Variation in dopamine genes influences responsivity of the human reward system

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In humans, dopamine neurotransmission is influenced by functional polymorphisms in the dopamine transporter (DAT1) and catechol-Omethyltransferase (COMT) genes. Here, we used event-related functional magnetic resonance imaging to directly investigate the neurofunctional effects of the Val¹⁵⁸Met COMT and variable number of tandem repeat DAT1 polymorphisms on distinct components of the reward system in humans. The results revealed a main effect of COMT genotype in the ventral striatum and lateral prefrontal cortex during reward anticipation (P < 0.001, uncorrected) and in the orbitofrontal cortex at the time of reward delivery (P < 0.005), met/met individuals exhibiting the highest activation. The main effect of DAT1 genotype was seen in robust blood-oxygen-level-dependent response differences in the caudate nucleus and ventral striatum during reward anticipation (P < 0.001) and in the lateral prefrontal cortex and midbrain at the time of reward delivery, with carriers of the DAT1 9-repeat allele showing the highest activity. Moreover, an interaction between the COMT and DAT1 genes was found in the ventral striatum and lateral prefrontal cortex during reward anticipation and in the lateral prefrontal and orbitofrontal cortices as well as in the midbrain at the time of reward delivery, with carriers of the DAT1 9-repeat allele and COMT met/met allele exhibiting the highest activation, presumably reflecting functional change consequent to higher synaptic dopamine availability. Taken together, these results indicate that genetically influenced variations in dopamine transmission modulate the response of brain regions involved in anticipation and reception of rewards and suggest that these responses may contribute to individual differences in reward-seeking behavior and in predisposition to neuropsychiatric disorders.

 $\mathsf{COMT} \mid \mathsf{DAT} \mid \mathsf{fMRI} \mid \mathsf{genetic} \ \mathsf{variation}$

he dopamine system and its projections sites, which include the striatum and the prefrontal cortex (PFC), play crucial roles in modulating motivated behavior, emotion, and high-order cognitive functions related to reward processing. The functions of reward include approach and consummatory behavior as well as the ability to predict the outcomes of potentially rewarding situations, serving goal-directed behavior and providing an evolutionary advantage for creatures facing unpredictable environments. The integrity of the dopamine system is important for efficient processing of reward information. Dysfunction of this system is involved in a variety of disorders, including schizophrenia, Parkinson's disease, pathological gambling, and drug addiction. Although there are clear individual genetic differences regarding susceptibility to and manifestation of these neuropsychopathologies, the influence of genetic predispositions and variations on activation of the human reward system remains poorly understood. Investigating the effects of interindividual differences in dopamine signaling on the response of the reward system is thus an important research question because these differences may contribute to heritable personality traits in the general population (1, 2) and to neuropsychiatric conditions involving abnormalities in catecholamine neurotransmission, such as substance abuse (3), mood disorders (4), obsessive compulsive disorder (5), attention deficit hyperactivity disorder, and schizophrenia (6).

Two important proteins contribute to terminating the action of intrasynaptic dopamine in the brain: Catechol-O-methyltransferase

(COMT), which catabolizes released dopamine, and the dopamine transporter (DAT), which plays a crucial role in determining the duration and amplitude of dopamine action by rapidly recapturing extracellular dopamine into presynaptic terminals after release.

In humans, the *COMT* gene contains a common and evolutionarily recent functional polymorphism that codes for the substitution of valine (val) by methionine (met) at codon 158. The COMT enzyme is involved in the metabolic degradation of catecholamines, catalyzing transfer of the methyl group from *S*-adenosyl methionine to a hydroxyl group of a catecholic substrate, thereby converting dopamine into 3-methoxytyramine and norepinephrine into normetanephrine. Because the COMT protein containing methionine is relatively thermolabile, its activity is lower at body temperatures than the COMT valine protein, which is fully active at body temperature. Hence, individuals with 2 copies of the met allele (met/met) have 25–75% reduction in COMT enzyme activity compared to individuals with 2 copies of the val allele (val/val), and therefore presumptively more baseline synaptic dopamine (7, 8).

