Influence of COMT Gene Polymorphism on fMRI-assessed Sustained and Transient Activity during a Working Memory Task

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Abstract

■ The catechol *O*-methyltransferase (COMT) gene—encoding an enzyme that is essential for the degradation of dopamine (DA) in prefrontal cortex (PFC)—contains a single nucleotide polymorphism (val/met) important for cognition. According to the tonic–phasic hypothesis, individuals carrying the low-enzyme-activity allele (met) are characterized by enhanced tonic DA activity in PFC, promoting sustained cognitive representations in working memory. Val carriers have reduced tonic but enhanced phasic dopaminergic activity in subcortical regions, enhancing cognitive flexibility. We tested the tonic–phasic DA hypothesis by dissociating sustained and transient brain activity during performance on a 2-back working memory test using mixed blocked/event-related functional magnetic resonance imaging. Participants were men recruited from a random sample of the population (the Betula study) and consisted of 11 met/met and 11 val/val carriers aged 50 to 65 years, matched on age, education, and cognitive performance. There were no differences in 2-back performance between genotype groups. Met carriers displayed a greater transient medial temporal lobe response in the updating phase of working memory, whereas val carriers showed a greater sustained PFC activation in the maintenance phase. These results support the tonic–phasic theory of DA function in elucidating the specific phenotypic influence of the COMT val^{158}met polymorphism on different components of working memory. ■

INTRODUCTION

The protein encoded by the catechol *O*-methyltransferase (COMT) gene is essential for the degradation of dopamine (DA). A single nucleotide polymorphism (SNP) in the coding region of the COMT gene—leading to a val to met substitution (val^{158}met)—has gained attention because of its importance for dopaminergic activity in prefrontal cortex (PFC) and hippocampus (Matsumoto et al., 2003). In vitro studies suggest this SNP to influence activity and thermal stability of the enzyme (Lachman et al., 1996; Lotta et al., 1995), the variant of the protein encoded by the met allele displaying one quarter of the activity of that encoded by the val allele. Thus, met carriers are assumed to be characterized by enhanced DA activity in PFC, where the inactivation of DA to a great extent is conducted by COMT-catalyzed breakdown rather than by reuptake.

The COMT val^{158}met polymorphism has been associated with interindividual differences in working memory (Weickert et al., 2004; Goldberg et al., 2003) and other forms of higher-order cognition (de Frias et al., 2004, 2005), with met allele carriers usually performing better than val allele carriers (Malhotra et al., 2002; Egan et al., 2001; Weinberger et al., 2001). In line with this pattern, brain imaging studies indicate that val carriers need greater prefrontal activity for comparable levels of working memory performance, indicating that cognitive processing is less efficient in these individuals (Apud et al., 2007; Ho, Wassink, O'Leary, Sheffield, & Andreasen, 2005; Gallinat et al., 2003; Mattay et al., 2003; Egan et al., 2001).

However, to fully understand the effects of the COMT genotype on cognition, it may be necessary to consider whether a task requires tonic or phasic dopaminergic activation (Grace, Floresco, Goto, & Lodge, 2007; Bilder, Volavka, Lachman, & Grace, 2004; Grace, 1991). The tonic component is characterized by a constant, slow, irregular firing of DA neurons, whereas the phasic component is characterized by transient, high-amplitude activity, so-called burst firing. By promoting the stability of neural networks involved in cognitive processing, and by preventing uncontrolled spontaneous switches, tonic DA transmission is assumed to facilitate performance in tasks requiring sustained attention by activation of D_1 receptors in PFC. By contrast, phasic DA activity in subcortical regions is important for transient activation states (as modulated by D_2 receptors), and thus, for the updating and gating of relevant

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novel information (Bilder et al., 2004; Wilkerson & Levin, 1999). Whereas the met allele is assumed to increase stability-enhancing tonic DA activity, hence, reducing burst firing of DA neurons, the val allele is assumed to decrease tonic DA activity and thereby increase plasticity-enhancing phasic DA activity.

