

Midbrain dopamine and prefrontal function in humans: interaction and modulation by *COMT* genotype

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Using multimodal neuroimaging in humans, we demonstrate specific interactions between prefrontal activity and midbrain dopaminergic synthesis. A common V(108/158)M substitution in the gene for catecholamine-O-methyltransferase (*COMT*), an important enzyme regulating prefrontal dopamine turnover, predicted reduced dopamine synthesis in midbrain and qualitatively affected the interaction with prefrontal cortex. These data implicate a dopaminergic tuning mechanism in prefrontal cortex and suggest a systems-level mechanism for cognitive and neuropsychiatric associations with *COMT*.

Prefrontal cortex (PFC) and midbrain are precisely synaptically interconnected¹, and the activity of dopamine neurons in the midbrain is under both excitatory and inhibitory control of the PFC^{2,3}. These interactions are critical for motivated behavior, working memory⁴ and reward-related learning, and have been proposed to contribute to the pathogenesis of several neuropsychiatric disorders, including schizophrenia^{3,5}. *COMT*, a major enzyme in dopamine catabolism, influences cortical dopamine flux, especially in PFC as a result of the paucity of dopamine transporters in this region⁶; and a marked increase in prefrontal dopamine pools is seen in *COMT*-knockout mice⁷. A common mutation in the *COMT* gene causing a valine-to-methionine substitution, V(108/158)M, leads to a significant reduction in the activity of the enzyme in brain and lymphocytes (codon number depends on protein variant)⁶. This polymorphism has been implicated in a number of neuropsychiatric phenotypes, particularly risk for schizophrenia⁸, and has been shown to influence PFC activation⁸, PFC-dependent neuropsychological function⁸ and response to amphetamine in humans⁹. A recent postmortem study¹⁰ has shown that the number of valine-encoding alleles predicted increased dopamine synthesis in the midbrain and suggested that this functional polymorphism can modulate prefrontal-midbrain interaction. With respect to working memory, previous work^{8,9} indicates that valine-allele carriers and methionine homozygotes are positioned on different

limbs of a putative ‘inverted-U’ curve showing the relationship between activation of PFC neurons and dopaminergic, especially D1 receptor, stimulation^{2,4} (**Supplementary Fig. 1** online). This suggested that *COMT* genotype might have a qualitative effect on the interaction of prefrontal activity and midbrain dopaminergic function.

Because no human *in vivo* data on the regulation of midbrain dopamine synthesis existed, we used multitracer PET methodology¹¹ to measure both regional cerebral blood flow (rCBF; with H₂¹⁵O) and presynaptic dopaminergic function using the tracer 6-[¹⁸F]DOPA in two sessions on a GE Advance 3D scanner (see also **Supplementary Methods** online). Twenty-four healthy subjects (age range 22–40 years) participated after giving informed consent according to the guidelines of the National Institute of Mental Health Institutional Review Board and the Radiation Safety Committee. Thirteen methionine homozygotes were matched with 11 valine-allele carriers for age, gender and performance on the *n*-back working memory task¹² (**Table 1** and **Supplementary Table 1** online). We determined the subjects’ *COMT* V158M genotypes using the 5′-exonuclease Taqman assay⁶.

Uptake of labeled F-DOPA was measured in the resting state over 90 min after pretreatment with 200 mg of carbidopa, with 25 images acquired starting 90 s after tracer infusion. Data were analyzed as previously described¹¹. Uptake in the midbrain was reliably detected. *COMT* valine-allele carriers had significantly ($P < 0.03$, $t_{22} = -2.3$) higher uptake rates (0.00320 ± 0.0004 ; mean \pm s.d.) than methionine-allele homozygotes (0.00275 ± 0.0005), consistent with previous postmortem results¹⁰ (**Fig. 1a**). It is likely that F-DOPA uptake in the midbrain reflects, at the timescale of the present experiment, overall demands on dopamine synthesis, because availability of dopamine within the cell is the primary metabolic regulator of dopamine synthesis¹³. Because methionine alleles result in reduced *COMT* activity⁵, methionine homozygotes are predicted to have relatively greater prefrontal synaptic dopamine for a given level of dopamine neuronal activity in the midbrain, suggesting that the lower dopamine synthesis in the midbrain in methionine-allele carriers observed here and in postmortem brain studies¹⁰ may be a consequence of the effect of *COMT* genotype on dopamine flux in PFC.

