

# Catechol O-methyltransferase Val<sup>158</sup>Met Genotype and Neural Mechanisms Related to Affective Arousal and Regulation

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**Context:** Catechol O-methyltransferase (COMT), the major enzyme determining cortical dopamine flux, has a common functional polymorphism (val<sup>158</sup>met) that affects prefrontal function and working memory capacity and has also been associated with anxiety and emotional dysregulation.

**Objectives:** To examine COMT val<sup>158</sup>met effects on corticolimbic circuitry reactivity and functional connectivity during processing of biologically salient stimuli, as well as the relationship to the temperamental trait of novelty seeking.

**Design:** Within-subject functional magnetic resonance imaging study.

**Setting:** National Institute of Mental Health, Genes, Cognition, and Psychosis Program, Bethesda, Md.

**Patients:** One hundred one healthy subjects of both sexes.

**Results:** We found that the met allele was associated with a dose-dependent increase in hippocampal formation and ventrolateral prefrontal cortex activation during viewing of faces displaying negative emotion. In met/met homozygotes, limbic and prefrontal regions showed increased functional coupling. Moreover, in these same subjects, the magnitude of amygdala-orbitofrontal coupling was inversely correlated with novelty seeking, an index of temperamental inflexibility.

**Conclusions:** Our results indicate that heritable variation in dopamine neurotransmission associated with the met allele of the COMT polymorphism results in heightened reactivity and connectivity in corticolimbic circuits. This may reflect a genetic predisposition for inflexible processing of affective stimuli, a mechanism possibly accounting for aspects of arousal and behavioral control that contribute to emotional dysregulation previously reported in met/met individuals.

*Arch Gen Psychiatry.* 2006;63:1396-1406

**D**OPAMINE NEUROTRANSMISSION plays a critical role in the regulation of neural circuits supporting both cognitive and affective behavioral processes. Animal models reveal that dopamine may bias affective responses by augmenting excitatory sensory input and attenuating inhibitory prefrontal input to the amygdala<sup>1</sup> and medial striatum,<sup>2</sup> thereby altering the functional dynamics of key nodes of a neural circuit implicated in affect generation and regulation.<sup>3,4</sup> Furthermore, dopamine plays a critical role in hippocampal processing,<sup>5</sup> which is also implicated in emotion regulation.<sup>6,7</sup> Consistent with these observations, abnormalities in dopamine neurotransmission contribute to affective disturbances in a broad range of psychiatric disorders.<sup>8</sup> Moreover, individual differences in dopamine neurotransmission can bias the functional re-

sponsivity of these brain systems and, in turn, both normal and pathological variability in associated behaviors,<sup>9</sup> possibly by influencing the signal-to-noise ratio of neuronal ensembles, resulting in more efficient processing of task-relevant information.<sup>10</sup> Thus, identifying genetic polymorphisms that impact dopamine signaling and related brain functions will contribute to elucidating biological mechanisms of individual differences in affective behavior and possibly the risk for affective disorders.

While there are candidate polymorphisms in several dopamine subsystem genes, the most comprehensively studied and well characterized is a relatively frequent single nucleotide polymorphism (G → A) in the human catechol O-methyltransferase (COMT) gene, which catabolizes catechol chemicals including epinephrine, norepinephrine, and dopamine. The polymorphism results in a

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methionine (met) to valine (val) substitution at codon 158.<sup>11,12</sup> The val allele is associated with high enzymatic activity and consequently low extracellular dopamine levels, while the met allele is associated with significantly reduced enzyme activity and high extracellular dopamine levels.<sup>11-13</sup> The effects of the *COMT* val<sup>158</sup>met polymorphism and associated changes in synaptic dopamine availability have been explored in a series of behavioral and neuroimaging studies of prefrontal cortical cognition.<sup>14-18</sup> These studies have revealed that the low-activity *COMT* met allele is characterized by greater task-dependent prefrontal efficiency (ie, decreased magnitude and extent of activation for a given level of task performance) and associated cognitive advantages (eg, working memory enhancement). These collective results have been posited to reflect the dependence of prefrontal neuronal function on dopamine signaling, as well as the critical nature of *COMT* activity in regulating prefrontal dopamine availability.<sup>18,19</sup>

Although these data strongly suggest that the low-activity *COMT* met allele is advantageous for prefrontal function and related cognitive behaviors, several recent behavioral association studies have reported negative mood and affective disorders to be associated with the met allele. Specifically, the met allele has been linked with increased levels of anxiety in women,<sup>20</sup> obsessive-compulsive disorder in men,<sup>21</sup> panic disorder,<sup>22</sup> type 1 alcoholism,<sup>23</sup> aggressiveness and anger-related traits,<sup>24,25</sup> decreased novelty seeking (NS)<sup>26,27</sup> and increased reward dependence,<sup>27</sup> higher sensitivity to pain,<sup>28</sup> bipolar affective disorder,<sup>29</sup> and major depression.<sup>30</sup> While there have been inconsistencies<sup>31</sup> and failures to replicate these associations,<sup>32</sup> they nevertheless raise the intriguing possibility of a functional trade-off between genetic variation that concurrently results in more efficient cognitive behaviors and abnormal affective behaviors. A recent imaging study in a small group of healthy subjects suggested that increased anxiety associated with the met allele may be the result of relatively increased amygdala and hippocampal activation<sup>33</sup>; however, the relationship between patterns of brain activation and affective behaviors associated with the *COMT* genotype remains unexplored.

Given the challenges of linking the subtle and nuanced biological effects of genetic variation with differences in complex, dynamic emergent behavioral phenomena,<sup>34</sup> we used functional magnetic resonance imaging (fMRI) to directly examine the effects of the *COMT* val<sup>158</sup>met polymorphism on the reactivity and connectivity of corticolimbic circuitry, which mediates aspects of affective behaviors, in a large cohort of healthy volunteers of both sexes. We have also explored the relationship between genotype effects on this circuitry and NS, a heritable personality measure<sup>35</sup> related to temperamental inflexibility (low scores indicate rigidity and regimentation)<sup>36</sup> that has been previously associated with dopamine gene variants<sup>37,38</sup> including *COMT*.<sup>26,27</sup> Our uniquely large sample also allowed us to control for *5-HTTLPR* genotype status, which is known to bias the functional reactivity of this circuitry,<sup>39</sup> as well as additional potential confounds such as age and sex. We report evidence that the *COMT* val<sup>158</sup>met genotype affects

the functional dynamics of corticolimbic circuits implicated in affective arousal and regulation.