The DAT1 gene (SLC6A3) includes 15 exons, with a variable number of tandem repeat (VNTR) polymorphism in the 15th exon, a region encoding the transcript's 3' UTR (9). The 40-bp VNTR element is repeated between 3 and 13 times but occurs with greatest frequency in the 9- and 10-repeat forms. Expression of the DAT1 9-repeat allele is lower than the 10-repeat allele (10-12), although one study reported the opposite (13). Thus, the DAT1 10-repeat allele, associated with increased expression of the gene, presumably leads to relatively decreased extrasynaptic striatal dopamine levels, consistent with a human single-photon-emission computed tomography report of increased striatal DAT availability in 9-repeat carriers relative to 10-repeat carriers (ref. 14, but see ref. 12). Mice lacking the DAT1 gene show extensive adaptative changes in the dopaminergic system, the DAT controlling the duration of extracellular dopamine signals and regulating presynaptic dopamine homeostasis (15).

Importantly, animal studies indicate differential functional localization of the COMT and DAT proteins. The COMT enzyme plays a particular role in modulating dopamine in PFC, where *DAT*1 expression is sparse (16, 17). *COMT* is expressed more abundantly in cortical neurons than in the striatum (18), but it is unclear to what extent *COMT* modulates catecholamine function outside the cortex. Recent studies in COMT knockout mice suggest that COMT has little if any role in striatal DA levels (19). In contrast, animal research and human postmortem studies indicate

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that the DAT1 is expressed abundantly in midbrain, striatum, and hippocampus but sparsely in the PFC (20, 21).

Electrophysiological experiments on nonhuman primates have demonstrated that midbrain dopamine cells fire both during anticipation of uncertain rewards and at the time of unexpected reward delivery (22). In parallel with these fundamental results, fMRI studies in healthy humans have documented that distinct reward anticipation- and outcome-processing phases are associated with differential patterns of specific midbrain dopaminergic postsynaptic targets (23-25). Specifically, anticipation of reward robustly activates the ventral striatum (23, 24), particularly during anticipation of rewards with maximal uncertainty (i.e., reward probability = 0.5) (25) whereas rewarded outcomes activate the lateral and orbital parts of the PFC (23, 25). Despite the direct involvement of the COMT and DAT proteins in dopamine transmission, the influences of COMT and DAT1 functional polymorphisms on distinct components of the reward system have not been as systematically explored as have been the domains of working and episodic memory (21, 26, 27). Here, we used an event-related fMRI reward paradigm to directly investigate the relationship between COMT and DAT1 functional polymorphisms and the response of the reward system during anticipation of uncertain reward and at the time of reward delivery, bridging the gap between basic molecular genetics, fundamental electrophysiological findings and functional neuroimaging in humans.

Because COMT primarily modulates dopamine in the PFC whereas the effects of *DAT*1 predominate in the striatum (16, 17, 20), our central hypotheses were that DAT1 and COMT polymorphisms would influence reward-related activity in the ventral striatum and PFC, respectively, and that there may also be gene-gene interactions. More specifically, because reward anticipation activates the ventral striatum and reward delivery involves the PFC (23–25), we tested the following hypotheses: (i) individuals with the 9-repeat DAT1 allele, associated with lower expression of the gene and relatively increased extrasynaptic striatal dopamine levels will exhibit higher ventral striatal activation during anticipation of uncertain rewards; (ii) compared to individuals homozygous for the *COMT* val allele, those homozygous for the met allele, who have relatively lower COMT enzyme activity and presumably higher levels of prefrontal synaptic dopamine, will exhibit stronger response in the PFC at the time of reward delivery; and (iii) interactive effects between DAT1 and COMT polymorphisms may be observed at both striatal and prefrontal sites.

Functional Imaging Data. All participants performed at ceiling (>97% correct). No significant response time difference was observed between genotyped groups, indicating that between-group brain activation differences cannot simply be attributed to general attentional, perceptual, or cognitive phenomena. Because our a priori hypotheses concerned the roles of the PFC and the striatum, we focus on results in these brain regions. A complete list is provided in supporting information (SI) Tables S1 and S2.