During the working memory challenge, we made 14 PET rCBF measurements (seven each of the 0-back and 2-back tasks in alternation, with injection of 10 mCi H₂¹⁵O per scan) and analyzed the results using SPM99 software as described previously¹². We found reliable activation in bilateral dorsolateral prefrontal cortex, inferior parietal lobule and cerebellum (see **Supplementary Fig. 2** and **Supplementary Tables 2** and **3** online). There were no differences between genotypes in blood flow and activation in a functionally defined PFC region of interest (ROI; see **Supplementary Table 1** online). The rCBF in this region was highly correlated with midbrain dopamine uptake in a way that was dependent on *COMT* genotype (**Table 1**; **Fig. 1b,c**): during

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Table 1 Task and subject parameters by genotype group

	Methionine homozygotes (<i>n</i> = 13)	Valine carriers (<i>n</i> = 11)	Difference between genotypes
Age (years)	27.8 ± 5.5	29.3 ± 6.2	<i>P</i> = 0.73
Gender	7 M, 6 F	7 M, 4 F	<i>P</i> = 0.83
Performance, 0-back (%)	99.25 ± 1.21	99.85 ± 0.20	<i>P</i> = 0.11
Performance, 2-back (%)	86.21 ± 15.04	86.22 ± 11.44	<i>P</i> = 0.99
Reaction time, 0-back (ms)	517.3 ± 104.0	497.3 ± 112.5	<i>P</i> = 0.66
Reaction time, 2-back (ms)	379.0 ± 230.7	378.5 ± 232.0	<i>P</i> = 0.85
Striatum F-DOPA <i>K_i</i>	0.0095 ± 0.0014	0.0093 ± 0.0010	<i>P</i> = 0.70
Striatum rCBF 0-back	50.05 ± 0.69	50.23 ± 0.55	<i>P</i> = 0.50
Correlation of midbrain <i>K_i</i> with DLPFC ROI 0-back	-0.85 (<i>P</i> < 0.001)	0.96 (<i>P</i> < 0.001)	<i>P</i> < 0.001
Correlation of midbrain <i>K_i</i> with DLPFC ROI 2-back	-0.82 (<i>P</i> < 0.001)	0.96 (<i>P</i> < 0.001)	<i>P</i> < 0.0001
Correlation of midbrain <i>K_i</i> with DLPFC ROI activation	0.59 (<i>P</i> < 0.05)	-0.51 (<i>P</i> < 0.05)	<i>P</i> < 0.02

Performance information and striatal dopamine synthesis rate, by genotype group (mean ± s.d.). DLPFC, dorsolateral prefrontal cortex. Difference significance values obtained by two-tailed *t*-test except for gender (χ^2) and significance of difference of correlation coefficients between groups (Williams-Pearson). For further information, see **Supplementary Table 1** online.

both 0-back and 2-back tasks, higher F-DOPA uptake in the brainstem was predicted by lower rCBF in methionine homozygotes but by higher rCBF in carriers of the valine allele. The inverse relationship held for activation (2-back task compared to 0-back control), where positive correlations were found for methionine homozygotes (**Fig. 1d**) but negative correlations for valine-allele carriers. All within-group correlations were significant, as were differences in correlation indices between genotype groups (**Table 1**). Voxelwise mapping of correlations of 0-back rCBF with midbrain F-DOPA uptake rates throughout the brain, by genotype, showed significant correlations with PFC only, in dorsolateral, ventrolateral and medial PFC (**Fig. 2, Supplementary Fig. 2** and **Supplementary Table 4** online). Because no other brain regions showed activity linked to midbrain dopamine, these data extend to humans the results of numerous anatomical¹ and physiological² studies that show a predominant role of PFC in dopaminergic regulation³.