Hippocampal D_2 receptors modulate PFC by the hippocampal–PFC pathway, and facilitate quick learning and making comparisons to guide future actions (Goto & Grace, 2008; Takahashi et al., 2007, 2008), whereas striatal D_2 receptor availability and binding are implicated in motor, perceptual, and cognitive speed (Bäckman, Nyberg, Lindenberger, Li, & Farde, 2006; Bäckman et al., 2000; Volkow et al., 1998); both regions may function to assist in efficient updating. Although the striatum has been implicated in updating processes (Dahlin, Neely, Larsson, Bäckman, & Nyberg, 2008; O'Reilly, 2006), it has not been associated with COMT (Taylor et al., 2007), as COMT is not expressed in this brain region. By contrast, COMT has been directly linked to hippocampal activation (Smolka et al., 2005; Masuda, Tsunoda, & Imai, 2003; Matsumoto et al., 2003).

Previous studies addressing the relationship between working memory and brain activity have reported maintenance in working memory to be associated with dorsal PFC (Owen et al., 1999; Courtney, Ungerleider, Keil, & Haxby, 1997) and phasic, item-specific mnemonic working memory processes to be associated with hippocampus (Cabeza, Dolcos, Graham, & Nyberg, 2002; Rypma & D'Esposito, 2000). Accordingly, Marklund, Fransson, Cabeza, Larsson, et al. (2007) and Marklund, Fransson, Cabeza, Petersson, et al. (2007) studied brain activity during a 2-back working memory task using a mixed fMRI design (Donaldson, 2004), and found the activation of dorsolateral PFC (DLPFC) to be sustained and the activation of the hippocampal region to be transient and associated with an increased demand on updating in working memory (see also Sakai, Rowe, & Passingham, 2002). Furthermore, with respect to working memory maintenance, research demonstrates recruitment of DLPFC for tasks involving high memory demands, whereas ventrolateral PFC may suffice in tasks with low memory demands (Rypma & D'Esposito, 2003).

In previous fMRI studies on the influence of COMT genotype on brain activity, blocked designs were used, which cannot distinguish between sustained and transient activation. The aim of the present study was to address the tonic–phasic hypothesis regarding the influence of COMT genotype on cognition by using a mixed blocked/event-related fMRI design that enables the dissociation of sustained from transient brain activity during working memory performance. On the basis of the tonic–phasic theory (Bilder et al., 2004; Grace, 1991) and select previous findings (Marklund, Fransson, Cabeza, Larsson, et al., 2007; Marklund, Fransson, Cabeza, Petersson, et al., 2007; Taylor et al., 2007; Mattay et al., 2003; Egan et al., 2001), we predicted that (a) met homozygotes would require greater transient frontal activation and greater transient hippocampal activation during a 2-back task, reflecting lower efficiency in updating (i.e., a comparison of whether the item on-line was a repeat of the item presented two trials earlier) contents of working memory; (b) val carriers would display greater sustained frontal activation, reflecting lower efficiency in holding information in working memory; and (c) COMT genotype would not influence striatal brain activity.

METHODS

Participants

Parent Sample

The 231 nondemented participants were recruited from two random samples (Sample 1: S1 and Sample 3: S3) of adults from the most recent data collection of the Betula project (Wave 4), a longitudinal study of health and aging (see Nilsson et al., 1997, 2004). The youngest available cohort at Wave 4 (15-year follow up) was 50 years old. Met/met and val/val carriers were matched with respect to age, sex, and education. Met/met carriers (79 women, 62 men) were aged 50 to 65 years (M = 58.44 years, SD = 5.61) and had a mean of 11.92 years of education (SD = 3.47). Val/val carriers (48 women, 42 men) were also aged 50 to 65 years (M = 58.61 years, SD = 5.81) and averaged 12.51 years of formal schooling (SD = 3.96).

fMRI Sample

A subsample of 22 nondemented male participants (11 met/met carriers; 11 val/val carriers) for which COMT genotype information was available volunteered to participate in the imaging experiment. The two groups were matched with respect to age (50 to 65 years), education, global cognitive functioning (Mini Mental State Exam or MMSE; Folstein, Folstein, & McHugh, 1975), and specific cognitive functions (free recall, vocabulary, and visuospatial ability). Met/met carriers had a mean age of 55.9 years (SD = 5.4) and 12.0 years of education (SD = 3.5), and val/val carriers had a mean age of 56.8 years (SD = 5.1) and 13.6 years of education (SD = 4.5). Cognitive test results stratified by COMT genotype are displayed in Table 1. Vision was normal or corrected to near normal using scanner-compatible glasses or contact lenses.

