Catechol-*O*-Methyltransferase Valine^{158}Methionine Polymorphism Modulates Brain Networks Underlying Working Memory Across Adulthood

Fabio Sambataro, Jeff D. Reed, Vishnu P. Murty, Saumitra Das, Hao Yang Tan, Joseph H. Callicott, Daniel R. Weinberger, and Venkata S. Mattay

Background: Cognitive abilities decline with age with large individual variability. Genetic variations have been suggested to be an important source for some of this heterogeneity. Among these variations, those related to the dopaminergic system, particularly the valine^{158}methionine polymorphism in catechol-*O*-methyltransferase (COMTval^{158}met), have been implicated in modulating age-related changes in executive function.

Methods: We studied 75 subjects (age 21–90 years) using functional neuroimaging while they performed a low-level working memory (WM) task to explore the effects of aging, of the COMTval^{158}met polymorphism, and their interactions on the physiological patterns of interconnected cortical activity engaged by WM.

Results: Our results show that val homozygotes and older subjects showed increased activity in dorsolateral prefrontal cortex (DLPFC) and decreased activity in ventrolateral prefrontal cortex (VLPFC) relative to met homozygotes and younger subjects, respectively. Interestingly, there were also independent effects of the COMTval^{158}met polymorphism and age on the strength of connectivity between brain regions within the left prefrontal-parietal network; val homozygotes and older subjects showed greater connectivity between the DLPFC and other brain regions within the network and met homozygotes showed greater connectivity between the VLPFC and other brain regions within the network. Furthermore, the greater functional connectivity strength of DLPFC in val homozygotes relative to met homozygotes was much more pronounced in older adults

Conclusions: Our findings suggest that the COMTval^{158}met polymorphism modulates both the activity and functional connectivity of brain regions within WM networks and most importantly that this effect is exaggerated with increasing age, contributing to the variability in age-related decline in executive cognition.

Key Words: Aging, COMT, functional magnetic resonance imaging, independent component analysis, working memory

Evidence from cellular, animal, postmortem, drug, and neuroimaging studies indicates generalized age-dependent changes in the function of the dopaminergic system (1). D1 binding, D2/D3 receptor density, and dopaminergic synthesis decline from early to late adulthood in frontal, temporal, and parietal areas (2). In keeping with these changes, cognitive abilities relying on dopaminergic signaling in these cortical areas, including working memory (WM) and executive functions, decline with increasing age (3).

Working memory is critically dependent on the integrity of prefrontal cortex (PFC) (4). In the PFC, dopamine levels are thought to be regulated by catabolic activity of catechol-*O*-methyltransferase (COMT) (5,6). The activity of this enzyme in humans is affected by a common single nucleotide polymorphism (G→A) that leads to a substitution of valine (val) into methionine (met) at codon 158 (COMTval^{158}met). The met variant is thermolabile and shows two to four times lower dopamine-degrading activity relative to the val variant, thus resulting in greater dopamine levels. Increased availability of this neurotransmitter translates into better performance in executive function tasks along with an increase in efficiency of information processing within the PFC, i.e., lower neural activity for similar levels of task performance (7,8). However, the effect of COMTval^{158}met polymorphism on functional coupling between the brain regions within the executive cognition network has been explored to only a limited degree (9). The COMTval^{158}met polymorphism also has been reported to affect cognitive abilities in older subjects, with better performance on WM and executive function tasks in met homozygotes relative to val homozygotes (2,10–13). Additionally, Nagel *et al.* (14) showed that met homozygotes were faster than val homozygotes during the performance of the Wisconsin Card Sorting Test and a spatial WM task and this difference was greater in older adults relative to young adults. Although these findings suggest that aging increases the effect of COMTval^{158}met polymorphism on executive cognition, the neural bases of this process remain to be clarified.

In the present study, we tested the effects of the COMTval^{158}met polymorphism on age-related changes of both the activity and the functional connectivity of the brain regions in the networks underlying WM across adulthood (from young adulthood to old age). We used multivariate statistical approaches (independent component analysis [ICA]) together with univariate analyses (general linear model [GLM]) to test the following hypotheses: 1) the COMTval^{158}met polymorphism modulates cortical activation as well as functional networks underlying WM across adulthood; 2) advancing age modulates activity and connectivity across brain regions subserving WM; and 3) the effect of the COMTval^{158}met polymorphism on information processing within the PFC is magnified in older relative to younger adults, i.e., there is an interaction of age and COMT genotype on this measure of cortical function.

From the Genes, Cognition, and Psychosis Program, Clinical Brain Disorders Branch, National Institute of Mental Health Intramural Research Program, National Institutes of Health, Bethesda, Maryland.

Address correspondence to Venkata S. Mattay, M.D., Genes, Cognition, and Psychosis Program, NIMH, NIH, Building 10, Room 3C-108, Bethesda, MD 20892; E-mail: vsm@mail.nih.gov.

Received Dec 24, 2008; revised Apr 7, 2009; accepted Apr 8, 2009.

Table 1. Demographics – Entire Sample

	Val/Val	Val/Met	Met/Met	Significance
N	20	31	24	
Male:Female Ratio	11:13	15:16	10:10	χ^2 (2) = .08; p = .9
Age (M ± SD, years)	42.9 ± 15.1	45.8 ± 19.4	38.5 ± 16.9	F (2,72) = 1.07, p = .4
Handedness (% right)	95.0 ± 9.0	93.5 ± 10.2	92.3 ± 13.0	F (2,72) = .36, p = .7
Education (M ± SD, years)	16.5 ± 2.0	16.3 ± 2.6	16.2 ± 2.1	F (2,72) = .11, p = .9
1-Back WM Accuracy (M ± SD, %)	97.0 ± 3.4	96.7 ± 5.3	95.0 ± 8.2	F (2,72) = .74, p = .5
1-Back WM RT (M ± SD, msec)	429.4 ± 242.3	424.6 ± 228.8	448.0 ± 245.9	F (2,72) = .06, p = .9

Met, methionine; RT, reaction time; Valine, valine; WM, working memory.

Materials and Methods

Subjects

Seventy-five subjects (age range = 21–90 years) (Tables 1 and 2; details in Supplement 1) were genotyped for rs4680.

Task

All the subjects were scanned with functional magnetic resonance imaging (fMRI) while performing a low WM load task (15), i.e., the 1-back WM task condition (details in Supplement 1). This task was chosen to control for performance differences between older and young subjects.

Data Analysis

Image Analysis. Following preprocessing, all the fMRI data were analyzed using both ICA and GLM approaches (Supplement 1). The use of ICA can yield complementary information to that obtained from GLM analysis on neurophysiological processes, especially in the study of older subjects who may show deviations from the classical hemodynamic response (16). While ICA, a multivariate statistical approach, can identify multiple spatially independent and temporally synchronous activity patterns in brain regions (functional covariance), GLM analysis allows the detection of changes in brain activity based on a priori task design and hemodynamic response function. Hence, while GLM analysis is more sensitive to detect functional specificity (measured by activation), ICA is more sensitive in defining patterns of functional connectivity within multiple networks (17). Independent component analysis also has advantages in the estimation of functional connectivity over the classical univariate seeded correlation approach. First, ICA is able to identify noise-related and artifactual components (18) that can be removed, thus allowing a cleaner estimation of the coupling of brain regions. Additionally, it allows the estimation of higher-order functional connectivity (19), thus identifying association between voxels that show the same dependency of temporal variation and not just a pairwise correlation with respect to time. This allows the identification of multiple networks during the same task, each of which subserves a specific subprocess.