Genotype Effects During Anticipation of Uncertain Rewards. First, during anticipation of uncertain rewards, we identified brain areas showing robust correlation between the number of val alleles (0, 1, or 2) and the blood-oxygen-level-dependent (BOLD) response. Met/met carriers activated the ventral striatum, the left superior prefrontal gyrus, and the dorsolateral PFC (DLPFC) more than val/val individuals, with heterozygotes showing an intermediate response (Fig. 1). That is, individuals homozygous for the met allele exhibited more activity than val/met heterozygotes, who in turn showed greater activity than val homozygotes. The opposite analysis revealed no significant findings in the PFC or striatum.

Second, we compared brain activity between 9- and 10-repeat allele carriers during anticipation of reward. Confirming our first hypothesis (see Introduction), more robust bilateral ventral striatal

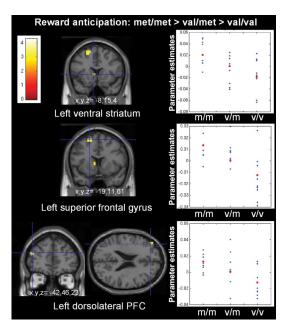


Fig. 1. Main effect of COMT genotype during anticipation of reward with maximal uncertainty. (Left) Statistical maps showing BOLD fMRI responses in the ventral striatum, left superior PFC, and dorsolateral PFC overlaid onto structural MRI coronal and axial planes. (Right) Negative relationship between COMT val allele dosage (0_met/met, 1_val/met, or 2_val/val) and BOLD response in these brain regions during anticipation of reward with maximal uncertainty.

and caudate nuclei activities were observed in 9-repeat compared to 10-repeat DAT1 individuals (Fig. 2). No voxel in the PFC or striatum survived the opposite comparison.

Third, we assessed gene-gene interactions during reward anticipation by using 2 covariates: the DAT1 genotype [2 levels: 9-repeat (including *DAT*1 9-repeat and 9/10 repeat) and *DAT*1 10-repeat] and COMT genotype (3 levels: met/met, val/met, and val/val). Confirming our third hypothesis, an interaction (9-repeat > 10repeat and met/met > val/met > val/val) was found between COMT and DAT1 in the bilateral ventral striatum, caudate nuclei, and in the left anterior lateral PFC region (Fig. 3). The opposite interaction effect (9 < 10 and met/met < val/met < val/val) was only present in the temporal cortex bilaterally.

Genotype Effects at the Time of Reward Outcome. As in the reward anticipation phase, we next identified, at the time of reward receipt, brain areas showing correlations between the number of *COMT* val alleles (0, 1, or 2) and the BOLD response. As predicted by our second hypothesis, met/met carriers activated the orbitofrontal cortex (OFC) bilaterally more than val homozygotes, with heterozygotes showing an intermediate response (Fig. 4). Regression analysis in the opposite direction revealed no significant effect in the OFC or striatum, but a small area in the left DLPFC was affected.

Individuals with the 9-repeat DAT1 allele showed a more robust BOLD response in the right DLPFC and the right anterior PFC compared to 10-repeat carriers (Fig. 5 Top). In the opposite comparison, 10-repeat DAT1 allele did not show higher BOLD response than 9-repeat carriers in the frontal lobe or the basal ganglia. In addition, because a recent episodic memory encoding study reported higher midbrain activation in 9-repeat compared to 10-repeat allele carriers (21), we searched for between-group differences in this region with a less stringent (P < 0.01)statistical threshold. We found higher midbrain activation in 9-repeat compared to 10-repeat carriers (Fig. 5 Bottom).