Table 1. Means and Standard Deviations (in Parentheses) for Matched Cognitive Measures

Measures	*Met/Met*	*Val/Val*
MMSE	28.4 (2.1)	28.0 (1.3)
Free recall	10.3 (2.1)	9.6 (1.4)
Vocabulary	23.7 (3.7)	23.9 (2.8)
Block design	36.3 (7.3)	30.9 (7.2)

Participants were paid for their participation and informed consent was obtained in accordance with the guidelines of the Swedish Research Council.

Behavioral Task

Working memory was assessed using the 2-back task. Participants were asked to monitor the identity of a series of verbal stimuli (common nouns) and to indicate whether the item currently presented on the screen was identical to the one presented two trials back. In the fMRI experiment (fMRI sample), the participant was asked to press "yes" when they thought the item presented was the same as the one presented two trials earlier in the sequence, and to press "no" to indicate that it was not. The maximum 2-back score in this task was 32. In the behavioral experiment (parent sample), participants were asked to verbally indicate their responses to words read aloud by the person who administered the cognitive test. Participants received one of 20 parallel word lists. Here, the maximum score was 20.

fMRI Data Acquisition

Data were collected on a 1.5-T Philips Intera scanner (Philips Medical Systems, Netherlands). Functional T2*-weighted images were obtained with a single-shot gradient-echo EPI sequence used for BOLD imaging. The sequence had the following parameters: echo time = 50 msec; repetition time = 3000 msec (33 slices acquired); flip angle = 90°, field of view = 22 × 22 cm; 64 × 64 matrix; and 4.4 mm slice thickness. To eliminate signals arising from progressive saturation, five dummy scans were performed prior to the image acquisition. The stimulus material comprised single-word items separated by intertrial intervals (ITIs) of varying time length filled with a centered cross-hair. A small circle was presented during gaps between task blocks instructing subjects to rest while maintaining their gaze on the circle. All stimuli were projected on the center of a semi-transparent screen that the subjects viewed through a tilted mirror attached to the head coil. Two 4-button Lumitouch fMRI optical response keypads (Lightwave Medical Industries, Canada) were used to collect responses. Behavioral performance (i.e., accuracy data and reaction time [RT] data) were handled by a PC running E-Prime 1.1 (Psychology Software Tools, Pittsburgh, PA). After functional imaging, high-resolution T1- and T2-weighted structural images were acquired.

fMRI Data Analysis

Functional imaging data were preprocessed in a number of steps prior to statistical analyses using the SPM2 software package (Wellcome Department of Cognitive Neurology, London, UK) on Matlab 6.5.1 (Mathworks, Sherborn, MA). All image volumes were first corrected for variability in slice timing acquisition. Image realignment and unwarping were then performed. The image volumes were then normalized to an approximate Talairach space (Talairach & Tournoux, 1988) as defined by the SPM2-weighted MNI template, and finally smoothed with an isotropic 8-mm full width at half maximum Gaussian kernel.

The four task blocks contained eight items each. The first two items from each block were obligatory "no responses" and were dropped before calculating the final score. Word items were intermixed with a fixation cross-hair at the center of the screen and a circle was presented between blocks (i.e., a resting baseline that lasted 21 sec). In mixed blocked/event-related designs, the regressors modeling event-related responses will invariably exhibit some correlation with their corresponding state-related regressor. A high degree of correlation indicates low parameter estimation efficiency (Visscher et al., 2003; Otten, Henson, & Rugg, 2002). In the present study, we decorrelated the event and state regressors by introducing a jittering of the intervals between stimuli within each block. The stimulus onset asynchrony (SOA) was pseudorandomized between 2.5 and 25 sec according to an approximate Poisson distribution [i.e., for approximately 60% of the items, the SOA was 2.5 sec; for 30% of the items, the SOA was 20 sec; and for a minority of items, the SOA was 22.5 (9%) or 25 (1%) sec long]. As a result, the correlation between event and epoch-related regressors did not exceed .6. This degree of relationship allows a reasonably efficient estimation of event- and state-related responses, while at the same time not compromising the test properties. There was no variation in ITIs for the 2-back task used in the parent sample in which an experimenter read the sequence of words aloud at a fixed rate of one word every 3 sec and the participants gave their responses orally.

Sustained and transient effects were modeled separately within the framework of the general linear model (Friston et al., 1998) as implemented in SPM with a select focus on differential effects in PFC (primarily dorsal), medial temporal lobe (MTL), and striatum. Event-related transient responses were modeled as regressors containing delta functions representing onsets of the stimuli, whereas state-related sustained responses were modeled with a boxcar function (Friston et al., 1998). Both regressor types were convolved with a canonical hemodynamic response function.