Table 2. Neuropsychological Status of the Older Adults

	M (SD)
Cognitive Status	
MMSE	30
CDR	0
WAIS	118.1 (9.4)
Executive Function	
Trail Making Test B (sec)	52 (11.1)[a]
Word Fluency Test (letters)	48.6 (9.8)[b]
Category Fluency Test (animals)	54.5 (8.4)[b]
Letter and Number Sequencing	12.7 (2.3)[c]
WAIS picture completion	12 (2.4)[d]
WAIS arithmetic	11.8 (3)[d]
WAIS similarity	15.2 (4)[d]
Memory	
WMS Logical Memory Immediate Recall	13.1 (1.8)[a]
WMS Logical Memory Delayed Recall	14.4 (2.3)[c]
Processing Speed	
Trail Making Test A (sec)	51.5 (16.2)[a]
WAIS Digit Symbol Test	16.3 (10.7)[d]

There was no effect of COMTval^{158}met genotype on any of these measures.

CDR, Clinical Dementia Rating; MMSE, Mini-Mental State Examination; WAIS, Wechsler Adult Intelligence Scale; WMS, Wechsler Memory Scale.

[a]Information was available on 22 subjects.
[b]Information was available on 19 subjects.
[c]Information was available on 20 subjects.
[d]Information was available on 25 subjects.

General Linear Model Analysis. One-way analysis of variance (ANOVA) was used to compare brain activation differences across the genotype groups. A simple correlation analysis was also performed to assess the effect of increasing age on brain activation across adulthood.

To evaluate the effect of COMTval^{158}met on age-related changes in information processing within the PFC, we used a 2 × 2 factorial ANOVA selecting subjects belonging to the tails of the age range (i.e., young adults <35 years of age and older adults > 55 years of age; Tables 1 and 2 in Supplement 1) and homozygotes for val and met allele, respectively.

Independent Component Analysis. A group spatial ICA was performed on preprocessed data using the Group ICA of fMRI Toolbox (GIFT; Medical Image Analysis Lab, University of New Mexico, Albuquerque, New Mexico; http://icatb.sourceforge.net) (20). Each subject's components of interest (COI) were entered into SPM5 (Wellcome Department of Cognitive Neurology, London, United Kingdom; http://www.fil.ion.ucl.ac.uk) and analyzed using second-level random-effects analyses to identify the effect of COMTval^{158}met genotype and age. A two-way ANOVA including age (young and older adults) and COMTval^{158}met was used to assess the COMTval^{158}met by age interaction. A multiple regression was performed outside image space to confirm these results, removing the effect of nuisance variables.

Correlations between individual time courses (TCs) and a regressor associated to the 1-back condition were estimated using a GLM for each subject and COI. The beta values of this temporal correlation represent the extent to which the TC of a COI is modulated by the WM task relative to the sensory-motor control task. These beta values were then entered in a regression with genotype, age, their interaction, and RT as regressors to evaluate the effect of these variables on the TC modulation by

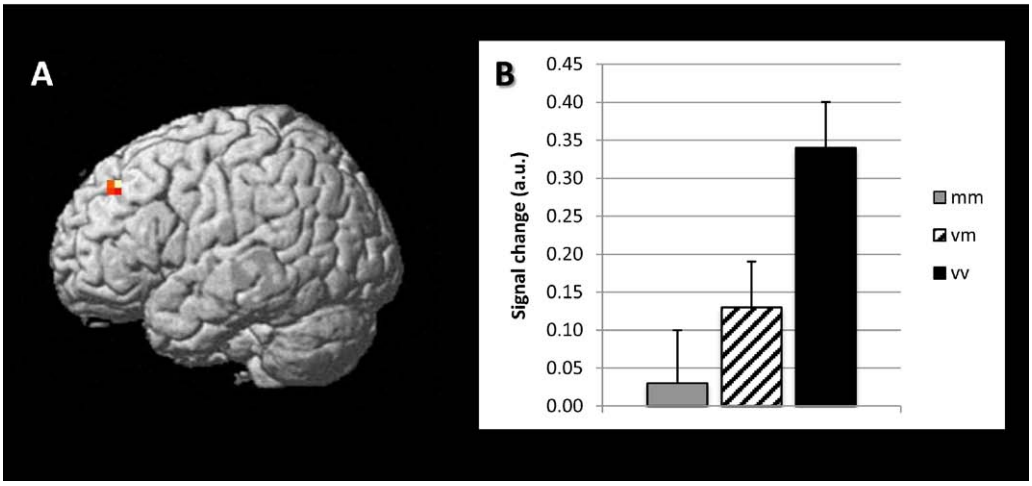

Figure 1. Effect of COMTval[158]met polymorphism on brain activation during a 1-back working memory task. Individuals homozygous for val allele (val/val, $n = 24$) show greater signal change relative to heterozygote subjects (val/met, $n = 31$), who in turn show greater signal change relative to subjects homozygous for met allele (met/met, $n = 20$). **(A)** Thresholded statistical t-map (val/val > val/met > met/met) of DLPFC activation (1-back−0-back) surface-rendered on the MNI brain template ($p < .005$); **(B)** Percent signal change during working memory (1-back−0-back) in the left DLPFC (MNI coordinates of peak cluster: BA 9; x = −38, y = 41, z = 42 mm) as a function of COMTval[158]met genotype. Bar graphs represent mean percent signal change of the BOLD response for each genotype group. Error bars indicate one standard error of the mean. BA, Brodmann area; BOLD, blood oxygenation level-dependent; COMT, catechol-*O*-methyltransferase; DLPFC, dorsolateral prefrontal cortex; met, methionine; MNI, Montreal Neurological Institute; val, valine.

WM processes. Correlations of TC task design with age were compared between val and met homozygotes using Fisher's r-to-z transformation.

A statistical threshold of $p < .05$ with false discovery rate (FDR) (21) small volume correction (SVC) was used to identify significant differences within regions of interest (ROI). False discovery rate correction for multiple comparisons was implemented with $\alpha = .05$. Regions of interest were defined as 20-mm radius spheres centered around the peak coordinates of the regions activated during the N-back WM task as reported in meta-analysis of 24 N-back studies (22), namely dorsolateral prefrontal cortex (DLPFC), ventrolateral prefrontal cortex (VLPFC), anterior frontopolar cortex, and parietal cortex. We also used a more stringent threshold of $p < .01$ FDR to test the effects of age and genotype and an age × genotype interaction at the level of whole brain to identify differences in brain regions not a priori. All coordinates are reported in Montreal Neurological Institute (MNI) system. In the a priori ROI analyses, we tested all the effects but we only reported those results that survived statistical correction.

Results

GLM Results

There was a main effect of COMTval[158]met in the left DLPFC (Brodmann area [BA] 9; x = −38, y = 41, z = 42, $Z = 3.88$; $p < .001$ FDR-corrected; Figure 1) with greatest activation in val/val individuals, followed by val/met and then met/met individuals.