Finally, consistent with our third hypothesis, at the time of the rewarded outcome, an interaction between the COMT and DAT1 genes (comparison 9-repeat >10-repeat and met/met > val/

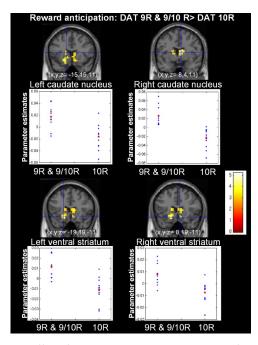


Fig. 2. Main effect of *DAT*1 genotype during anticipation of reward with maximal uncertainty. (*Top*) Statistical maps showing BOLD fMRI responses in the bilateral caudate nuclei. More robust BOLD response was observed in 9-repeat carriers (including *DAT*1 9-repeat and 9/10) compared to 10-repeat individuals during reward anticipation. (*Bottom*) Statistical maps showing BOLD fMRI responses in the bilateral ventral striatum. Again, 9-repeat carriers exhibited higher BOLD response than 10-repeat individuals.

met > val/val) was observed in 2 right lateral PFC regions (Fig. 6 Top), near to those found with the main effect of DAT1 genotype. An interaction between the COMT and DAT1 genes was also observed in the midbrain and the right OFC with a less stringent (P < 0.01) statistical threshold (Fig. 6 Middle and Bottom). No voxel in the PFC or striatum survived the opposite interaction (9 < 10 and met/met < val/met < val/val).

Discussion

Our study identified influences of *COMT* and *DAT1* functional polymorphisms, and interactions between them, on specific components of the reward system. A gene–gene interaction was present in ventral striatum during reward anticipation and in the PFC at the time of reward delivery, demonstrating the influence of variation in dopamine-regulating genes on two brain regions respectively involved in anticipation versus receipt of reward (23, 24).

Effects of COMT Val¹⁵⁸Met Genotype on Reward-Related Processes. First, during anticipation of rewards with maximal uncertainty, the number of met alleles positively correlated with response of the ventral striatum-lateral PFC circuit (Fig. 1), which may be due to the fact that individuals with the met/met genotype have lower COMT enzyme activity and relatively more baseline dopaminergic signaling at PFC synapses than val homozygotes. Although the action of *COMT* genotype on the PFC was hypothesized and could be predicted on the basis of the animal literature, we also observed a main effect of *COMT* genotype on the striatum during reward anticipation. That is, higher *COMT* activity conferred by the val allele and associated with reduced PFC dopamine may indirectly impact dopaminergic function in brain regions other than the PFC, likely as a manifestation of changes in *COMT* prefrontal activity leading to downstream adaptive changes (28).

A primary role for *COMT* genotype in PFC functions is supported by a wealth of findings, such as the fact that *COMT* is expressed more abundantly in cortical neurons than in the striatum

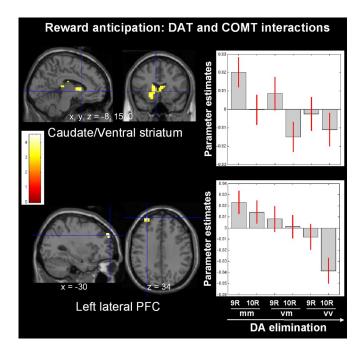


Fig. 3. Interaction between *COMT* and *DAT*1 genotypes with *COMT* Met homozygous and *DAT*1 9-repeat homozygous subjects showing augmented neural response during anticipation of reward with maximal uncertainty. (*Left*) Statistical maps of the BOLD signal interaction showing stronger engagement of ventral striatum and anterior lateral PFC. (*Right*) Interaction between the *COMT* and *DAT*1 genotypes in the prefronto-striatal system during anticipation of reward. Combined heritable variation in dopamine neurotransmission associated with the *COMT* met/met and 9-repeat and 9/10 VNTR *DAT*1 alleles produces hyperresponsivity of the reward system.

(17, 18) and accounts for more than 60% of dopamine degradation in rodent PFC, but less than 15% in the striatum (16), likely because of the scarcity of cortical dopamine transporters relative to the striatum (20). Recently, a real-time study of evoked dopamine overflow showed that removal of dopamine was twofold slower in the PFC of mice lacking *COMT* than in wild-type mice, whereas dopamine overflow/decline in the dorsal striatum was not affected (19), thus demonstrating the significant contribution of COMT in modulating dopamine dynamics in rodent PFC.