## METHODS

### SUBJECTS

From September 2000 to March 2004, 213 subjects recruited as "normal volunteers" were scanned with fMRI as part of a large ongoing study exploring the genetics of cognition and emotion. All subjects gave written informed consent and participated in the study according to the guidelines of the National Institute of Mental Health institutional review board. Subjects underwent extensive reviews of medical history, physical examination, and in-depth clinical interviews, including the Structured Clinical Interview for *DSM-III-R* for lifetime psychiatric diagnosis.<sup>40,41</sup> Fifty-seven subjects were excluded for preexisting neurological, psychiatric, and/or substance abuse problems and history of other medical problems and/or treatment relevant to cerebral metabolism and blood flow.<sup>17</sup> To control for confounding effects of population substructure and ethnic heterogeneity, we limited our data analyses to 101 white subjects with a known *COMT* genotype (met/met=24, val/met=57, val/val=20) from the larger sample of healthy volunteers.

### CORTICOLIMBIC REACTIVITY TASK

During fMRI, subjects completed a simple perceptual task involving the matching of fearful and angry facial expressions known to robustly engage the amygdala and interconnected circuitry.<sup>42-45</sup> In this paradigm, 2 blocks of a face-processing task were interleaved with 3 blocks of a sensorimotor control task. During the face-processing task, subjects viewed a trio of faces and were instructed to select 1 of the 2 faces (bottom) that was identical to a target face (top). Each face-processing block consisted of 6 images, 3 of each sex and target affect (angry or afraid), all derived from a standard set of pictures of facial affect,<sup>46</sup> presented sequentially for 5 seconds. During the sensorimotor control block, subjects viewed a trio of geometric shapes (circles and vertical and horizontal ellipses) and selected 1 of the 2 shapes (bottom) that matched a target shape (top). Each control block consisted of 6 different images presented sequentially for 5 seconds. Different variations of face tasks have been used to successfully activate the limbic system and are frequently cited in functional neuroimaging paradigms in the study of emotion processing.<sup>47,48</sup> This task, as a putative index of biological salience, reliably and robustly activates corticolimbic circuitry.<sup>42-45</sup> Thus, in contrast to tasks that compare positive and negative emotional expressions but generally show greater variability in engaging this circuitry,<sup>49</sup> we preferred this task for our purposes of investigating genotype-specific differences in activation of affect-related brain circuitry.

### MOOD AND PERSONALITY ASSESSMENT

The NS subset of the Tridimensional Personality Questionnaire was administered as an index of behaviors, such as impulsiveness and rigidity, putatively influenced by dopaminergic neurotransmission<sup>50</sup> and previously associated with various dopamine gene variants<sup>37,38</sup> including *COMT*.<sup>26,27</sup> The harm avoidance (HA) subset of the Tridimensional Personality Questionnaire was also administered as a putative index of behaviors such as fear and anxiety, given its previous association with genetically driven variation in corticolimbic network activation.<sup>51</sup> Analysis of variance was used to identify genotype, sex, and genotype  $\times$  sex effects on total NS and HA scores, respectively. Tridimensional Personality Questionnaire scores were

available in 79 of the 101 subjects included in the final fMRI analysis.

## fMRI ACQUISITION PARAMETERS

Each subject was scanned using a 3-T scanner (GE Signa, Milwaukee, Wis) with a real-time functional imaging upgrade. An automated shim procedure was applied to minimize possible magnetic field inhomogeneities. Blood oxygenation level-dependent (BOLD) fMRIs were acquired with a gradient-echo Echo Planar Imaging sequence and covered 24 axial slices (4 mm thick, 1-mm gap) that began at the cerebral vertex and encompassed the entire cerebrum and the majority of the cerebellum (repetition time=2000 milliseconds, echo time=28 milliseconds, field of view=24 cm, 64 × 64 matrix). Before the collection of fMRI data for each subject, we acquired a reference Echo Planar Imaging scan and visually inspected it for artifacts (eg, ghosting) as well as for good signal across the entire volume of acquisition, including the medial temporal lobes.

## fMRI DATA ANALYSIS

Analysis of the BOLD fMRI data was performed using the general linear model within SPM99 (Wellcome Department of Imaging Neuroscience, London, England; <http://www.fil.ion.ucl.ac.uk/spm>) and with analysis of variance in SPSS (version 11.5.0; SPSS Inc, Chicago, Ill). Briefly, images for each subject were realigned to the middle volume in the time series to correct for head motion, spatially normalized into a standard stereotaxic space (Montreal Neurological Institute template; McGill University, Montreal, Quebec) using a 12-parameter affine model followed by nonlinear matching to a customized template image, and smoothed to minimize noise and residual differences in gyral anatomy with a gaussian filter, set at 8 mm full width at half maximum. Voxelwise signal intensities were ratio normalized to the whole-brain global mean.

Predetermined condition effects for the entire functional brain volume were calculated using a *t* statistic, producing a statistical image for the contrast of the face-processing task vs the sensorimotor control for each subject (N=101). These individual contrast images were then used in a second-level random-effects model, which accounts for both scan-to-scan and subject-to-subject variability, to identify regions significantly activated by the task. To identify genotype effects, the same first-level images were contrasted between genotype groups using a 2-groups analysis of covariance model, covarying for age and sex to further ensure that results indicated differences due to genotype and were independent of these factors. All statistical result maps are shown at an uncorrected threshold of  $t=2.7$ . All voxels reported as showing significant effects furthermore survived a multiple comparison correction at the  $P<.05$ , corrected, level. For the main effect of task and functional connectivity analyses, whole-brain cluster correction was used. For genotype effects on BOLD response, we used a small-volume correction in hippocampal and prefrontal regions where *COMT* effects were hypothesized a priori based on messenger RNA expression studies.<sup>13,52</sup> The small-volume correction was conducted using anatomical masks created with the WFU PickAtlas (Wake Forest University, Winston-Salem, NC) for regions that overlapped with the main effect of task, resulting in a 35-voxel ventrolateral prefrontal cortex (vlPFC) mask and a 59-voxel hippocampal formation mask.