There was a significant positive correlation between brain activation and age in left DLPFC (x = −45, y = 15, z = 34, $Z = 3.47$; $p < .001$ FDR-corrected), bilateral inferior parietal lobule (BA 39/40; $p < .001$ FDR-corrected), left cerebellum, and right middle temporal gyrus (BA 21) (Figure 2).

There was a significant negative correlation ($p < .001$ FDR-corrected) between activation and age, i.e., decreasing activation with increasing age, in the left globus pallidus, right precuneus, right inferior frontal gyrus, cingulate gyrus (BA 24/32), right visual cortex, left putamen, left precuneus, left postcentral gyrus, left inferior parietal lobule (BA 40), right precentral gyrus, and left temporal. These changes may be related to the age-related decline of the nigrostriatal dopaminergic system (23).

ICA Results

Components of Interest. Three positive COIs (A, B, and C) showed a strong temporal correlation between the independent component TCs and 1-back task design as defined above. The identified COIs with their spatial maps, TCs, and temporal correlation are shown in Figure 3 and are described as follows. Component of interest A (COI-A) primarily identified a prefrontal-parietal (PFC-Par) network markedly greater in the left hemi-

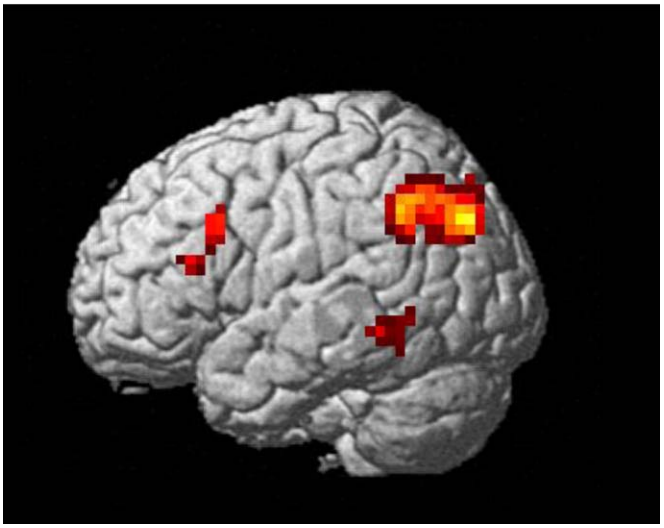

Figure 2. Effect of age on brain activations during a 1-back working memory task. Older adults show increased activation in left DLPFC (MNI coordinates of peak cluster: x = −45, y = 15, z = 34 mm) and bilateral posterior parietal cortices. Thresholded statistical t-map of left DLPFC activation (1-back−0-back) surface-rendered on the MNI brain template ($p < .005$). DLPFC, dorsolateral prefrontal cortex; MNI, Montreal Neurological Institute.

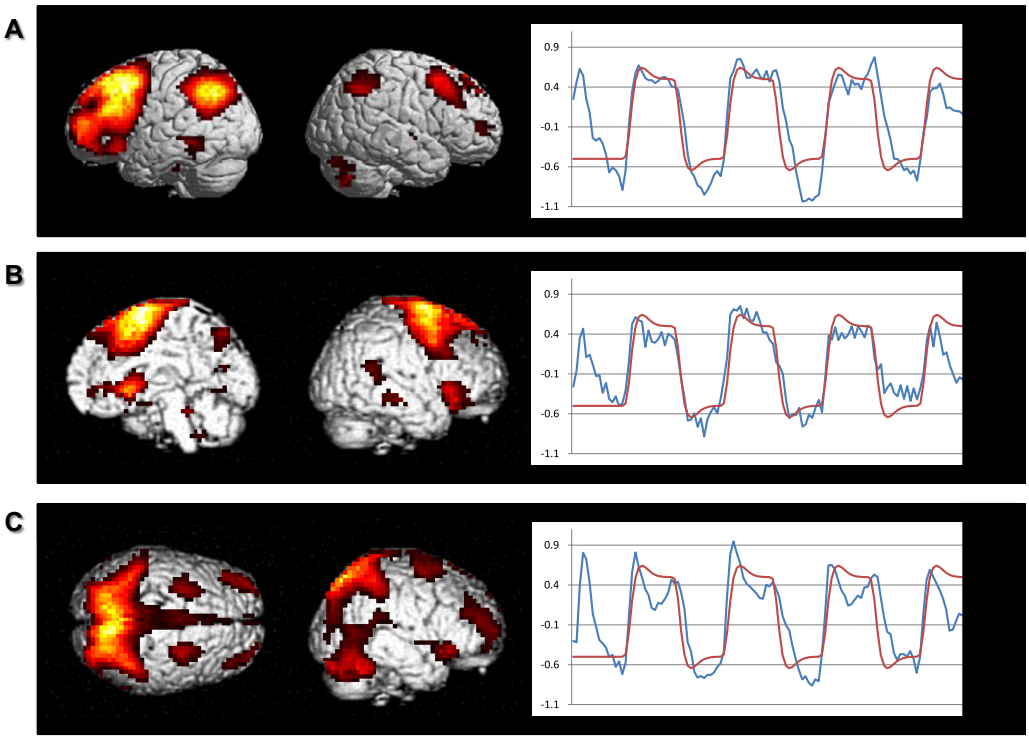

Figure 3. Working memory task-related functional networks. Three COIs were identified: **(A)** left frontoparietal ($r = .60$); **(B)** medial frontal ($r = .50$); and **(C)** posterior parietal ($r = .50$). Surface-rendered maps display the spatial pattern of the independent components identified. Time courses represent the temporal profile of each component (blue) overlaid on the paradigm "box-car" design (red). *R* values indicate the Pearson's correlation between the time course of the component and the task design. All images are thresholded at $p < .05$ FDR-corrected for the whole brain. COI, component of interest; FDR, false discovery rate.

sphere that in the right hemisphere. This pattern included DLPFC (BA 9/46) and VLPFC (BA 44/47) and inferior parietal lobule (BA 40) (Figure 3A), regions implicated in the maintenance and manipulation of information during WM. Component of interest B (COI-B) identified a medial frontal cortex component comprising medial frontopolar cortex (BA 10)/dorsal cingulate (BA 32) and presupplementary motor area (BA 6), with additional activity in the dorsal premotor cortex (BA 4), right inferior frontal junction (BA 44), anterior insula (BA13), and head of caudate bilaterally (Figure 3B). This network has been implicated in monitoring actions and performance outcomes and subsequent adjustments. Component of interest C (COI-C) identified the superior posterior parietal cortex (BA 7) bilaterally with additional activity in bilateral cerebellar and right superior temporal gyrus regions (Figure 3C). These regions play a crucial role in attention, specifically in spatial and nonspatial attention switching.