Applying the general linear model to the imaging data resulted in least square estimates of the regressors on a subject-specific level. Task-related sustained and transient effects were calculated as linear combinations of the individual regressors and stored as subject-specific contrast images. To detect whether there was an association between COMT genotype and fMRI activation in PFC, MTL, and striatum, the contrast images of all 22 (i.e., 11 met/met and 11 val/val) participants were included in regression analyses using SPMs. These were entered into a second-level model using two-sample *t* tests to test for differential activations between groups for each contrast of interest. This two-stage mixed effects procedure is equivalent to a

random effects analysis treating subjects as a random variable (Holmes & Friston, 1998).

Whole-brain SPMs were generated for all between-group comparisons (val/val > met/met; met/met > val/val) of task-related sustained and transient BOLD signal changes relative to baseline in PFC and MTL. For transient effects, different types of behavioral responses (i.e., 2-back matches with "yes" responses and 2-back mismatches with "no" responses) were collapsed and analyzed together. Given the a priori hypothesis regarding specific anatomical loci of interest, we adopted a region-of-interest (ROI) approach to limit the scope of our analyses. These loci were PFC for the sustained response (Bilder et al., 2004; Owen et al., 1999; Courtney et al., 1997) and both MTL and striatum for the transient response (Marklund, Fransson, Cabeza, Larsson, et al., 2007; Bäckman et al., 2006; Rypma & D'Esposito, 2000).

ROIs covering PFC, MTL, and striatum were anatomically defined and created on the basis of the WFU PickAtlas software (Maldjian, Laurienti, Kraft, & Burdette, 2003). PFC ROI encompassed both lateral (superior, inferior, and middle frontal gyrus) and medial (including anterior cingulate cortex) portions of the frontal lobes. The subregions were combined to create one PFC ROI. Although effects in dorsal PFC were primarily expected, COMT-related differences in activation might be expressed throughout the frontal lobes because the 2-back task has been associated with activity in a number of different PFC subregions (Owen, McMillan, Laird, & Bullmore, 2005). An additional reason to collapse across subregions was to reduce the number of multiple comparisons. The MTL ROI included hippocampus and parahippocampal gyrus. The striatal ROI included caudate nucleus and putamen. For each between-group contrast, the respective ROI was used as a mask for interrogation of differentially activated voxels within SPMs thresholded at $p < .005$, uncorrected. The extent threshold was set to 5 contiguous voxels. Bonferroni corrections for multiple ROI comparisons (collapsing left and right hemispheres) were applied (0.005/3 ROIs), yielding a corrected threshold of $p < .00167$. For the whole-brain analysis, SPM statistical significance was thresholded at $p < .001$, uncorrected, and the extent threshold was set to 10 voxels.

Genetics Analyses

Genomic DNA was isolated from whole blood by Qiagen Genomic DNA Purification Kit (Qiagen, Chatsworth, CA). Polymerase chain reactions were carried out using HotstarTaq polymerase (Qiagen) in a total volume of 20 μl containing 1.5 mM $MgCl_2$, 0.15 μM primers (fw: 5′-TCA CCA TCG AGA TCA ACC CC-3′, rev: 5′-ACA ACG GGT CAG GCA TGC A-3′), and approximately 50 ng genomic DNA. After an initial 15 min denaturation step at 95°C, 45 cycles were performed including 30 sec at 94°C, 30 sec at 62°C, and 30 sec at 72°C. PCR products were genotyped with a Pyrosequencer PSQ 96 and the PSQ 96 SNP Reagent Kit (Pyrosequencing, Uppsala, Sweden; Nordfors et al., 2002), using the sequence primer 5′-TGG TGG ATT TCG CTG-3′.

RESULTS

Behavioral Findings

A comparison of met/met and val/val carriers revealed no significant influence of COMT genotype on the outcome of the 2-back tasks in the parent sample [$F < 1$] or in the fMRI sample [$F(1, 20) = 1.99, p > .05$]. RT differences for short and long ITIs were examined as a function of COMT genotype. The effect of ITI length on RT was not moderated by COMT genotype ($p > .05$ for both short and long ITIs).

