI, Fenker DB, Lauer CJ, Bunzeck N, Bernstein HG *et al* (2006). The dopaminergic midbrain participates in human episodic memory formation: evidence from genetic imaging. *J Neurosci* **26**: 1407–1417.

Smolka MN, Schumann G, Wrase J, Grusser SM, Flor H, Mann K *et al* (2005). Catechol-O-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *J Neurosci* **25**: 836–842.

Tan HY, Callicott JH, Weinberger DR (2009). Prefrontal cognitive systems in schizophrenia: towards human genetic brain mechanisms. *Cogn Neuropsychiatry* **14**: 277–298.

Tan HY, Chen Q, Goldberg TE, Mattay VS, Meyer-Lindenberg A, Weinberger DR *et al* (2007). Catechol-O-methyltransferase Val158Met modulation of prefrontal-parietal-striatal brain systems during arithmetic and temporal transformations in working memory. *J Neurosci* **27**: 13393–13401.

Tunbridge EM, Bannerman DM, Sharp T, Harrison PJ (2004). Catechol-o-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. *J Neurosci* **24**: 5331–5335.

Tunbridge EM, Harrison PJ, Weinberger DR (2006). Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biol psychiatry* **60**: 141–151.

Weissman DH, Roberts KC, Visscher KM, Woldorff MG (2006). The neural bases of momentary lapses in attention. *Nat Neurosci* **9**: 971–978.

Winterer G, Musso F, Vucurevic G, Stoeter P, Konrad A, Seker B *et al* (2006). COMT genotype predicts BOLD signal and noise characteristics in prefrontal circuits. *NeuroImage* **32**: 1722–1732.

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