Effect of COMTval^{158}met Polymorphism and Age on COIs. *Left Frontoparietal Component (COI-A).* There was a significant main effect of COMTval^{158}met in the left DLPFC (BA 9; x = −34, y = 41, z = 34, $Z = 3.20$; $p < .001$ FDR-SVC-corrected) with greatest connectivity for val homozygotes, followed by heterozygotes and then met homozygotes (Figure 4A, 4B). Methionine homozygotes showed greater connectivity in left VLPFC (BA 44; x = −45, y = 15, z = 8, $Z = 3.35$; $p < .001$ FDR-SVC-corrected) relative to heterozygotes and val homozygotes (Figure 4C, 4D). There was a main effect of age with older subjects showing greater connectivity in left DLPFC extending to lateral BA 6/8, right VLPFC (BA 11/47), anterior cingulate (BA 24/32), and medial (BA 8/9), and younger subjects showing greater connectivity in left superior frontal gyrus (BA 8) and inferior parietal lobule (BA 40) ($p < .001$ FDR-corrected). The direct comparison of the COMTval^{158}met effect between younger and older groups yielded a cluster in the left DLPFC (BA 9; x = −34, y = 41, z = 34, $Z = 3.52$; $p < .001$) with older subjects showing a greater val/val > met/met effect when compared with younger subjects (Figure 5). This result was confirmed by a multiple regression analysis [adjusted-$R^2 = .16$; $F(6,51) = 2.808$, $p = .02$] that showed an effect of COMTval^{158}met [val/val > met/met, $r = .44$, $R^2 = .04$; $t(51) = 3.52$, $p < .001$] and a COMTval^{158}met by age interaction [$r = .26$, $R^2 = .04$; $t(51) = 1.93$, $p = .05$].

Medial Frontal Component (COI-B). There was a main effect of COMTval^{158}met in frontopolar cortex (BA 10; x = −15, y = 49, z = −8, $Z = 3.53$; $p < .001$ FDR-SVC-corrected) (Figure 6A) with met homozygotes showing greatest connectivity relative to heterozygotes and val homozygotes (Figure 6B). The inverse contrast revealed greater connectivity in medial superior frontal gyrus with val homozygotes showing the greatest connectivity (BA 8; x = −15, y = 34, z = 49, $Z = 3.95$; $p < .001$). There was an age-related increase of connectivity in bilateral superior frontal gyrus (supplementary motor area [SMA]; $p < .001$ FDR-corrected).

Posterior Parietal (COI-C). There was a significant main effect of COMTval^{158}met in left inferior parietal lobule (BA 40; x = −49, y = −49, z = 46, $Z = 3.95$; $p < .001$ FDR-SVC-corrected) with val homozygotes showing the greatest connectivity relative to heterozygotes and met homozygotes (Figure 1 in Supplement 1). Clusters of age-related increases in connectivity were identified in bilateral inferior parietal lobule and BA 10 ($p < .001$ FDR-corrected). Connectivity decreased with age in the left superior parietal cortex ($p < .001$ FDR-corrected).

To ensure that the effect of age-dependent changes on activity and connectivity strength was not driven by a difference in reaction time (RT) across age groups, we performed

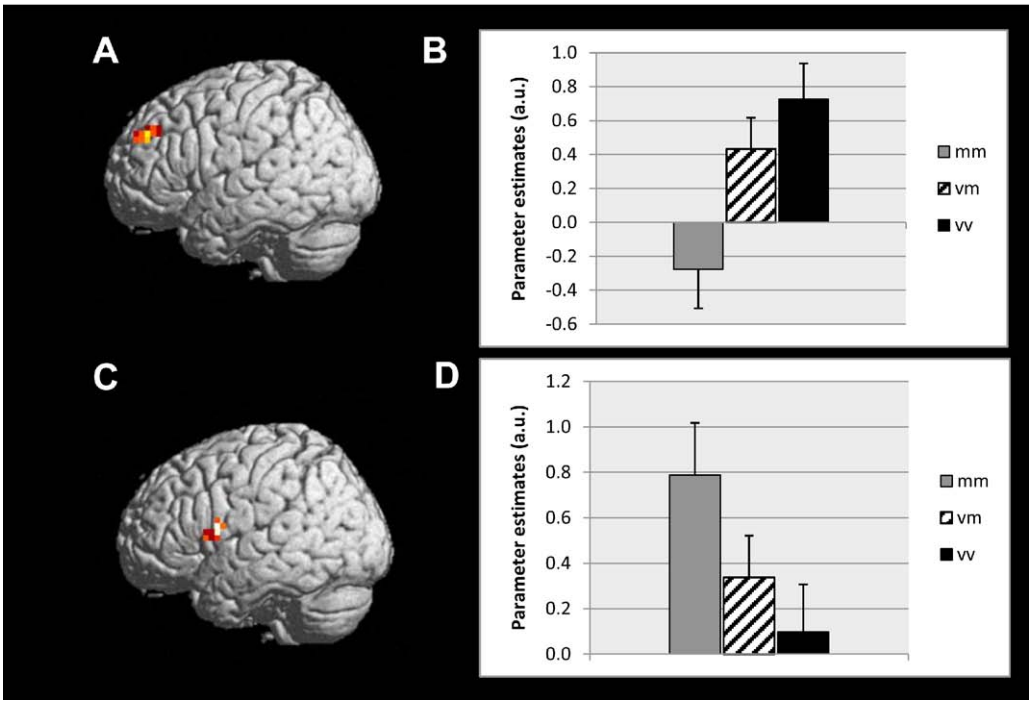

Figure 4. Effect of COMTval[158]met polymorphism on the left prefrontal-parietal COI. **(A)** and **(B)** Val homozygotes show greater connectivity in left DLPFC (MNI coordinates of peak cluster: BA 9; x = −34, y = 41, z = 34 mm) relative to heterozygotes and met homozygotes, whereas **(C)** and **(D)** met homozygotes show higher connectivity in left VLPFC (MNI coordinates of peak cluster: BA 44; x = −45, y = 15, z = 8 mm). Thresholded statistical t-maps of connectivity (val/val > val/met > met/met) **(A,C)** are surface-rendered on the MNI brain template ($p < .005$). Bar graphs **(B,D)** represent parameter estimates of the BOLD response for each genotype group measured in arbitrary units. Error bars indicate one standard error of the mean. BA, Brodmann area; BOLD, blood oxygenation level-dependent; COI, component of interest; COMT, catechol-*O*-methyltransferase; DLPFC, dorsolateral prefrontal cortex; met, methionine; MNI, Montreal Neurological Institute; val, valine; VLPFC, ventrolateral prefrontal cortex.

additional analyses using RT as covariate of no interest in both GLM and ICA. All results remained significant (data not shown).

Independent Component Time Course-Task Design Correlations. To examine the effects of age and COMTval[158]met on the recruitment of the COIs during the task, we studied the impact of these variables and their interaction on the correlation between the independent component TC and task design.

Medial Frontal Component (COI-B). The beta values of the TC-task design relationship of this COI were entered in a multiple regression model [adjusted-R^2 = .12; $F(4,70)$ = 3.484, p = .01]. These betas negatively correlated with age (r = −.35, R^2 = .25, p = .003). There was also a significant COMTval[158]met effect on these values (r = .22, R^2 = .03, p = .05) with val homozygotes showing greater TC-task design correlation for this component relative to heterozygotes and met homozygotes. Valine homozygotes showed a significant negative correlation (r = −.44, p = .03) (Figure 6D) with age, whereas met homozygotes did not (r = −.04, p = .85) (Figure 6C).