A plausible cellular mechanism for the observed strong dependence of the directionality of prefrontal-midbrain interaction on *COMT* genotype involves the action of extracellular dopamine in PFC: given the crucial role of *COMT* in dopamine catabolism in PFC⁶, a similar rate of midbrain neuronal activity and dopamine synthesis is predicted to lead, in methionine homozygotes, to considerably more prefrontal cortex extracellular dopamine because of reduced dopamine clearance. One would predict that this increase in 'tonic' stimulation of D1 receptors⁴ would place methionine homozygotes farther to the

right from valine-allele carriers on the inverted-U curve relating dopaminergic stimulation to PFC activity during working memory—a genotype effect also suggested by a previous experiment involving amphetamine stimulation⁹. The genotype-related difference we observed in the slope of the correlation of PFC activity and dopamine synthesis rate is as predicted from the positions of valine carriers and methionine homozygotes relative to the apex of the curve. In fact, assuming only that a given rate of midbrain cell activity and dopamine synthesis will result in greater cortical extracellular dopamine in methionine homozygotes than in valine-allele carriers⁶, our data show a notable fit to an inverted U-shaped curve (**Supplementary Fig. 3** online). Our data are consistent with the genotype effect on relative position being a trait-like characteristic, as we measured rCBF and F-DOPA uptake in two separate sessions. We measured the latter during a 90-min resting state, presumably capturing the basal dopamine synthesis rate rather than the dynamic variation of dopamine synthesis in response to working memory stimulation. Alternatively, or additionally, mechanisms at the level of the midbrain might be considered. However, local effects of genotype on dopamine synthesis seem unlikely because *COMT* mRNA expression in the midbrain itself is sparse¹⁴ and somatodendritic release—modulating dopamine D2 autoreceptors are absent in the ventral tegmental areas of humans and other primates¹⁵.

Earlier studies⁴ have shown that dopamine critically determines the ratio of task-related to task-unrelated neural firing, or 'tuning', of PFC

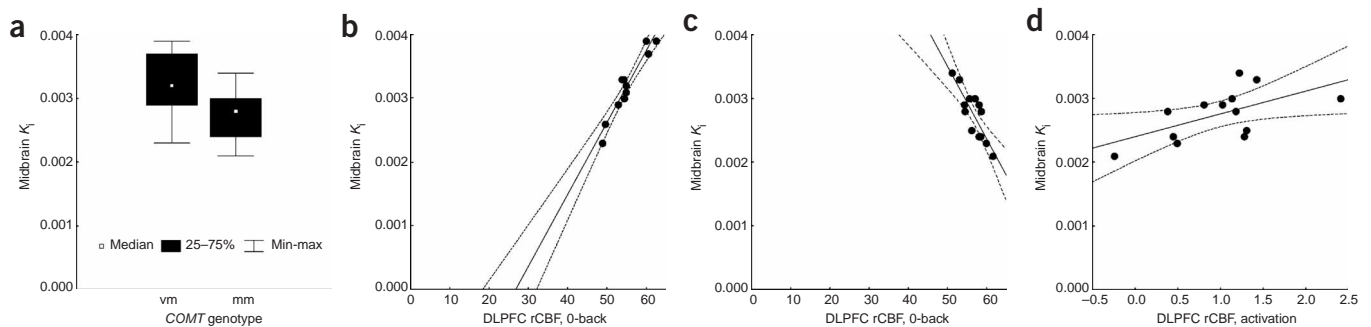


Figure 1 Midbrain dopamine and relationship with prefrontal rCBF. (a) Midbrain F-DOPA uptake (K_i) by genotype (bars reflect range; boxes, 25–75% of distribution). vm, valine carriers; mm, methionine homozygotes. (b) Correlation of midbrain F-DOPA uptake with DLPFC blood flow in valine carriers. (c) Correlation of midbrain F-DOPA uptake with DLPFC blood flow in methionine homozygotes. (d) Correlation of midbrain F-DOPA uptake with DLPFC activation in methionine homozygotes.