Confirming our second hypothesis at the time of the rewarded outcome the presence of val alleles was associated with less activation in the OFC (Fig. 4). Because this region is normally implicated in rewarded versus nonrewarded outcomes and in discriminating the relative value of rewards (23), the reduced excitability of the OFC to rewarded outcome in val/val carriers may reflect lower extrasynaptic dopamine levels and lower saliency of reward value in that group, which mirrors their lower ventral striatal activation during anticipation of uncertain rewards.

Relationship to Previous COMT Findings. A number of recent studies indicate that whether the val allele is beneficial or detrimental for prefrontal functions depends on a variety of factors, such as the nature of the task undertaken and the current dopaminergic state of the PFC (29). For example, whereas the met allele is beneficial for performance of working memory and PFC function (30), it is also associated with greater activity of limbic regions in response to unpleasant visual stimuli (28, 31), as well as with abnormal affective responses such as OCD in men (32), increased anxiety in women (2), and panic disorder (33) or negative states such as pain sensitivity (34). The present results extend this body of work to reward processing. The differences between findings in working memory versus processing of emotional and rewarding stimuli may reflect the importance of dopamine for intrinsic signal-to-noise

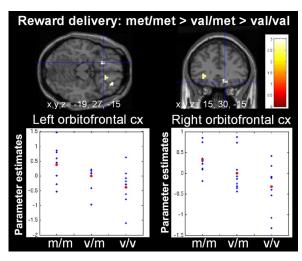


Fig. 4. Main effect of COMT genotype at the time of reward delivery $(Outcome_{20_All\ potentially\ rewarded\ slots} > Outcome_{0_Slot_D}\ where\ slot_D\ denotes\ the$ slot machine with sure knowledge to get no reward). (Upper) Statistical maps showing BOLD fMRI responses in the OFC. (Lower) Negative relationship between COMT val allele dosage and OFC activation at the time of reward delivery.

regulation in PFC for the former versus the primacy of subcortical dopamine in the latter. The impact of COMT genotype on PFC, thus, depends on the cognitive domain, the neural circuit recruited by the task as well as the role of the PFC in processing specific components of the task.

Our findings concerning the effect of COMT genotype on the reward system have important clinical implications. Indeed, the COMT Val¹⁵⁸Met polymorphism has been associated with substance abuse and neuropsychiatric conditions involving abnormalities in catecholamine neurotransmission, such as mood disorders (35), OCD (5), attention deficit hyperactivity disorder, and schizophrenia (6), long believed to involve a dysfunction of the dopaminergic system. Interestingly, smokers with genes associated with low resting dopamine tone (such as val/val individuals) have greater smoking-induced dopamine release than those with alternative genotypes (36). This finding suggests that polymorphisms of dopamine-regulating genes explain a significant proportion of the interindividual variability in smoking-induced dopamine release (37) and may confer genetic predisposition to smoking. Moreover, at the behavioral level, the COMT polymorphism may be associated with specific individual differences in personality traits, val/val carriers being more likely to have higher novelty-seeking and risk-seeking scores (1, 2) than met/met individuals. In contrast, in women, who are more prone to anxiety disorders than men, met/met individuals tend to have more anticipatory worry and fear of uncertainty (2). These personality differences, although not always found, may reflect relative differences in levels of synaptic dopamine, which modulates emotion and motivation.

Effects of DAT1 VNTR Genotype on Reward-Related Processes. Confirming our first hypothesis, during reward anticipation, DAT1 9-repeat carriers activated the ventral striatum more than 10-repeat individuals (Fig. 2), likely reflecting lower extrasynaptic striatal dopamine levels in 10-repeat carriers in whom DAT1 gene expression is greater both in vitro and in vivo (10, 11, 14), although see ref. 12 for opposite results. It should additionally be noted that uptake by the DAT is the most effective mechanism for terminating the synaptic action of dopamine in the striatum, and the role of COMT in dopamine elimination is minimal in this brain region (38).