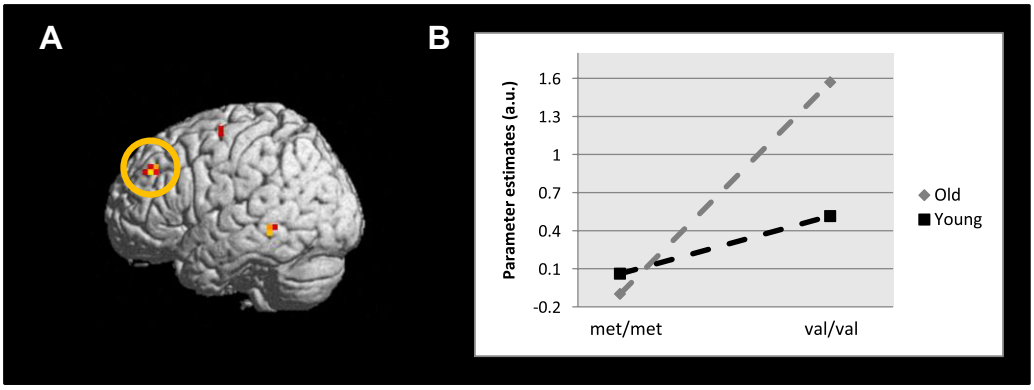

Figure 5. Interaction COMTval[158]met polymorphism by age on the left prefrontal-parietal COI. **(A)** Older adults show greater COMTval[158]met-related inefficiency in left-DLPFC (MNI coordinates of peak cluster: BA 9; x = −34, y = 41, z = 34 mm) relative to younger subjects. **(B)** COMTval[158]met effect, which is indicated by the slope of the lines, was greater in older subjects compared with younger subjects. Thresholded statistical t-map of activation **(A)** is surface-rendered on the MNI brain template ($p < .005$). Graph represents parameter estimates of the BOLD response for each genotype and age group measured in arbitrary units. BA, Brodmann area; BOLD, blood oxygenation level-dependent; COMT, catechol-*O*-methyltransferase; DLPFC, dorsolateral prefrontal cortex; met, methionine; MNI, Montreal Neurological Institute; val, valine.

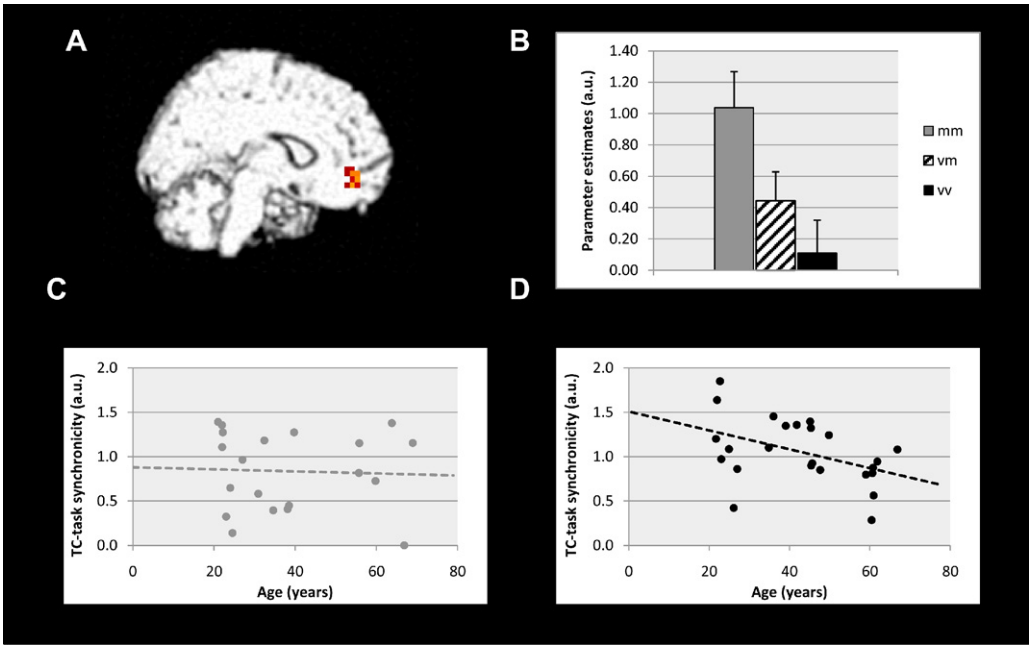

Figure 6. Effect of COMTval[158]met polymorphism on the medial frontal COI. **(A)** and **(B)** Met homozygotes show greater activity in medial BA 10/ACC (MNI coordinates of peak cluster: BA 9; x = −15, y = 49, z = −8 mm) relative to heterozygotes and val homozygotes. **(C)** Met homozygotes did not show an age-related effect on the correlation between the time course of the COI and the task design (Pearson's $r = -.05$, $p = .85$), whereas **(D)** val homozygotes show a decline with age of this correlation (Pearson's $r = -.43$, $p = .03$). Thresholded statistical t-map of activation **(A)** is surface-rendered on the MNI brain template ($p < .005$). Bar graphs **(B)** represent parameter estimates of the BOLD response for each genotype group measured in arbitrary units. Error bars indicate one standard error of the mean. Scatterplots report the age (measured in years) and time course-task design correlation (measured by the betas of this correlation). Lines indicate the best fit for the age by time course-task design relationship. ACC, anterior cingulated cortex; BA, Brodmann area; BOLD, blood oxygenation level-dependent; COI, component of interest; COMT, catechol-*O*-methyltransferase; met, methionine; MNI, Montreal Neurological Institute; val, valine.

We did not find a significant effect of age, COMTval[158]met genotype, or COMTval[158]met by age effect on TC-task design relationship in COI-A and COI-C. A simple regression analysis for COI-A identified a positive correlation between age and the beta values representing the TC-task design relationship ($r = .25$, $p = .03$).

Discussion

In this study, we investigated the interplay between COMTval[158]met polymorphism and aging on physiological activity within a cortical WM network. We found that during a low load WM task individuals homozygous for COMT val allele show greater activity and connectivity of the DLPFC when compared with heterozygotes and met homozygotes who showed greater connectivity of VLPFC within the left PFC-Par network (COI-A) across adulthood. Methionine homozygotes also showed greater connectivity in anterior PFC areas within a medial frontal network (COI-B). We also found an age-related modulation of brain regions with older adults showing greater DLPFC and posterior parietal activation and connectivity and young adults showing greater VLPFC activation. Additionally, older subjects showed greater COMTval[158]met effect (val/val > met/met) on DLPFC connectivity with other regions within the left PFC-Par network when compared with young subjects. Valine homozygotes also showed a negative association between TC-task design correlation and age in the medial PFC network (COI-B) in contrast to met homozygotes. These results show that COMTval[158]met polymorphism and age modulate the activity and functional connectivity of brain regions underlying WM and that the effects of COMTval[158]met polymorphism increase with advancing age.