Neuroimaging Findings

Main Effects of Task

The most pronounced sustained activity increases relative to baseline were exhibited in bilateral cerebellum, right dorsolateral PFC, right anterior insula, bilateral basal ganglia, left inferior/middle temporal gyrus, left superior parietal cortex, and left precentral gyrus (Figure 1, top row). These activation patterns are consistent with previous imaging evidence of brain regions engaged in *n*-back paradigms (Owen et al., 2005) and largely confirm the dissociable temporal dynamics of activity in distinct networks subserving maintenance and updating as revealed in prior hybrid fMRI studies using the 2-back task (Marklund et al., 2009; Marklund, Fransson, Cabeza, Larsson, et al., 2007; Marklund, Fransson, Cabeza, Petersson, et al., 2007). As would be expected, relative to baseline, the 2-back task evoked an extensive pattern of transient event-related activations in motor and visual cortex for the combined analysis of all 22 participants. Bilateral parietal cortices, ventrolateral PFC, dorsolateral PFC, basal ganglia, left hippocampus, and left temporal gyrus also showed robust transient activations (Figure 1, middle row).

ROI Analysis of Genotype Effects

Effect of COMT genotype on sustained activations. There was a significant effect of COMT genotype on sustained activations in right superior frontal gyrus [BA 8 (peak: $x = 18, y = 24, z = 40$); $t = 4.18$; 6 voxels], the activation being higher in val than in met carriers (Figure 2). Differential sustained effects of COMT genotype were also found for the striatal ROI where relatively greater activation in val carriers was demonstrated in the right caudate nucleus (peak: $x = 18, y = 24, z = 6$; $t = 3.21$; 22 voxels). However, this effect did not survive the Bonferroni correction ($p > .002$).

Effect of COMT genotype on transient activations. There was a significant effect of COMT genotype on transient activation in right hippocampus [BA 20 (peak: $x = 38$,

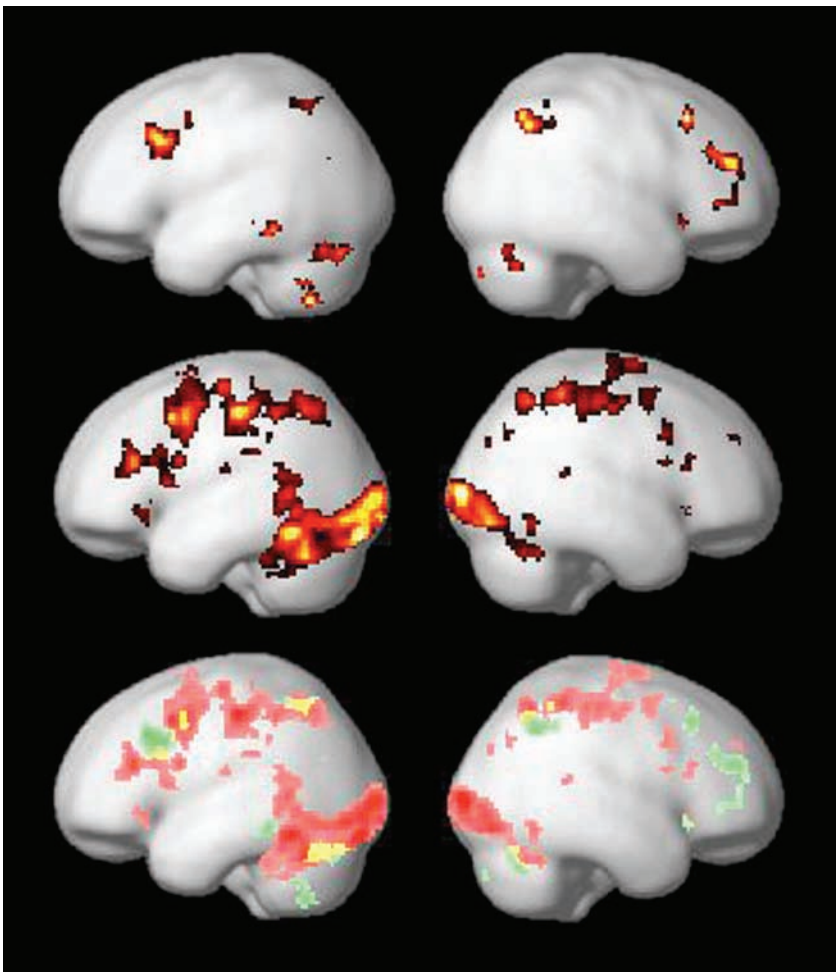

Figure 1. The main effects of task based on the whole-brain analysis across all participants. The top row shows sustained brain activity during 2-back relative to resting baseline (thresholded at $p < .002$ after false discovery rate correction). The middle row shows transient brain activity during 2-back relative to resting baseline (thresholded at $p < .05$ after family-wise error correction). The bottom row shows an illustration of the separation of cortical patterns of sustained (green) and transient (red) brain activity, with only a few overlapping areas (yellow).