## FUNCTIONAL CONNECTIVITY ANALYSIS

“Functional connectivity” is a measure of correlated activity, derived from BOLD fMRI data, between a reference and target

region that has been used widely in the imaging community as a simple and robust characterization of aspects of functional integration. Converging lines of evidence suggest that this measure reflects anatomically and functionally relevant coupling within neuronal circuitries.<sup>53</sup> Our methods to measure functional connectivity have been described previously.<sup>51,54</sup> Briefly, target regions were chosen from functional clusters identified by the main effects of task. Region of interest masks were created using the WFU PickAtlas for anatomical regions that overlapped with the main effect of task. After mean correction, median activity within this region of interest was correlated on a voxel-by-voxel basis with the preprocessed fMRI time series of all voxels showing significant ( $t=2.7$ , uncorrected) task activation, and the resulting correlation maps were analyzed using the general linear model, as described earlier, using whole-brain cluster correction.

## GENOTYPING

DNA isolation and analysis was conducted on blood samples using standard procedures. The *COMT* val<sup>158</sup>met genotype was determined by 5' exonuclease allelic discrimination TaqMan assay,<sup>55</sup> which uses the 5' nuclease activity of *Taq* DNA polymerase to detect a fluorescent reporter signal generated after polymerase chain reactions.<sup>13</sup> The individuals in the *COMT* genotype groups were also genotyped with a panel of 100 unlinked single nucleotide polymorphisms and showed no significant variation in frequency at any of these single nucleotide polymorphisms, including several that have been associated with variation in brain function and show considerable population variability (eg, *5-HTTLPR*, *BDNF*, *GRM3*) (available on request).

## RESULTS

### SAMPLE DEMOGRAPHICS

*COMT* genotype frequencies were in Hardy-Weinberg equilibrium in this sample of 101 healthy volunteers. Neither allele nor genotype frequencies differed between men and women ( $\chi^2=0.134$ ). A 2-factor (*COMT* genotype and sex) analysis of variance indicated no significant differences in age, IQ, handedness score, or fMRI task performance, as measured by accuracy and reaction time, between the genotype groups (**Table 1**). Since recent reports have indicated that the 5-HTTLPR polymorphism impacts amygdala reactivity<sup>45,56-58</sup> and limbic circuitry,<sup>51</sup> the groups were carefully balanced and did not significantly differ in their distribution of the 5-HTTLPR genotype ( $\chi^2=0.130$ ).

### COMT EFFECT ON BOLD RESPONSE

Consistent with our earlier reports,<sup>42,45,57</sup> direct comparison of the face-processing and sensorimotor control tasks revealed robust bilateral hippocampal, amygdala, fusiform, and prefrontal cortex (PFC) engagement across all 101 subjects (**Figure 1**). Subsequent analyses of genotype main effects were restricted to these brain regions. Statistical parametric maps for direct group comparisons of the BOLD imaging data revealed that met/met homozygotes showed greater right hippocampal formation activity than val/val homozygotes, with val/met heterozygotes exhibiting an intermediate BOLD re-

**Table 1. Demographic Data\***

	met/met	val/met	val/val	Total	P Value
Sample size	24	57	20	101	...
Sex (male/female)	10/14	27/30	14/6	51/50	.13†
Age, y	27.6 ± 6.55	32.4 ± 10.19	30.8 ± 7.45	30.3 ± 9.07	.10
IQ	106.0 ± 8.56	107.3 ± 9.30	105.8 ± 11.38	106.7 ± 9.52	.77
Education, y	16.9 ± 2.82	16.1 ± 2.4	16.5 ± 2.6	16.4 ± 2.6	.50
Handedness score	0.75 ± 0.42	0.71 ± 0.46	0.62 ± 0.64	0.70 ± 0.50	.68
Task accuracy, %	98.49 ± 3.29	99.35 ± 2.81	99.56 ± 1.91	99.19 ± 2.78	.39
Reaction time, ms	1585.2 ± 449.2	1621.3 ± 318.4	1653.1 ± 241.0	1619.2 ± 338.1	.82
Harm avoidance total score	9.5 ± 5.00	9.3 ± 4.93	9.2 ± 5.20	9.3 ± 4.94	.98
Novelty seeking total score	15.4 ± 3.9	15.4 ± 4.31	16 ± 5.59	15.6 ± 4.47	.91

\*Data are expressed as mean ± SD unless otherwise indicated.

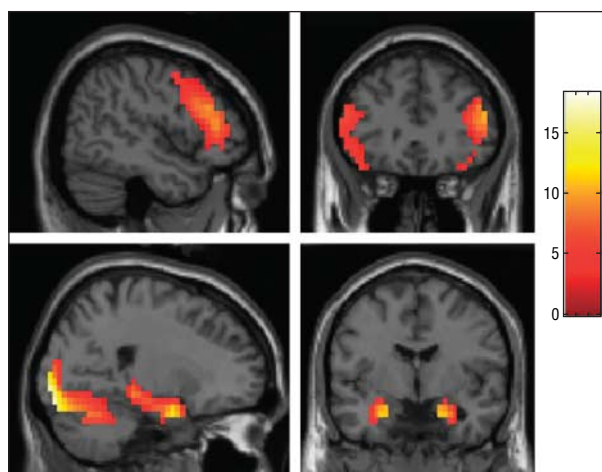
†Results from  $\chi^2$  analysis; all other *P* values obtained from 1-way analysis of variance.

response (**Figure 2**) (**Table 2**). Additionally, met/met subjects exhibited increased right vIPFC activation relative to val/val subjects, with val/met heterozygotes again showing an intermediate effect. The *COMT* genotype accounted for 16% ( $r=0.403$ ;  $P=.007$ ) of the interindividual variance in activation in the hippocampal formation and 13% ( $r=0.359$ ;  $P=.02$ ) of the variance in activation in the vIPFC. Importantly, these effects were independent of sex and age. There were no significant differences in amygdala activation by genotype even when thresholds were dropped below statistical significance. The reverse contrast (val/val > met/met) at the same threshold did not show significant differences in activation anywhere in the brain.