Our finding of higher lateral PFC activation in 9-repeat carriers at the time of rewarded outcome (Fig. 5), while not hypothesized, nevertheless extends to the reward domain a recent working

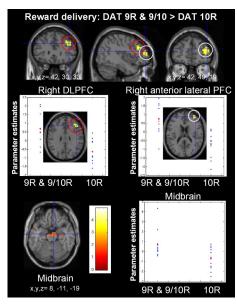


Fig. 5. Main effect of DAT1 genotype at the time of reward delivery. (Top) Statistical maps showing BOLD fMRI responses in the lateral prefrontal cortex. The graphs below show higher prefrontal BOLD signal in DAT1 9-repeat allele dosage compared to 10-repeat carriers at the time of reward delivery. (Bottom Left) Statistical map showing BOLD fMRI responses in the midbrain. (Bottom Right) Graph showing higher midbrain BOLD response in 9-repeat carriers as compared to 10-repeat individuals at the time of reward delivery.

memory report (27) of prefrontal modulation by DAT1 genotype and a report of higher reactivity of an episodic-memory network in 9-repeat carries (21). Thus, it seems that 9-repeat carriers, irrespective of the investigated domain, show hyperresponsivity of specific brain networks. DAT1 genotype influence on lateral PFC activity at the time of reward delivery may reflect: (i) local volume transmission of dopamine in PFC circuits; (ii) indirect influence of striatal DAT1 genotype on prefrontal activity via striato-cortical loops and/or (iii) direct influence via dopamine neurons, projecting to the PFC. The last is in part supported by our data because we found higher midbrain activity in carriers of the 9-repeat allele of the DAT1 VNTR when compared with 10-repeat homozygotes, extending a recent result obtained in the midbrain/substantia nigra during episodic memory encoding (21). That study, as ours, also observed no comparable effect in the midbrain for the COMT Val¹⁵⁸Met polymorphism, suggesting that the *DAT*1 polymorphism more reliably influences BOLD activity in this brain region.

Our findings regarding the effect of the *DAT*1 polymorphism on reward functions may shed light on the neural correlates of a number of neuropsychiatric disorders because the 10-repeat allele has been associated with attention deficit hyperactivity disorder and because the DAT is a target for psychoactive drugs and a gateway for several neurotoxins that destroy dopaminergic neurons.

Although the 3' VNTR polymorphism of the DAT gene is not associated with an amino acid variation and there is uncertainty about its functional effects, the observation of genotype-dependent differences in reward-related brain activity and in the availability of dopamine transporters implicates an effect of this polymorphism, or of a functional polymorphism to which it is linked, on the molecular mechanisms that account for availability of the DAT protein.

Interaction Between the COMT and DAT1 Genes. One important insight provided by our data is a clear demonstration of interaction between the DAT1 and COMT genes that controls a complex phenotype (activation of the reward system). This interaction likely reflects differences in dopamine level because of the combined effect of the COMT val/val and DAT1 10/10 genotypes on dopamine

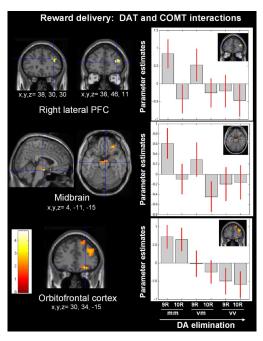


Fig. 6. Interaction between the *COMT* and *DAT*1 genotypes in the lateral PFC, midbrain, and OFC at the time of reward delivery. (*Left: Top, Middle, Bottom*) Statistical maps showing BOLD fMRI responses in the right lateral prefrontal cortex, midbrain, and right OFC. (*Right: Top, Middle, Bottom*) Effect of the interaction between *COMT* and *DAT*1 genotype on BOLD response in these brain regions at the time of reward delivery.

elimination in the fronto-striatal system. Interestingly, the effects of this presumed low dopamine level in val/val and 10-repeat carriers differ both according to brain regions and task phases (Figs. 3 and 6). Although an interaction between the *COMT* and *DAT*1 genes was found in the ventral striatum and left lateral PFC during reward anticipation, it was present in the right lateral and orbital PFC and in the midbrain at the time of reward delivery, carriers of the *DAT*1 9-repeat allele and *COMT* met/met allele exhibiting the highest activation, presumably reflecting functional change consequent to higher synaptic dopamine availability.