COMTval[158]met Polymorphism Affects Brain Function

Previous neuroimaging studies have reported a modulatory effect of the COMTval[158]met polymorphism on PFC activation during WM in young subjects (24,25). Valine homozygotes show an increase in DLPFC activation relative to met homozygotes, while heterozygotes show an intermediate response in the context of matched behavioral performance. These findings suggest cortical inefficiency in val carriers relative to met carriers, i.e., they engage greater prefrontal activation in the service of similar behavioral performance levels. Our results not only extend these findings from young adulthood to an older age group but also demonstrate the effect of COMTval[158]met polymorphism on the connectivity strength between brain regions within the PFC-Par network. Cortical efficiency of prefrontal networks during information processing has been linked to PFC dopamine levels, and val carriers are presumed to have lower levels of this neurotransmitter in this brain region relative to met carriers (5,26,27). Dopamine regulates firing of PFC pyramidal neurons and surrounding gamma-aminobutyric acid (GABA) inhibitory interneurons principally via dopamine D1 and D2 receptors (28). Through this modulation, dopamine focuses and stabilizes the response of PFC to the task at hand (29). Dopaminergic transmission also regulates brain networks by modulating the synchronization among different brain areas and membrane oscillation frequencies (29). It increases synaptic gain and task-related signals (30,31) and modulates functional connectivity strength by enhancing active connections and suppressing the least active ones (32,33). Therefore, higher dopamine levels in met subjects may result in greater stability of short-term memory and attentional networks thus decreasing trial-by-trial variability (noise) and increasing signal-to-noise ratio (34).

The lateral PFC plays a significant role in WM function, with ventral and dorsal regions playing different functional roles. Ventrolateral prefrontal cortex activity is involved in focusing attention either directly by selecting the content to focus on or by inhibiting irrelevant information (35–38), whereas the DLPFC plays a role in the comparison and strategic manipulation of WM content to decrease cognitive load (39,40).

Hence, the increased connectivity of VLPFC with the other brain regions within the left PFC-Par network that we found in met homozygotes relative to val homozygotes may reflect a more optimal strategy in the processing of a low WM load task. This notion of a more optimal strategy during prefrontal tasks by met relative to val allele carriers is also supported by the observations of Tan *et al.* (9,41), albeit during more demanding executive cognitive function tasks, e.g., higher load N-back WM task (2-back) and an arithmetic transformation task. In contrast, we used a low WM load task that probably relies on the lateral ventral prefrontal systems (vide supra) rather than on the dorsal system, which has been shown to be more consistently recruited during tasks with higher WM load (2-back and 3-back) (42). It is likely that met subjects may tend to perform the 1-back task as a mere attentional task focusing attention on new items, and as a consequence they favor regional allocation of neural resources in VLPFC. Conversely, adults homozygous for val allele may show a less efficient strategy for 1-back task, which in this case requires a manipulation of a sequence of numbers and therefore greater recruitment of the DLPFC to maintain performance. Alternatively, the increase of connectivity in specific brain regions within a network may represent a compensatory response. Given that a low WM task was necessary in this study to preclude performance changes from confounding the interpretation, correlations between differences in functional coupling and performance across groups are not feasible because of a ceiling effect on performance. Such correlations may be necessary to clarify the preferential use of VLPFC versus DLPFC networks in the performance of a task. Future studies with more challenging WM tasks may be helpful in addressing this.

There was a main effect of COMTval^{158}met polymorphism in anterior PFC (COI-B) and posterior parietal cortex (COI-C). Anterior PFC plays a pivotal role in cognitive branching, i.e., the ability to engage in multiple tasks not serially organized, a process that is crucial for N-back performance. More specifically, this brain region may allow switching among different processes while protecting representations of the postponed tasks from distracters and performance of the task at hand (43). Again, here greater connectivity in met homozygotes in BA 10/32 may suggest a better cognitive strategy for the performance of the task. Conversely, increased connectivity in BA 6/8 in val homozygotes suggests a decreased ability to maintain stimuli in WM. We observed COMTval^{158}met effects on posterior inferior parietal cortex in COI-C with val homozygotes showing greater cortical connectivity with the other regions within a bilateral posterior parietal network. A greater connectivity of this brain region in val carriers may be consistent with a greater demand on attentional processes due to inefficient PFC function in these individuals relative to met carriers.

Age-Related Changes in Prefrontal Function

We found age-related changes of cortical brain activity during WM tasks and most importantly in network connectivity. Older adults showed an over-recruitment of a network of regions, including left DLPFC and bilateral parietal cortices, whereas younger adults showed a greater recruitment of right VLPFC, postcentral gyrus, and inferior temporal gyrus. This pattern of age-related differences in DLPFC shows an intriguing similarity to the findings in patients with schizophrenia (42,44) that show an exaggerated response in this region during WM relative to normal control subjects. These findings, as well as the age-related changes in DLPFC (45), have been interpreted as resulting from inefficient information processing during WM, thus suggesting that differences in brain response in DLPFC may represent a nonspecific common manifestation of decreased neural efficiency that may, in turn, be a result of decreased dopaminergic signaling. Aging is associated with a generalized decline in the dopaminergic system, as suggested by a decrease of several dopaminergic markers and of dopaminergic synthesis in cortical areas including frontal, temporal, and parietal areas (46). Decreased PFC dopamine levels may result in inefficiency at the level of activity as well as connectivity (vide supra) within the PFC-Par executive network. These changes may be either due to a compensation mechanism, which is a reallocation of cortical resources to maintain task performance to compensate for decreased neural efficiency (47), or dedifferentiation mechanism, which is diminished cortical specificity resulting in increased neural noise and less distinctive neural representations (48). Rajah and D'Esposito (49), based on a meta-analysis, suggest that both compensation and dedifferentiation mechanisms may explain the age-related changes in VLPFC activity, whereas changes in DLPFC are more likely due to functional compensation. According to a hierarchical topographically organized model of PFC, this region functions along a rostrocaudal gradient of complexity with anterior PFC at the top, VLPFC at the bottom, and DLPFC in between (50). Hence, either decreased neural efficiency or dedifferentiation of the function of VLPFC may result in increased recruitment of higher hierarchical regions such as DLPFC and eventually anterior PFC. At a behavioral level, these neurobiological differences may translate into age-related changes in task strategies (51). The reduced speed of manipulation of the items in WM in older subjects may result in a greater overlap between manipulation and encoding of new items, thus increasing attentional load and DLPFC activation (52). Conversely, younger subjects who are able to manipulate items more rapidly—especially at low-task difficulty as in a 1-back task—may be engaged in continuous encoding resulting in greater VLPFC activity.

COMTval^{158}met Polymorphism Modulates Age-Related Changes in Prefrontal Function

We found an interaction between COMTval^{158}met and aging on the functional connectivity of DLPFC with the other regions of the left prefrontal-parietal network. In detail, there was an increased val/val > met/met genotype effect in older adults relative to young adults. Our data fit into a model in which age-related changes in the dopaminergic system result in a leftward shift of the inverted-U curve of prefrontal function-dopamine level relationship in older adults relative to younger adults, thus resulting in an exaggeration of COMTval^{158}met (val/val > met/met) effects on PFC function with increasing age (14). According to this model, older val homozygotes will have relatively lower dopamine levels and thereby greater cortical inefficiency relative to older met homozygotes. This difference would increase in older subjects relative to younger subjects, because the age-related decline in dopaminergic signaling shifts them to the left on the inverted-U response to dopaminergic signaling (Figure 7). These results, together with the observation of a lack of age-related decrease in synchronization between task