#### COMT EFFECT ON FUNCTIONAL CONNECTIVITY

To further investigate genotype effects on corticolimbic circuitry during emotion processing, functional clusters identified from the main effects of task analysis were used to create reference regions in the hippocampus and vIPFC, 2 regions in which *COMT* is highly expressed.<sup>52</sup> The amygdala, which expresses relatively little *COMT* but is densely interconnected with the PFC and hippocampus and thus may be affected indirectly by *COMT* effects on these regions, was also used as a reference region. Analyses of functional connectivity between these 3 reference regions were conducted with all other voxels significantly activated by the task. Since the 2 homozygote genotype groups (met/met and val/val) were identified as phenotypic extremes in the earlier-mentioned main effects of genotype analyses, functional connectivity analyses were restricted to analysis of variance comparisons between these 2 genotype groups, thus increasing the power to detect genotype effects on functional connectivity and limiting the number of statistical tests.

Analyses of variance using the right vIPFC (Brodmann area [BA] 45) as a reference region revealed greater coupling with the parahippocampal gyrus in met/met subjects relative to val/val homozygotes, as well as bilateral fusiform gyrus. Separate analyses for functional connectivity using each hippocampus as a separate seed region yielded the same results, and so the results were col-

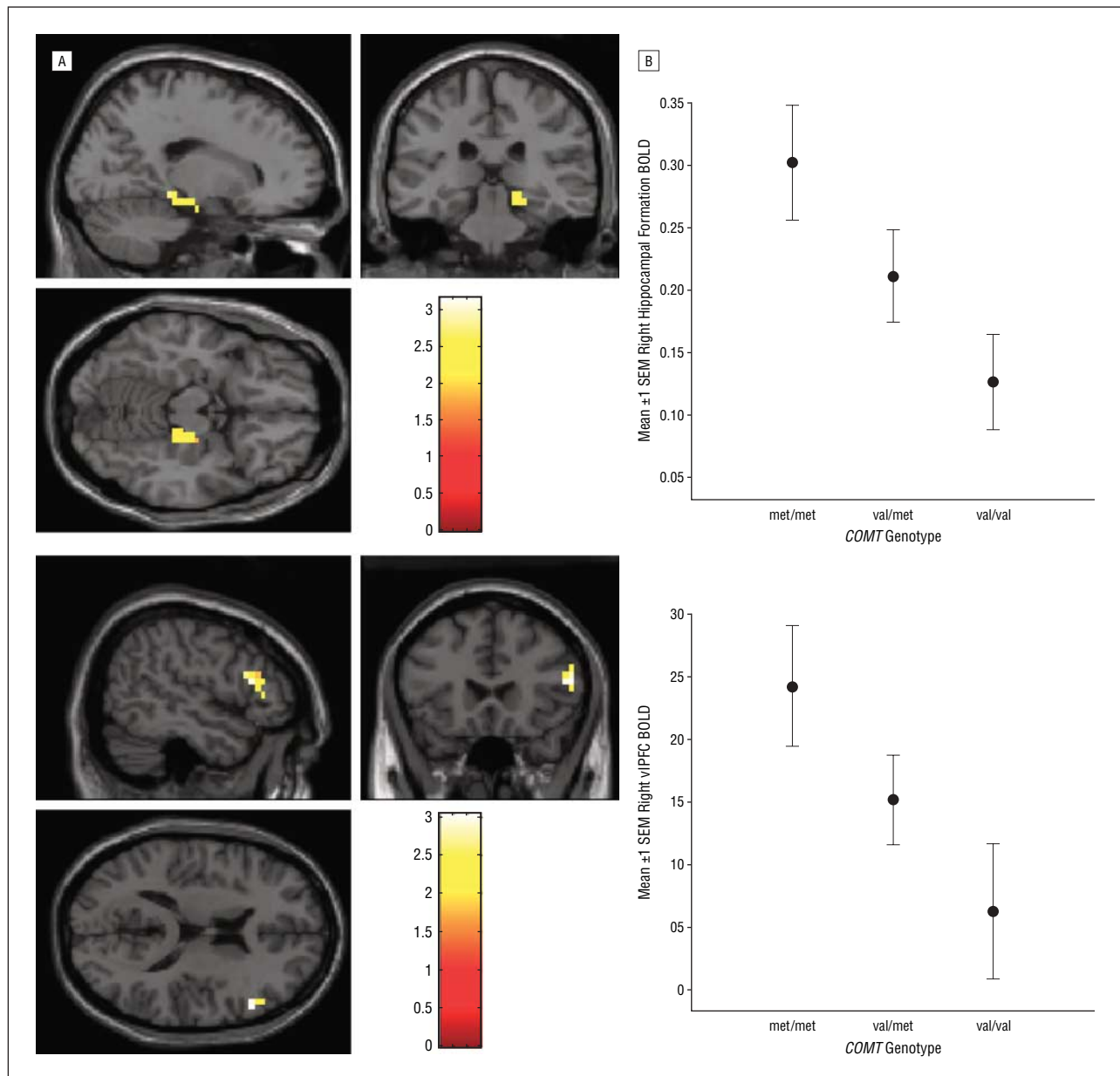


**Figure 1.** Main effects of task. Statistical parametric map of brain activation during the perceptual processing of fearful and threatening faces. Activations are shown overlaid onto an averaged structural magnetic resonance image. Color bar represents *z* scores for activations. Maps were thresholded at  $t=2.7$ ; see Table 2 for coordinates and statistical information.

lapsed across hemispheres by using a bilateral hippocampal mask. The bilateral hippocampus reference region revealed greater connectivity with the vIPFC (BA 45) and orbitofrontal cortex (OFC) (BA 11) in met/met subjects relative to val/val subjects. The right amygdala reference region also indicated greater coupling with bilateral OFC (right, BA 11; left, BA 47) and vIPFC (BA 45) in met/met relative to val/val subjects (**Figure 3**) (Table 2). The reverse contrasts (val/val > met/met) at the same thresholds for each of these comparisons did not show significant differences in functional connectivity anywhere in the brain. When we used a left amygdala and left vIPFC mask for connectivity we found no significant results.