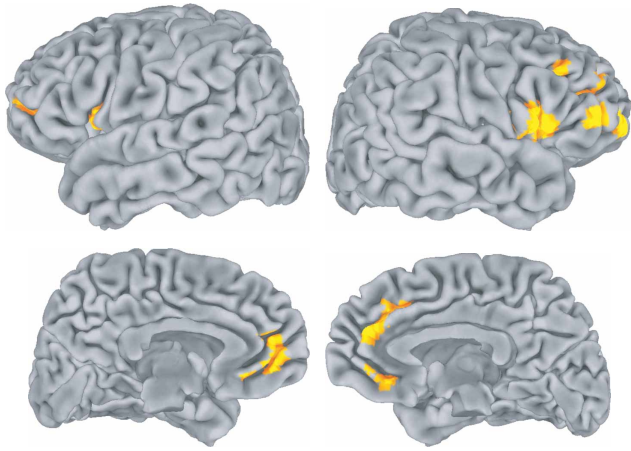


Figure 2 Interactions of cortical rCBF with midbrain dopamine. Significant ($P < 0.001$, uncorrected; $P < 0.05$, corrected, cluster-level) correlations of 0-back blood flow with midbrain F-DOPA uptake K_i , by *COMT* genotype (contrasting valine carriers with methionine homozygotes) were observed. See **Supplementary Table 4** online for coordinates and region labels.

neurons, with an optimum level of dopaminergic stimulation being necessary for the highest signal-to-noise ratios². Our PET approach allowed us to measure both task-unrelated rCBF (during the 0-back condition) and task-related activation and thus derive a measure of cortical tuning during cognitive processing. From this perspective, our data provide *in vivo* evidence for a dopaminergic tuning mechanism in humans, because, as predicted by the tuning concept, control (task-unrelated) rCBF and task-related activation were oppositely related to variations in midbrain dopamine in both genotype groups (absolute rCBF data during the 2-back condition were very similar to 0-back rCBF because activation-induced rCBF differences amount to only <2%). Multiple regression analysis using midbrain K_i and prefrontal 0-back rCBF as predictors of prefrontal activation showed that only midbrain K_i contributed significantly (at a significance level to enter into the regression equation of $P = 0.05$) to the variance, indicating that these inverse correlations were attributable to the effect of dopamine and could not be mainly explained by an inverse correlation between baseline rCBF and activation.

Limitations of our multitracer approach include the fact that amino acid decarboxylase, the enzyme whose activity is measured using 6-^[18F]DOPA, is not exclusive to dopaminergic synapses, and thus our results could have been contaminated by the effects of other neurotransmitter systems, such as the serotonin system. Because of the limited resolution of the PET scanner, we could not distinguish between ventral tegmental and nigral dopamine neurons in the midbrain. As expected from our previous work¹¹, we did not find an effect of *COMT* genotype on F-DOPA uptake in the striatum (**Table 1**).

However, working memory tasks are not optimal for investigating striatal function, and studies using other tasks capable of generating reliable striatal activation will be required to fully assess the potential influences of *COMT* genotype. Finally, because our data are cross-sectional and correlational, no inferences about causality are possible.

Our data were acquired in healthy humans who showed unimpaired performance during a working memory task and, as such, are within the realm of normal brain function. We demonstrated strong interactions between prefrontal cortex activity and dopamine synthesis that provide evidence for a cortical tuning mechanism in good agreement with current concepts about dopaminergic modulation of PFC function during working memory. Our results should provide a basis for studying interindividual differences in working memory function and performance, for example, as a function of age. In addition, our characterization of genotype-dependent prefrontal-subcortical feedback has potential implications for the pathogenesis of neuropsychiatric disorders affected by variation in *COMT* activity. In schizophrenia, where convergent evidence suggests the coexistence of prefrontal cortical dysfunction and subcortical dopaminergic disinhibition¹¹, our observations of increased midbrain dopamine synthesis and the accompanying positive regulatory mode for valine-allele carriers demonstrates a mechanism for increased risk for dopaminergic disinhibition conferred by this risk allele⁸.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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