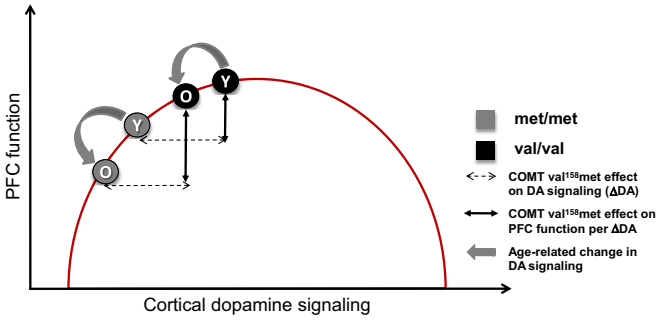

Figure 7. Theoretical inverted-U model describing the effects of COMTval^{158}met genotype and aging on PFC DA signaling and function across adulthood. Val homozygotes show lower prefrontal function relative to met homozygotes (↔). Age-related decline (⟸) in dopaminergic system results in a leftward shift on the x axis of the inverted-U curve resulting in a decline in PFC function. This leftward shift with aging puts older val homozygotes on the steeper portion of the inverted-U curve leading to an exaggerated effect of the COMTval^{158}met polymorphism on PFC function in older adults when compared with younger adults in response to similar COMTval^{158}met effects on DA levels (ΔDA). COMT, catechol-*O*-methyltransferase; DA, dopamine; met, methionine; PFC, prefrontal cortex; val, valine. The above figure is modified after Figure 1 in Nagel *et al.* (14).

and medial frontal network engagement (as measured by beta values of TC-task design correlation) in met homozygotes (Figure 6), suggests that carriers of the met allele may be more resilient to age-related decline in prefrontal function when compared with val homozygotes.

A caveat of our study is that differences in brain structure may affect functional results, thus limiting the spatial resolution of our findings. Since we did not have structural images in a majority of the subjects in this study, we could not correct for potential partial volume effects from age-related atrophy, if any, on our findings. Interestingly, in a recent brain morphometry study using voxel-based morphometry (VBM) to explore the COMTval^{158}met by age interaction on brain atrophy, Rowe *et al.* (53) reported only weak effects of COMTval^{158}met and COMTval^{158}met by age interaction on bilateral anterior insula and ventral frontal cortex with greater gray matter in val relative to met homozygotes in young subjects but not in older subjects. Within older subjects, val-homozygotes also showed a greater gray matter volume in the left premotor cortex relative to met homozygotes. In conclusion, although the findings of Rowe *et al.* (53) do not exclude an effect of brain atrophy on our COMTval^{158}met-related findings in the VLPFC, they suggest that it is unlikely that the results in the DLPFC were affected by this phenomenon, given the absence of a COMTval^{158}met or COMTval^{158}met by age interaction effect in this brain area.

Second, the age tails groups showed a significant difference in handedness. In this subsample selected to study the COMTval^{158}met by age interaction, although most of the subjects were right-handed, we found a statistical difference in handedness between age groups but neither across COMTval^{158}met groups nor, most importantly, across COMTval^{158}met by age groups. This difference in handedness would have been important when looking at age-related changes in brain activity (that we analyzed using the entire adulthood sample that was matched for handedness) but becomes secondary when analyzing the effects of COMTval^{158}met by age interaction on fMRI data where there were no differences in handedness. Nevertheless, we used handedness as a covariate of no interest in our analysis to control for the effect of handedness, if any.

Another limitation is that we explored the effects of age using a cross-sectional design. Future imaging studies combining longitudinal and cross-sectional approaches are warranted.

In this study, we demonstrate that COMTval^{158}met polymorphism modulates regional brain activity as well as multiple functional networks underlying WM tasks from young adulthood to old age. Our findings support a model in which COMTval^{158}met polymorphism modulates age-related changes in cortical physiology underlying cognitive functions with an exaggerated cortical functional decline in val homozygotes relative to met homozygotes, adding evidence that met homozygotes are more resilient to age-related changes in prefrontal cognition.

This work was supported by the National Institute of Mental Health Intramural Research Program. The study protocol was approved by the Intramural Review Board of the National Institute of Mental Health and appropriate procedures were used concerning the human subjects involved.

We thank Roberta Rasetti, M.D., for helpful discussions and revision of the manuscript and Morgan Prust, B.A., and Matthew Emery, B.S., for assistance in data analysis.

The authors report no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.

1. Volkow ND, Ding YS, Fowler JS, Wang GJ, Logan J, Gatley SJ, *et al.* (1996): Dopamine transporters decrease with age. *J Nucl Med* 37:554–559.
2. Bäckman L, Nyberg L, Lindenberger U, Li SC, Farde L (2006): The correlative triad among aging, dopamine, and cognition: Current status and future prospects. *Neurosci Biobehav Rev* 30:791–807.
3. Deary IJ, Wright AF, Harris SE, Whalley LJ, Starr JM (2004): Searching for genetic influences on normal cognitive ageing. *Trends Cogn Sci* 8:178–184.
4. Goldman-Rakic PS, Brown RM (1981): Regional changes of monoamines in cerebral cortex and subcortical structures of aging rhesus monkeys. *Neuroscience* 6:177–187.
5. Papaleo F, Crawley JN, Song J, Lipska BK, Pickel J, Weinberger DR, *et al.* (2008): Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress reactivity in mice. *J Neurosci* 28:8709–8723.
6. Tunbridge EM, Bannerman DM, Sharp T, Harrison PJ (2004): Catechol-o-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. *J Neurosci* 24:5331–5335.
7. Heinz A, Smolka MN (2006): The effects of catechol-O-methyltransferase genotype on brain activation elicited by affective stimuli and cognitive tasks. *Rev Neurosci* 17:359–367.
8. Savitz J, Solms M, Ramesar R (2006): The molecular genetics of cognition: Dopamine, COMT and BDNF. *Genes Brain Behav* 5:311–328.
9. Tan HY, Chen Q, Goldberg TE, Mattay VS, Meyer-Lindenberg A, Weinberger DR, *et al.* (2007): Catechol-O-methyltransferase Val158Met modulation of prefrontal-parietal-striatal brain systems during arithmetic and temporal transformations in working memory. *J Neurosci* 27:13393–13401.
10. de Frias CM, Annerbrink K, Westberg L, Eriksson E, Adolfsson R, Nilsson LG (2004): COMT gene polymorphism is associated with declarative memory in adulthood and old age. *Behav Genet* 34:533–539.
11. de Frias CM, Annerbrink K, Westberg L, Eriksson E, Adolfsson R, Nilsson LG (2005): Catechol O-methyltransferase Val158Met polymorphism is associated with cognitive performance in nondemented adults. *J Cogn Neurosci* 17:1018–1025.
12. Harris SE, Wright AF, Hayward C, Starr JM, Whalley LJ, Deary IJ (2005): The functional COMT polymorphism, Val 158 Met, is associated with logical memory and the personality trait intellect/imagination in a cohort of healthy 79 year olds. *Neurosci Lett* 385:1–6.
13. Starr JM, Fox H, Harris SE, Deary IJ, Whalley LJ (2007): COMT genotype and cognitive ability: A longitudinal aging study. *Neurosci Lett* 421:57–61.