#### CORRELATION OF FUNCTIONAL CONNECTIVITY AND NS

Our results indicate a strong effect of the *COMT* genotype on the coupling of the amygdala and OFC, hippocampus and OFC, and vIPFC and parahippocampal gyrus. Previous research has shown that functional connectivity values from key nodes in emotion process-



**Figure 2.** Main effects of genotype: met/met > val/val. A, Thresholded ( $P < .05$ , corrected for multiple comparisons within the hippocampus and prefrontal cortex) statistical maps showing the main effects of genotype (met/met > val/val) during the perceptual processing of fearful and threatening faces. Color bars represent z scores for activations. See Table 2 for coordinates and additional statistical information. B, Line graphs represent mean  $\pm$  SEM blood oxygen level–dependent (BOLD) signal change extracted from the right hippocampal formation and ventrolateral prefrontal cortex (vlPFC) functional cluster, respectively, identified in panel A. *COMT* indicates catechol *O*-methyltransferase.

ing circuits, rather than BOLD signal from single brain structures, is a better predictor of temperament traits.<sup>51</sup> Given the putative relationship of NS with dopaminergic neurotransmission,<sup>50</sup> and the prominent role of the OFC in emotion processing,<sup>59,60</sup> we performed a correlation analysis between total NS score and both amygdala–OFC and hippocampus–OFC functional connectivity measures.

While NS is not an index of mood,<sup>61</sup> low NS scores represent temperamental rigidity, reflection, reserve, and regimentation.<sup>36</sup> There was no main effect of *COMT* genotype or sex on NS scores in our sample. However, there was a significant negative correlation between total NS score and amygdala–OFC connectivity across the whole sample of met/met and val/val subjects ( $r = -0.373$ ;  $P = .03$ ),

such that increased coupling predicted lower NS scores. To parse out potential underlying *COMT* genotype effects, we conducted genotype group–specific correlation analyses. These analyses revealed a significant negative correlation in met/met subjects ( $r = -0.594$ ;  $P = .009$ ) but not in val/val subjects ( $r = -0.246$ ,  $P = .34$ ) between NS score and amygdala–OFC connectivity (**Figure 4**). This effect was more pronounced in met/met subjects than val/val subjects, although differences in the magnitude of this effect by genotype did not reach significance in post hoc comparisons using the Williams–Pearson test, suggesting that differences between *COMT* genotype groups reflect quantitative rather than qualitative differences.

**Table 2. fMRI Results\***

	Side	BA	x	y	z	Cluster Size	z Score	T Score	P Value	
<b>Main Effect of Task</b>										
Fusiform gyrus	R		34	-74	-13	1302	Inf	13.18	<.001	
Parahippocampal gyrus	R		15	-33	-3	1302	Inf	12.50	<.001	
	L		-26	-29	-7	1302	Inf	10.69	<.001	
Hippocampus	L		-26	-11	-16	1302	Inf	7.85	<.001	
	R		26	-11	-16	1302	Inf	6.23	<.001	
Amygdala	L		-22	-5	-21	1302	Inf	11.01	<.001	
	R		23	-5	-17	1302	Inf	9.24	<.001	
vIPFC	R	46	51	34	12	224	Inf	10.33	<.001	
	L	47	-41	28	-18	187	5.19	5.58	<.001	
	L	45	-49	22	17	187	6.98	7.95	<.001	
<b>Main Effect of Genotype: met/met &gt; val/val</b>										
Hippocampal formation (parahippocampal gyrus)	R		15	-33	-7	30	2.97	3.16	.001	
vIPFC	R	45	55	22	13	15	2.87	3.05	.002	
<b>Functional Connectivity: met/met &gt; val/val</b>										
Reference ROI	Activation Location									
R vIPFC			26	-26	-7	50	4.59	5.27	<.001	
		Parahippocampal gyrus	R							
		Fusiform gyrus	R	19	30	-73	-5	32	3.54	<.001
B hippocampus		Fusiform gyrus	L	19	-34	-73	-5	32	4.00	<.001
		vIPFC	R	45	52	22	13	13	3.65	<.001
		Inferior frontal gyrus (OFC)	R	11	38	33	-10	17	3.64	<.001
R amygdala		Inferior frontal gyrus (OFC)	R	11	38	33	-10	13	3.64	<.001
		Inferior frontal gyrus (OFC)	L	47	-45	37	-6	13	3.88	<.001
		vIPFC	R	45	51	19	13	10	3.24	.001

Abbreviations: B, bilateral; BA, Brodmann area; fMRI, functional magnetic resonance imaging; inf, infinite; L, left; OFC, orbitofrontal cortex; R, right; ROI, region of interest; vIPFC, ventral prefrontal cortex.

\*Voxels reported were significant at  $P < .05$  corrected for multiple comparisons, as described in the experimental procedures. Coordinates have been transformed from Montreal Neurological Institute (McGill University, Montreal, Quebec) space to that of Talairach and Tournoux.