Dopaminergic innervation to the striatum plays an important role in drug-seeking behavior and both the Val¹⁵⁸Met genotype and the *DAT1* VNTR polymorphisms (3) show association with drug abuse. Because gambling, with its intrinsic reward uncertainty characteristics, may share common reinforcing neural mechanisms with addictive drugs, the highest functional reactivity of the ventral striatum and OFC observed in met/met and 9-repeat carriers may boost susceptibility to drug addiction. At the behavioral level, the robust striatal and PFC (lateral and orbital parts) activations observed with the number of *COMT* met and *DAT1* 9-repeat alleles may be associated with greater motivation during reward anticipation and higher hedonic response at the time of reward delivery. Thus, our data demonstrate that variation in genes coding for enzymes crucial for dopamine transmission interactively modulate response of distinct components of the reward system.

A recent study used a guessing task to investigate how individual variation in *COMT* and *DAT*1 genes influences reward processing (39). In accordance with our results, that study reported that, during reward anticipation, lateral PFC and ventral striatum activities were *COMT* genotype-dependent: met homozygotes showed larger response in these brain regions than val homozygotes. This effect was observed when averaging all probabilities and magnitudes against baseline, but no main effect of *COMT* genotype was observed on ventral striatal sensitivity to reward uncertainty and no main effect of *DAT*1 genotype on striatal activity during reward anticipation

was reported despite the well-established abundancy of DAT in the striatum. A gene-gene interaction between *COMT* and *DAT*1 was observed in the ventral striatum when sorting genotypes from met/met *DAT*1 10-repeat allele to val/val 9-repeat allele, interpreted as consistent with the notion that basal dopaminergic tone, regulated by *COMT*, interacts with phasic dopamine release, regulated by the *DAT* (40). It is difficult to directly compare our findings to these previous results because *COMT* and *DAT*1 genotypes may both directly influence distinct components of the human reward system (COMT modulating the DLPFC and DAT, the striatum) and differentially affect their neurofunctional balance in a task-dependent manner. Finally, because this previous study did not report results at the time of reward delivery, it remains unclear whether distinct phases of this guessing task induce differential brain activity dependent on *COMT* and *DAT*1 polymorphisms.

Patterns of genetic interactions among unlinked loci that produce impaired synaptic function or impaired development of homeostatic response may account for the epistatic component of genetic risk for neuropsychiatric disorders. One interesting possibility inferred from our finding of interaction of met/met and 9-repeat alleles is that *COMT* may have a general homeostatic role in regulating several genes, such as *DAT*1, to enhance dopaminergic signaling. This hypothesis extends recent transcriptional profiling and pharmacological manipulations identifying a transcriptional and behavioral interaction between the proline dehydrogenase (*Prodh*) and *COMT* genes, which may represent a homeostatic response to enhanced dopaminergic signaling in the frontal cortex (41).

Possible Future Directions. Our work may have implications for potential genotype-based targeted pharmacological treatments aimed at modifying activity of the reward system in neuropathogical disorders, such as drug addiction, depression, schizophrenia, and Parkinson's disease. For example, whereas no applications related to reward have yet been attempted, treatment with tolcapone, a specific *COMT* inhibitor, affected measures of verbal episodic memory in a *COMT* genotype-specific manner, such that individuals with val/val genotypes improved, whereas individuals with met/met genotypes worsened (5). A similar gene-based pharmacological intervention on reward-related neural activity would extend the current results.

Conclusion

It should be noted that our study cannot establish the neurophysiological mechanisms underlying the relationship between dopamine release and BOLD signal increase. However, the present work directly links genotype-dependent synaptic dopamine availability with BOLD signal change in humans and suggests more activity at prefronto-striatal sites, conferred by specific genotypes, is associated with greater dopamine synaptic availability (i.e., less dopamine elimination), in agreement with recent studies observing: (i) that in young adults there is a tight coupling between increased midbrain dopamine synthesis and reward-related increase BOLD signal in the PFC both during reward anticipation and at the time of reward delivery (42); and (ii) that in animals, injection of dopaminereleasing agents increases BOLD signal in mesolimbic regions (frontal cortex, striatum, cingulate cortex) with a time course that parallels changes observed by microdialysis measurements of striatal dopamine release (43).