14. Nagel IE, Chicherio C, Li S, von Oertzen T, Sander T, Villringer A, *et al.* (2008): Human aging magnifies genetic effects on executive functioning and working memory. *Front Hum Neurosci* 2:1.
15. Callicott JH, Mattay VS, Bertolino A, Finn K, Coppola R, Frank JA, *et al.* (1999): Physiological characteristics of capacity constraints in working memory as revealed by functional MRI. *Cereb Cortex* 9:20–26.
16. Sambataro F, Murty VP, Callicott JH, Tan HY, Das S, Weinberger DR, Mattay VS (2008): Age-related alterations in default mode network: Impact on working memory performance [published online ahead of print July 30]. *Neurobiol Aging*. doi:10.1016/j.neurobiolaging.2008.05.022.
17. Esposito F, Bertolino A, Scarabino T, Latorre V, Blasi G, Popolizio T, *et al.* (2006): Independent component model of the default-mode brain function: Assessing the impact of active thinking. *Brain Res Bull* 70:263–269.
18. McKeown MJ, Makeig S, Brown GG, Jung TP, Kindermann SS, Bell AJ, *et al.* (1998): Analysis of fMRI data by blind separation into independent spatial components. *Hum Brain Mapp* 6:160–188.
19. Yang K, Rajapakse JC (2004): ICA gives higher-order functional connectivity of brain. *Neural Inform Process Lett Rev* 2:27–32.
20. Calhoun VD, Adali T, Pearlson GD, Pekar JJ (2001): A method for making group inferences from functional MRI data using independent component analysis. *Hum Brain Mapp* 14:140–151.
21. Genovese CR, Lazar NA, Nichols T (2002): Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage* 15:870–878.
22. Owen AM, McMillan KM, Laird AR, Bullmore E (2005): N-back working memory paradigm: A meta-analysis of normative functional neuroimaging studies. *Hum Brain Mapp* 25:46–59.
23. Reeves S, Bench C, Howard R (2002): Ageing and the nigrostriatal dopaminergic system. *Int J Geriatr Psychiatry* 17:359–370.
24. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, *et al.* (2001): Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A* 98:6917–6922.
25. Mattay VS, Goldberg TE, Fera F, Hariri AR, Tessitore A, Egan MF, *et al.* (2003): Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc Natl Acad Sci U S A* 100:6186–6191.
26. Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, *et al.* (2004): Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): Effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet* 75:807–821.
27. Slifstein M, Kolachana B, Simpson EH, Tabares P, Cheng B, Duvall M, *et al.* (2008): COMT genotype predicts cortical-limbic D1 receptor availability measured with [11C]NNC112 and PET. *Mol Psychiatry* 13:821–827.
28. Seamans JK, Durstewitz D, Christie BR, Stevens CF, Sejnowski TJ (2001): Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. *Proc Natl Acad Sci U S A* 98:301–306.
29. Seamans JK, Yang CR (2004): The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog Neurobiol* 74:1–58.
30. Fries P, Reynolds JH, Rorie AE, Desimone R (2001): Modulation of oscillatory neuronal synchronization by selective visual attention. *Science* 291:1560–1563.
31. Tiesinga PHE, Fellous JM, Salinas E, Jose JV, Sejnowski TJ (2004): Synchronization as a mechanism for attentional gain modulation. *Neurocomputing* 58–60:641–646.
32. Bamford NS, Zhang H, Schmitz Y, Wu NP, Cepeda C, Levine MS, *et al.* (2004): Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. *Neuron* 42:653–663.
33. Nagano-Saito A, Leyton M, Monchi O, Goldberg YK, He Y, Dagher A (2008): Dopamine depletion impairs frontostriatal functional connectivity during a set-shifting task. *J Neurosci* 28:3697–3706.
34. Rolls ET, Loh M, Deco G, Winterer G (2008): Computational models of schizophrenia and dopamine modulation in the prefrontal cortex. *Nat Rev Neurosci* 9:696–709.
35. Aron AR, Fletcher PC, Bullmore ET, Sahakian BJ, Robbins TW (2003): Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. *Nat Neurosci* 6:115–116.
36. Cools R, Clark L, Owen AM, Robbins TW (2002): Defining the neural mechanisms of probabilistic reversal learning using event-related functional magnetic resonance imaging. *J Neurosci* 22:4563–4567.
37. Petrides M (1994): Frontal lobes and behaviour. *Curr Opin Neurobiol* 4:207–211.
38. Rushworth MF, Nixon PD, Eacott MJ, Passingham RE (1997): Ventral prefrontal cortex is not essential for working memory. *J Neurosci* 17:4829–4838.
39. Bor D, Duncan J, Wiseman RJ, Owen AM (2003): Encoding strategies dissociate prefrontal activity from working memory demand. *Neuron* 37:361–367.
40. D'Esposito M, Aguirre GK, Zarahn E, Ballard D, Shin RK, Lease J (1998): Functional MRI studies of spatial and nonspatial working memory. *Brain Res Cogn Brain Res* 7:1–13.
41. Tan HY, Chen Q, Sust S, Buckholtz JW, Meyers JD, Egan MF, *et al.* (2007): Epistasis between catechol-O-methyltransferase and type II metabotropic glutamate receptor 3 genes on working memory brain function. *Proc Natl Acad Sci U S A* 104:12536–12541.
42. Callicott JH, Bertolino A, Mattay VS, Langheim FJ, Duyn J, Coppola R, *et al.* (2000): Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. *Cereb Cortex* 10:1078–1092.
43. Koechlin E, Hyafil A (2007): Anterior prefrontal function and the limits of human decision-making. *Science* 318:594–598.
44. Tan HY, Sust S, Buckholtz JW, Mattay VS, Meyer-Lindenberg A, Egan MF, *et al.* (2006): Dysfunctional prefrontal regional specialization and compensation in schizophrenia. *Am J Psychiatry* 163:1969–1977.
45. Mattay VS, Fera F, Tessitore A, Hariri AR, Berman KF, Das S, *et al.* (2006): Neurophysiological correlates of age-related changes in working memory capacity. *Neurosci Lett* 392:32–37.
46. Ota M, Yasuno F, Ito H, Seki C, Nozaki S, Asada T, *et al.* (2006): Age-related decline of dopamine synthesis in the living human brain measured by positron emission tomography with L-[beta-11C]DOPA. *Life Sci* 79:730–736.
47. Davis SW, Dennis NA, Daselaar SM, Fleck MS, Cabeza R (2008): Que PASA? The posterior-anterior shift in aging. *Cereb Cortex* 18:1201–1209.
48. Li SJ, Biswal B, Li Z, Risinger R, Rainey C, Cho JK, *et al.* (2000): Cocaine administration decreases functional connectivity in human primary visual and motor cortex as detected by functional MRI. *Magn Reson Med* 43:45–51.
49. Rajah MN, D'Esposito M (2005): Region-specific changes in prefrontal function with age: A review of PET and fMRI studies on working and episodic memory. *Brain* 128:1964–1983.
50. Koechlin E, Ody C, Kouneiher F (2003): The architecture of cognitive control in the human prefrontal cortex. *Science* 302:1181–1185.
51. Emery L, Myerson J, Hale S (2007): Age differences in item manipulation span: The case of letter-number sequencing. *Psychol Aging* 22:75–83.
52. Emery L, Heaven TJ, Paxton JL, Braver TS (2008): Age-related changes in neural activity during performance matched working memory manipulation. *Neuroimage* 42:1577–1586.
53. Rowe JB, Hughes L, Williams-Gray CH, Bishop S, Fallon S, Barker RA, Owen AM (2008): The val(158)met COMT polymorphism's effect on atrophy in healthy aging and Parkinson's disease [published online ahead of print August 26]. *Neurobiol Aging*. doi:10.1016/j.neurobiolaging.2008.07.009.