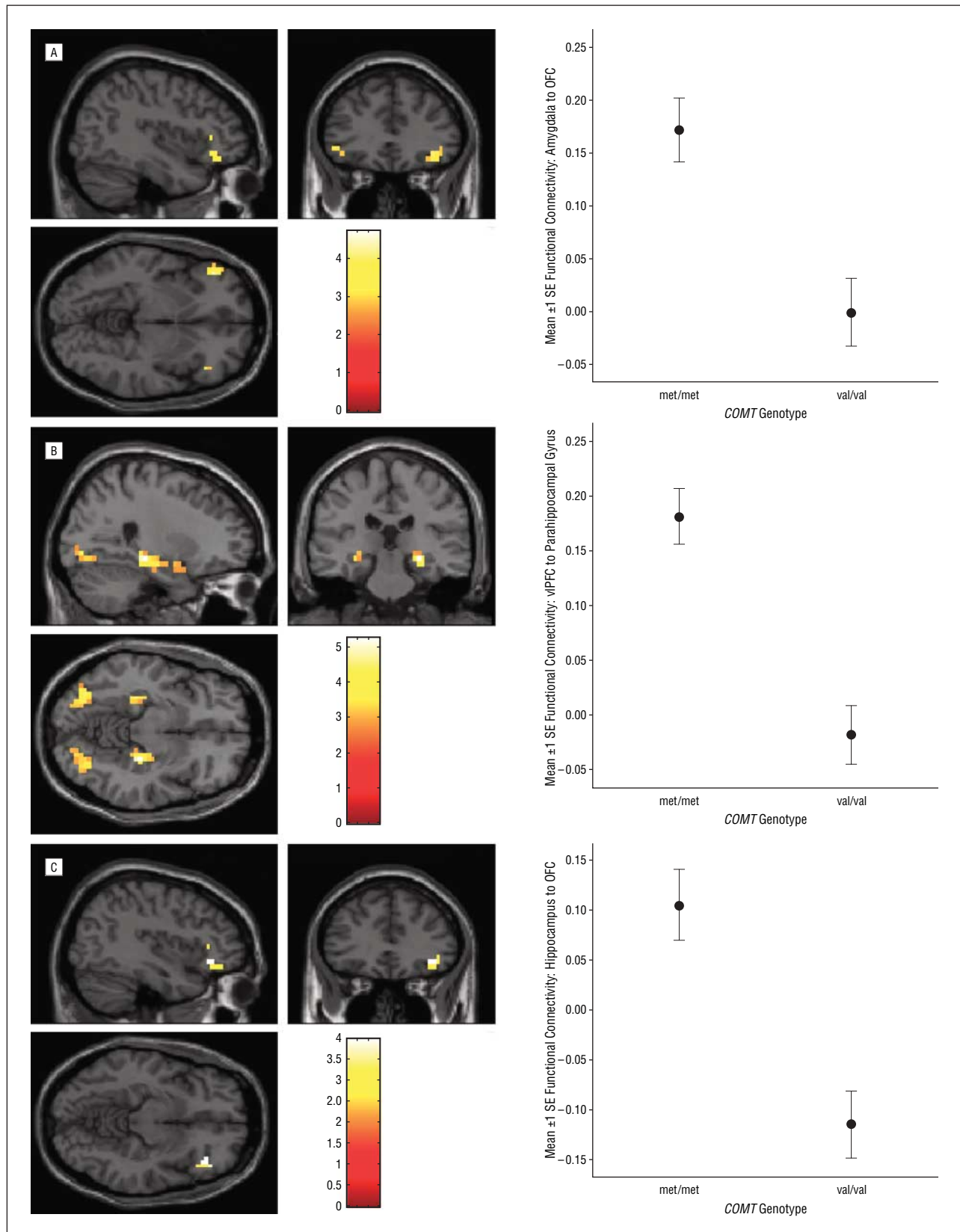
Given the association of another temperamental characteristic, HA, with mood disorders and with connectivity in an amygdala-cingulate circuit related to the 5-HTTLPR genotype,<sup>51</sup> we also investigated the relation of HA and the functional connectivity of these limbic-OFC circuits. There was no correlation between HA score and any of the functional connectivity measures across the whole sample. Furthermore, there was no correlation between NS and HA in this sample, confirming that they are distinctly separate indexes of temperament.<sup>62</sup>

**COMMENT**

Our results, using a well-characterized fMRI paradigm known to robustly engage corticolimbic circuitry,<sup>42,45,57</sup> indicate that heritable variation in dopamine neurotransmission associated with the COMT val<sup>158</sup>met polymorphism impacts the functional reactivity of limbic and prefrontal circuitry implicated in the regulation of emotional arousal. Group analyses revealed that met/met homozygotes exhibited greater hippocampal formation and vIPFC reactivity than val/val homozygotes and val/met heterozygotes. The intermediate response of val/met heterozygotes in the hippocampal formation and vIPFC, demonstrating an allele load effect, is consistent with both in vitro thermostability studies indicating that the alleles act codominantly<sup>63</sup> and in vivo imaging studies reporting similar allele load effects on PFC activation.<sup>17,18,33</sup> To examine COMT genotype effects on brain circuitry involved

in arousal and behavioral control, we conducted functional connectivity analyses using 3 target regions that were robustly engaged by our fMRI task: the amygdala, hippocampus, and vIPFC. These analyses revealed that subjects with the met/met genotype had greater functional connectivity between the amygdala and OFC/vIPFC, the hippocampus and OFC/vIPFC, and vIPFC and parahippocampal gyrus relative to val/val homozygotes. Furthermore, the degree of coupling between the amygdala and OFC negatively correlated with NS scores, but only in COMT met/met individuals.

Importantly, these genotype effects were observed in a large cohort of healthy subjects carefully screened for lifetime history of psychiatric illness or treatment and under stringent controls for the potential confounding effects of age, sex, IQ, and ethnicity between genotype groups. Moreover, our uniquely large sample allowed us to ensure that COMT genotype groups did not differ in 5-HTTLPR allele frequencies, a polymorphism known to impact amygdala activation<sup>45,56-58</sup> and amygdala-PFC connectivity<sup>39</sup> during this particular task. The fact that the groups also did not differ in allele frequencies at 100 unlinked single nucleotide polymorphisms across the genome suggests that the groups did not differ in terms of genetic admixture.<sup>64</sup> Thus, the current results suggest that the effect of the COMT val<sup>158</sup>met polymorphism on corticolimbic circuitry represents a heritable trait related to the biology of this gene and independent of such non-COMT factors.