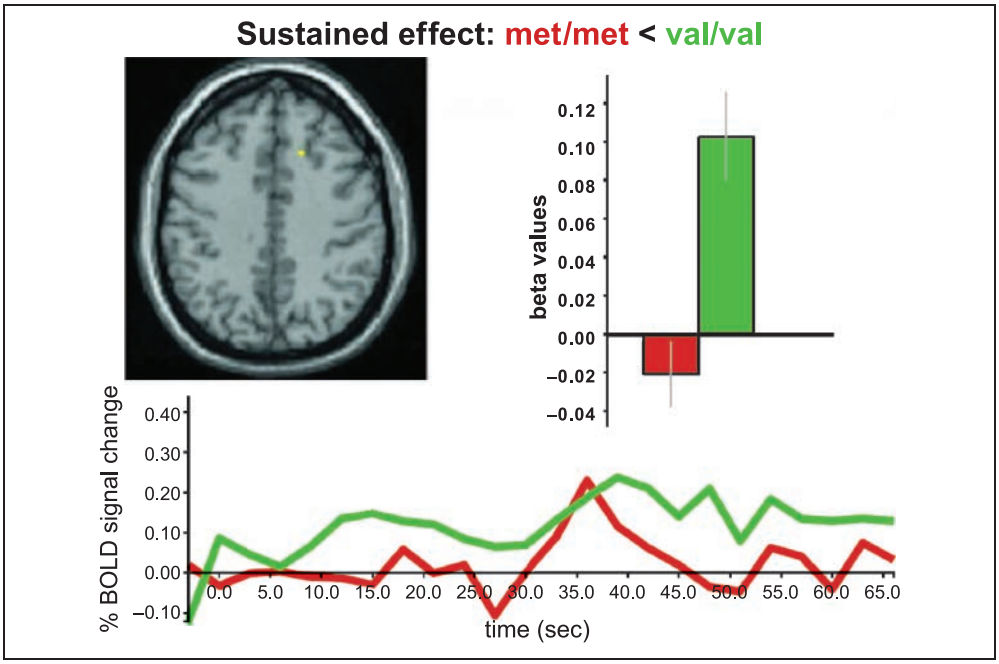

Figure 2. The region associated with a sustained response (tonic DA; ROI analyses), which increased during working memory for val carriers relative to met carriers was right superior frontal gyrus (BA 8, MNI coordinates: $x = 18$, $y = 24$, $z = 40$). The time course and BOLD bars for this region are shown.