Taken together, our fMRI results indicate that responsivity of a prefronto-striatal reward-related network is directly influenced by heritable variation in dopamine neurotransmission associated with the *COMT* and *DAT1* polymorphisms. The increased BOLD responses observed in met/met and 9-repeat individuals at sites receiving dopaminergic projections may correspond to greater synaptic dopamine availability. Such genetically driven variations in dopamine function and consequent reactivity of the reward system have important implications for clinical manifestations of diseases

that involve disordered catecholamine regulation and that may clarify biological mechanisms underlying individual differences.

Interestingly, both the val/met and the DAT1 VNTR polymorphisms may be evolutionarily recent, as a VNTR homologue has been observed in humans, chimpanzees, and cynomologous macaques, but not in lower mammals, including the rat and mouse. Because no equivalent polymorphism of the human COMT gene has been found in any other species examined to date (44), the met variant appears to be specific to humans. As previously discussed, the met allele is associated not only with beneficial effects in the cognitive domain, but also with detrimental phenotypes, such as impaired emotional processing and OCD (28, 31). Thus, these multiple and complex associations may explain the persistence in the human population of the val and met alleles as well as their synergistic action with the DAT1 VNTR polymorphisms.

Materials and Methods

Subjects. Twenty-seven right-handed young subjects (mean age = $27.3 \pm [SD] 5.7$, 16 males) with known COMT genotype provided written informed consent following procedures approved by the National Institute of Mental Health Institutional Review Board. Twenty-two had available DAT1 genotype. Because many $factors\,other\,than\,genetic\,polymorphisms\,contribute\,to\,variance\,in\,the\,fMRI\,data$ and must be minimized to identify genetic effects, the three COMT genotype groups on one hand, and the two DAT1 groups on the other hand, were matched for age, sex, handedness, and educational background. All subjects (except one) were of European ancestry. The final \emph{COMT} sample consisted of 9 val/val subjects (age = 25.5 \pm 3.8 years; years of education = 16.8 \pm 2.9; 4 women), 9 val/met carriers (age = 28.6 \pm 6; years of education = 15.8 \pm 2; 3 women) and 9 met/met

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individuals (age = 27.8 \pm 3.8; years of education = 16.6 \pm 1.7; 4 women). The DAT1 sample was composed of 11 10/10 subjects (age = 26.2 \pm 5.5 years; 5 women) and 11 9-repeat allele carriers (age = 29.5 ± 4.7 ; 4 women, 3 of these 11 subjects had 9/9 DAT1 genotype and 8 were 9/10 DAT1 carriers). All subjects were free of neurologic, psychiatric, and substance abuse problems. They had no history of gambling and no medical problems or medical treatment that could affect cerebral metabolism and blood flow. Smokers were also excluded. Subjects were paid for participating and earned extra money for performing the task described below. They were told that they would earn only a percentage of each of the cash values presented on the screen.

Experimental Paradigm. Experimental trials were divided into 2 phases: Reward anticipation and outcome. During reward anticipation, a slot machine was presented on the screen and the words: "Chance to win \$ XX" (where XX stood for \$0, \$10, or \$20) remained visible on top of each slot machine with a pie chart indicating the probability of winning the indicated amount of money. There were four slot machines (see SI Methods) and subjects indicated which slot machine was presented by pressing a specific response button on a diamondshaped four-response button device at the time of slot presentation and again at the time of the outcome (regardless of winning or not).

Image Analysis. See SI Methods for details. We used a threshold of P < 0.005, uncorrected in the PFC and striatum (random effects model) in all comparisons because of our strong a priori hypotheses concerning these reward-related brain regions. Findings outside these hypotheses-driven brain regions are reported if they met a statistical threshold of P < 0.001, uncorrected.

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