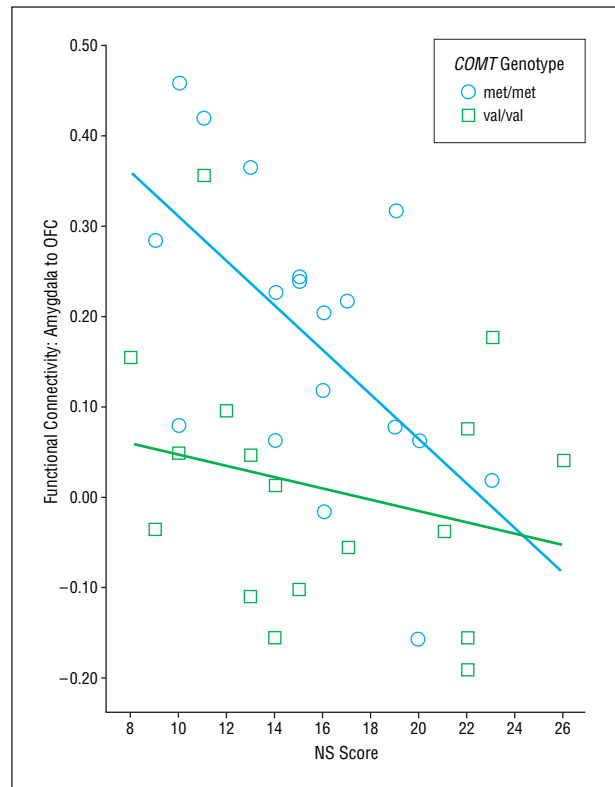


**Figure 3.** Functional connectivity: met/met > val/val. Thresholded ( $t > 2.7$ ) statistical maps showing the functional connectivity associated with the right amygdala (A), right ventrolateral prefrontal cortex (vIPFC) (B), and bilateral hippocampus (C) reference regions for met/met > val/val. Corresponding line graphs represent mean  $\pm$  SEM functional connectivity values extracted from the peak voxel. For the amygdala reference region, values were extracted from  $x = -45$ ,  $y = 37$ , and  $z = -6$ ; for the vIPFC region, values were extracted from  $x = 26$ ,  $y = -26$ , and  $z = -7$ ; and for the hippocampus reference region, values were extracted from  $x = 38$ ,  $y = 33$ , and  $z = -10$ . See Table 2 for coordinates of all voxels significant at  $P < .05$ , corrected for multiple comparisons across the whole brain, and additional statistical information. COMT indicates catechol *O*-methyltransferase; OFC, orbitofrontal cortex.

Interestingly, the 2 regions identified in this study as having the largest main effect of *COMT*, the PFC and hippocampal formation, also show the most abundant expression of *COMT*, both in terms of messenger RNA density<sup>13,52</sup> and enzyme activity,<sup>13,65</sup> thus reinforcing that this functional finding represents changes in *COMT*-mediated dopamine neurotransmission. It might be considered somewhat surprising that we found no evidence of *COMT* effects on amygdala reactivity. However, our results are consistent with human postmortem<sup>66</sup> studies illustrating that *COMT* is minimally expressed in the amygdala where termination of dopamine effects are largely mediated by the dopamine transporter,<sup>67</sup> in contrast to the PFC and hippocampus. Notably, in *COMT* knockout mice and dialysis studies of rats treated with the *COMT* inhibitor drug, tolcapone, cortical dopamine levels were altered while norepinephrine levels were not, implicating dopamine neurotransmission as the basis of our findings.<sup>68,69</sup> Thus, we have limited our discussion of *COMT* effects to dopamine-related changes.

Our findings are consistent with a recent study from Smolka and colleagues,<sup>33</sup> which found increased met allele dose-dependent activation in the right vLPFC and hippocampus in response to unpleasant vs neutral affective pictures. However, Smolka and colleagues also found increased right amygdala activation during their task, which may reflect differences in task design (discriminating valence of complex visual scenes vs general biologically salient arousal) as well as differences in data processing specifications, which might impact anatomical resolution (12-mm smoothing kernel vs our 8-mm kernel). Additionally, the sample in the present study is almost 3 times larger than the sample of Smolka and colleagues and thus has increased power to detect *COMT* genotype effects on brain function and allowed us to more completely control for important confounding effects such as sex and genetic admixture.

Our results indicate that the *COMT* val<sup>158</sup>met polymorphism impacts a neural circuitry that has been implicated in anxiety and negative mood. Evidence from human structural<sup>70</sup> and functional neuroimaging studies,<sup>71</sup> as well as animal lesion models,<sup>6,72</sup> implicates a specific role for the hippocampus in mediating anxious states and behaviors, as well as regulating responses to arousal.<sup>7,73</sup> Recent neuroimaging data have demonstrated a role for dopamine in modulating hippocampal-dependent processing of affectively relevant stimuli.<sup>74</sup> Moreover, a positive correlation between aggressive behavior and hippocampal *COMT* gene expression has been reported in mice<sup>75</sup> and the *COMT* knockout mouse exhibits increased aggressiveness in males and increased anxiety-like behaviors in females.<sup>68</sup> Consequently, *COMT*-mediated dopamine increases in the hippocampus could underlie the amplified responsivity to affectively salient stimuli seen in met/met individuals in an effort to contextualize or regulate an affective response. This may render these individuals more sensitive to negative environmental cues, which, though apparently compensated at the level of clinical phenotypes in these healthy subjects, may lead to exaggerated arousal responses in real-life situations. Likewise, the right vLPFC has been implicated in the processing of emotional stimuli and context and has been



**Figure 4.** Correlation between novelty seeking (NS) and amygdala-orbitofrontal cortex (OFC) connectivity. The graph shows significant negative correlation between amygdala-OFC connectivity and total NS scores across the whole sample ( $r = -0.373$ ;  $P = .03$ ). Within genotypes, the correlation is only significant in met/met subjects ( $r = -0.594$ ;  $P = .009$ ) and not in val/val subjects ( $r = -0.246$ ;  $P = .34$ ). *COMT* genotype met/met,  $R^2$  linear = 0.353. *COMT* genotype val/val,  $R^2$  linear = 0.06. *COMT* indicates catechol *O*-methyltransferase.

specifically linked to alterations in amygdala reactivity to affective stimuli.<sup>43,76-82</sup> Thus, our findings potentially reflect a dopamine-mediated bias in met/met individuals toward dedicating PFC resources to the cognitive processing of affective stimuli. Earlier studies of *COMT* genotype effects on prefrontal processing of working memory revealed greater efficiency associated with the met allele.<sup>17,18</sup> These contrasting findings underscore the potential biological tradeoffs of *COMT* differences in cortical dopamine function depending on the specificity of information being processed.