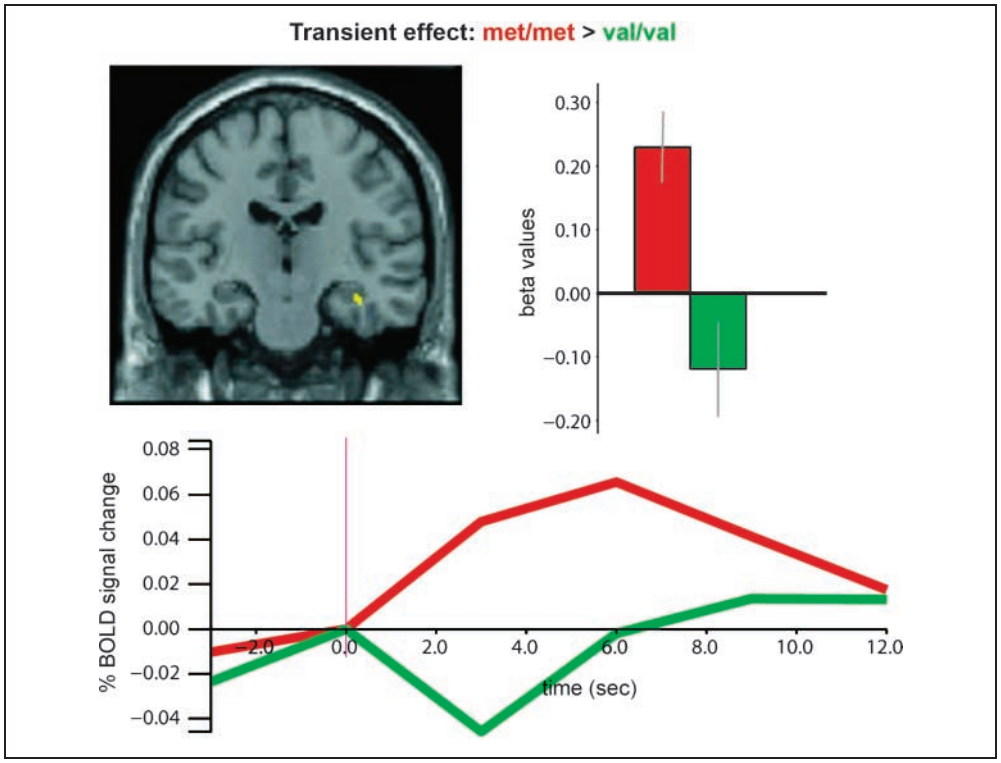

Figure 3. The region associated with a transient response (phasic DA; ROI analyses), which increased during working memory for met carriers relative to val carriers was right hippocampus (MNI coordinates: $x = 38, y = -18, z = -18$). The time course and BOLD bars for this region are shown.

$y = -18, z = -18$); $t = 4.07$; 17 voxels], with higher activation in met than in val allele carriers (Figure 3).

Explorative Whole-brain Analysis of Genotype Effects

Effect of COMT genotype on sustained activations. There was a significant effect of COMT on sustained activations in right parietal cortex including angular gyrus [BA 39 (peak: $x = 42, y = -60, z = 28$); $t = 5.11$; 63 voxels] and brain stem [(peak: $x = 2, y = -26, z = -44$); $t = 4.56$; 58 voxels], the activation being higher in met than in val allele carriers. There was also a significant effect of COMT on sustained activations in left posterior cingulate gyrus [(peak: $x = -18, y = -42, z = 20$); $t = 4.72$; 22 voxels] and left hippocampus [(peak: $x = -22, y = -40, z = 4$), $t = 4.09$; 15 voxels]. Here, activation was higher in val than in met allele carriers. Furthermore, the whole-brain SPM also picked up the differential sustained response in PFC revealed in the ROI analysis, showing greater activation in superior frontal gyrus for val carriers (peak: $x = 18, y = 24, z = 40$). Although this effect only comprised three voxels, it substantiated the result from the ROI analysis.

Effect of COMT genotype on transient activations. No group comparisons at $p < .001$, uncorrected (extent threshold = 10), showed significant clusters. Still, at a more lenient extent threshold, the whole-brain analysis once again recovered the finding from the ROI analysis by demonstrating a differential transient effect in right hippocampus (met > val) encompassing five voxels (peak: $x = 38, y = -18, z = -18$; $p < .0003$, uncorrected).

DISCUSSION

The observation that COMT genotype modulated brain activity in PFC and hippocampus during a working memory task is in line with previous imaging studies supporting a role of DA in human working memory (Takahashi et al., 2007, 2008; Bäckman et al., 2006; Aalto, Brück, Laine, Någren, & Rinne, 2005; Meyer-Lindenberger et al., 2005) as well as with related animal work (Castner & Goldman-Rakic, 2004; Seamans & Yang, 2004; Wang, Vijayraghavan, & Goldman-Rakic, 2004; Glickstein, Hof, & Schmauss, 2002; Reid, Lloyd, & Rao, 1999). The most important finding was that the association between COMT genotype and brain activation was dependent on the temporal dynamics of the BOLD signal, as reflecting sustained versus transient responses, linked to maintenance and updating operations, respectively. A comparison of val and met homozygotes matched for age, sex, education, and cognitive performance showed a greater transient MTL response reflecting the updating component of working memory among met carriers, and greater sustained PFC activation reflecting the maintenance component of working memory among val carriers. Of note is that the maintenance phase was defined and modeled by an epoch regressor that spanned across each task block encompassing eight trials with varying ITIs, with an average individual maintenance period of approximately 7 sec, rather than by separately modeled delay periods (cf. Wang et al., 2004).

Consistent with our results regarding PFC activation during sustained activation, Marklund, Fransson, Cabeza, Larsson, et al. (2007) and Marklund, Fransson, Cabeza, Petersson, et al. (2007) found greater activation of PFC

for the sustained component of working memory. Similarly, Owen et al. (1999) reported greater activity in PFC when a task demanded the maintenance of information. Our observation that the sustained response in PFC was larger in val/val carriers is in line with the notion that the stability of cortical activation states depends on tonic (sustained) DA transmission that prevents uncontrolled spontaneous switches (Bilder et al., 2004), and that val/val carriers require more activation during tonic (sustained) DA transmission because they have faster DA breakdown, hence, lower D_1 activation in PFC.

The whole-brain analyses confirmed this frontal effect, and additionally revealed a region in left hippocampus where val carriers, but not met carriers, showed a sustained effect. Although this effect was not part of our initial prediction, it is in agreement with recent observations of COMT expression in hippocampus (Redell & Dash, 2007; Matsumoto et al., 2003) and of delayed activity in hippocampus during working memory maintenance (Piekema, Kessels, Mars, Petersson, & Fernandez, 2006). Thus, the faster DA breakdown in val carriers may affect functional activity in integrated fronto-hippocampal networks that are involved in maintenance of information in working memory. Furthermore, the ROI analyses show greater sustained striatal activation in val carriers. Although this effect did not hold after Bonferroni correction, it is in line with previous neuroimaging studies supporting the role of striatal DA and sustained striatal activity in working memory maintenance (Landau, Lal, O'Neil, Baker, & Jagust, 2009; Chang, Crottaz-Herbette, & Menon, 2007; Gazzaley, Rissman, & D'Esposito, 2004). Moreover, increased working memory load has been associated with greater sustained activity in the caudate (Marklund, Fransson, Cabeza, Larsson, et al., 2007), which suggests that the effect observed here is related to exerted effort displayed by val carriers corresponding to less neural efficiency.

The tonic–phasic hypothesis regarding the influence of DA on cognition further states that val carriers, by displaying lower tonic DA activity, are relatively less resistant to phasic DA activity, and thus, more cognitively flexible. Our finding that met carriers required more activation in MTL during the stimulus-locked phase of the task, likely demanding phasic DA transmission, supports this assertion. Additional support for hippocampal dopaminergic innervation and, more specifically, the recruitment of hippocampal D_2 (transient) activity in working memory performance, has been observed in other human (Aalto et al., 2005; for review, see Marklund, Fransson, Cabeza, Larsson, et al., 2007; Marklund, Fransson, Cabeza, Petersson, et al., 2007; Piekema et al., 2006; Lisman & Grace, 2005) and animal (Gasberri, Sulli, & Packard, 1997; Wilkerson & Levin, 1999) studies.

A possible role of hippocampal DA in working memory (especially updating) relates to novelty detection (Lisman & Grace, 2005). The presentation of novel stimuli produces an increase in hippocampal activity (e.g., Dudokovic & Wagner, 2007; Düzel et al., 2003). Novelty involves comparing incoming and stored information, a skill needed to successfully complete the *n*-back task. Novelty also enhances long-term potentiation through DA release in hippocampus (Lisman & Grace, 2005), a mechanism that may aid updating in more challenged met carriers.

Two limitations of the study are worth noting. First, the sample size was modest ($n = 22$ with 11 persons in each allelic group). The relatively small sample size may be one reason why we did not find greater transient frontal activations for the met group, as would be predicted by the tonic–phasic theory (Bilder et al., 2004). Second, although the lack of COMT-related behavioral differences is in agreement with the claim that greater recruitment of a region helps to ameliorate or avoid (i.e., compensate for) behavioral shortcomings (e.g., Mattay et al., 2003), it is also possible that the 2-back task is not sensitive enough to detect a genotype effect. That said, recent meta-analytic evidence suggests that differences between COMT genotypes in more cognitively challenging working memory tasks are negligible (Barnett, Scoriels, & Munafò, 2008). Future research should test the tonic–phasic theory using different paradigms in a mixed blocked/event-related design.

In summary, by using a mixed fMRI design to differentiate maintenance from updating functions in working memory, we provide additional support for the tonic–phasic theory regarding the influence of DA on cognition (Grace et al., 2007; Bilder et al., 2004; Grace, 1991). Specifically, whereas met/met and val/val carriers had the same level of performance on the 2-back task, our imaging data suggest that they activate different brain regions to different extents while performing this task.

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