Recent speculation by Bilder and colleagues<sup>83</sup> provides an intriguing model elucidating a potential mechanism by which the *COMT* val<sup>158</sup>met polymorphism may drive corticolimbic reactivity. According to their tonic-phasic dopamine hypothesis model, the met allele is associated with increased tonic (and decreased phasic) dopamine neurotransmission subcortically and increased phasic dopamine neurotransmission cortically. While such shifts in dopamine tone are hypothesized to result in increased stability of neural networks, they may also render these same networks inflexible to information processing routines.<sup>83</sup> Supporting this notion, Nolan and colleagues<sup>84</sup> have reported significant deficits in task switching in *COMT* met allele carriers. Such basic physiologic and behavioral findings have led Bilder and colleagues<sup>83</sup> to argue that much of the negative emotional-



ity associated with the met allele is related to this fundamental inflexibility. This argument may also elucidate recent findings that met/met individuals have (1) an adverse physiologic response in the PFC to amphetamine,<sup>18</sup> (2) qualitatively different relationships between cortical function and brainstem dopamine metabolism,<sup>14</sup> and (3) decreased frontal P300 amplitude, putatively indicating decreased “noise” in the PFC and, hence, increased stability (and potentially decreased flexibility) of frontal neuronal processing.<sup>85</sup>

Previous literature indicates a key role for the OFC in processing emotional salience and changes in reinforcement contingencies and updating and integrating affective information to guide behavior.<sup>59,60,86</sup> Animals with OFC lesions cannot inhibit a prepotent affective response,<sup>87</sup> and humans with OFC lesions are inflexible and emotionally disinhibited.<sup>88</sup> Additionally, there is evidence indicating robust bidirectional anatomical<sup>89</sup> and functional<sup>90</sup> connections between the OFC and amygdala, and a recent neuroimaging study has found increased OFC and decreased amygdala activation when down-regulating emotion.<sup>82</sup> Although amygdala reactivity and associated arousal are unaffected by *COMT* genotype in our paradigm, the increased OFC-amygdala connectivity seen in met/met subjects during this task may reflect increased sensitivity of OFC circuits to excitatory inputs from the amygdala as a result of a higher signal-to-noise ratio associated with increased cortical dopamine availability in met/met individuals. The hippocampus is also densely functionally interconnected with the OFC, and this circuitry has been deemed “the working memory of viscerio-emotional processing.”<sup>91</sup> Increased hippocampal-OFC connectivity in met/met subjects may reflect increased monitoring of stimulus salience and integrating context with affective response. Thus, in contrast to the demonstrated direct effects of the *5-HTTLPR*-associated changes in serotonin function on amygdala reactivity and coupling with the ventral cingulate,<sup>51</sup> our results suggest that the *COMT* val<sup>158</sup>met genotype exerts effects on emotional behavior by altering a complementary circuitry: the functional coupling of the amygdala and hippocampus with the OFC. We suggest that this effect biases the cortical integration and regulation of limbic structures associated with arousal and stress.

A litmus test of the relevance of our connectivity analyses is whether the results can be related to aspects of temperament. In our data set, met/met subjects show greater amygdala-OFC connectivity that is correlated with low NS, which reflects rigidity and regimentation.<sup>36</sup> This pattern of connectivity, as well as increased hippocampus-OFC connectivity in these subjects, may reflect relative inflexibility in processing and integrating affectively relevant information (ie, an affective perseveration). We propose that met/met individuals are more easily locked into an affective processing set, reverberating emotional information through this reentrant loop. Such affective processing biases do not necessarily result in dysfunctional behavior, as all our subjects were psychiatrically healthy. However, this implicit processing bias in the context of provocative environments (eg, acute or chronic stressors) may lead to increased susceptibility for negative mood states and affective disorders in *COMT* met/met individuals.

There are several limitations of the present study. First, while our task reflects the effect of biologically salient stimuli on the engagement of corticolimbic circuitry, it does not allow for a dissection of neural circuitry reflecting affect-specific processes (eg, anger, disgust, fear). Furthermore, while sample homogeneity facilitated the investigation of subtle contributions of genetic effects to corticolimbic information processing, it limits the generalizability of the findings to diverse populations. Additionally, the blocked design paradigm offers limited temporal resolution, and further examination using more temporally sensitive measures (event-related fMRI or magnetoencephalography) are necessary to disambiguate the time course of activation of specific brain structures during affect processing. Moreover, investigation of interactions between additional functional gene variants in biasing the response dynamics of affective brain circuits, as well as the modulation of these genotype effects by stressful life events, is necessary.

In summary, the genetic predisposition of *COMT* met/met individuals to focus attention on a relevant set of stimuli and inhibit interference from other stimuli, while advantageous in the context of working memory challenges, may have deleterious effects in the context of environments representing multiple biologically provocative stimuli that require rapid and flexible processing. This is evident in the *COMT* met/met phenotype of increased reactivity and connectivity of brain circuitry implicated in generating and regulating affective responses. Additional studies in more diverse samples, including patient populations, are required to further determine the role of such *COMT*-driven rigidity in corticolimbic information processing contributing to risk for affective disorders.

**Submitted for Publication:** May 9, 2005; final revision received December 19, 2005; accepted January 30, 2006.

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**Financial Disclosure:** None reported.

**Funding/Support:** This work was supported by the National Institute of Mental Health Intramural Research Program.

**Acknowledgment:** We thank J. W. Buckholtz, BS, for comments on the manuscript, T. E. Goldberg, PhD, for providing psychological data, and J. H. Callicott, MD, for data acquisition support.

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