ARCHIVAL REPORT

Influence of the *COMT* Genotype on Working Memory and Brain Activity Changes During Development

Iroise Dumontheil, Chantal Roggeman, Tim Ziermans, Myriam Peyrard-Janvid, Hans Matsson, Juha Kere, and Torkel Klingberg

Background: The Valine158Methionine (Val158Met) polymorphism of the *COMT* gene leads to lower enzymatic activity and higher dopamine availability in Met carriers. The Met allele is associated with better performance and reduced prefrontal cortex activation during working memory (WM) tasks in adults. Dopaminergic system changes during adolescence may lead to a reduction of basal dopamine levels, potentially affecting Met allele benefits during development.

Methods: We investigated the association of *COMT* genotype with behavioral ($n = 322$) and magnetic resonance imaging data ($n = 81–84$) collected during performance of a visuospatial WM task and potential changes in these effects during development (reflected in age $\times$ genotype interactions). Data were collected from a cross-sectional and longitudinal typically developing sample of 6- to 20-year-olds.

Results: Visuospatial WM capacity exhibited an age $\times$ genotype interaction, with a benefit of the Met allele emerging after 10 years of age. There was a parallel age $\times$ genotype interaction on WM-related activation in the right inferior frontal gyrus and intraparietal sulcus (IPS), with increases in activation with age in the Val/Val group only. Main effects of *COMT* genotype were also observed in the IPS, with greater gray matter volumes bilaterally and greater right IPS activation in the Val/Val group compared with the Met carriers.

Conclusions: These results suggest that *COMT* genotype effects on WM brain activity and behavior are not static during development. The full developmental picture should be considered when trying to understand the impact of genetic polymorphisms on the mature cognition of healthy adult or psychiatric populations.

The gene coding for the catechol-*O*-methyltransferase (COMT) enzyme, which mediates the degradation of catecholamines, in particular dopamine, has attracted considerable attention as a promising candidate gene for studying variance in cognitive function and mental illness (1,2). Developmental studies of this gene could inform us on the typical development of the dopamine system and provide a reference for the study of developmental disorders that have been linked to dopamine system dysfunction, such as schizophrenia (3), for which COMT function may be relevant (2,4,5), although the evidence is inconsistent (e.g., [1]).

The common rs4680 Valine158Methionine (Val158Met) single nucleotide polymorphism (SNP) leads to a reduction of COMT enzymatic activity in Met carriers (6–8). The effect of rs4680 on cognition has been extensively studied in adults, both in typical and neuropsychiatric populations. Better working memory (WM) performance (e.g., in N-back tasks), fewer perseverative errors, and higher IQ tend to be associated with the Met allele in healthy individuals (9–11), although some studies have failed to replicate these effects (10). In functional magnetic resonance imaging (fMRI) studies, Val/Val adults tend to exhibit greater prefrontal cortex (PFC) activation during WM tasks than Met/Met individuals (12,13). Structural imaging studies report less consistent results (14–16).

In children and adolescents, effects of *COMT* genotype in specific age groups have been reported on brain structure (17–19), resting brain perfusion (20), and brain activation in response to emotional stimuli (17). Behavioral findings are mixed (21–23). This lack of consistency may arise from age differences in the effect of *COMT* genotype on cognition.

Dopamine effects on behavior follow an inverted U-shaped dose-response curve, with both deficient and excessive amounts of dopamine activity predicting poor cognitive task performance (24,25). The Met/Met adults are thought to be near the apex of this curve, while Val carriers lay toward the lower end because of the increased dopamine metabolism rate associated with the Val allele (2,26,27).

There is some evidence that the dopamine system undergoes changes during childhood and adolescence. In the rhesus monkey, dopamine concentration in the PFC and dopamine synthesis in the PFC and parietal cortex peak around puberty (2–3 years), while dopamine concentration in the parietal cortex remains stable from 5 months of age (28,29). Dopaminergic input in the PFC also peaks around puberty, both in terms of length of the axons and density of varicosities (30–32). In humans, postmortem studies of the PFC have shown that dopamine concentration is highest during early postnatal development (33), while D1 receptor density peaks in adolescents (age 14–18) and young adults compared with neonates, infants, adults, and aged adults (33). In the living brain, a decrease in D1 receptor binding was observed in the PFC and parietal cortex over an age span of 10 to 30 years (34). These results overall suggest a decrease in dopamine levels from puberty to adulthood (23). Little information is available regarding potential sex differences.

A decrease in basal dopamine level between childhood and adulthood could affect the position of the Val158Met genotypes on the inverted U-shaped curve and lead to a differential effect of rs4680 during development. We tested the hypothesis of a change

From the Neuroscience Department (ID, CR, TZ, TK), Karolinska Institutet, Stockholm; Department of Biosciences and Nutrition (MP-J, HM, JK), Karolinska Institutet, Huddinge; and Science for Life Laboratory (JK), Department of Biosciences and Nutrition at Novum, Karolinska Institutet, Stockholm, Sweden; and Department of Medical Genetics (JK), Haartman Institute, University of Helsinki, and Folkhälsan Institute of Genetics, Helsinki, Finland.

Address correspondence to Iroise Dumontheil, Ph.D., Karolinksa Institutet, Neuroscience Department, Retzius väg 8, SE-171 77 Stockholm, Sweden; E-mail: i.dumontheil@gmail.com.
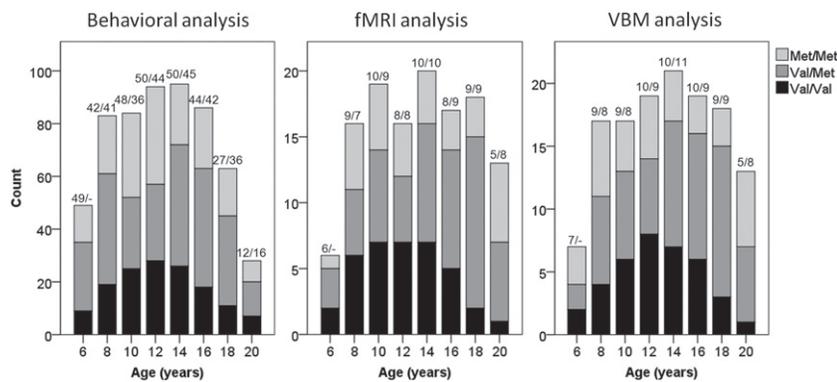
Figure 1. Distribution of the data points across round 1 and round 2 according to age and *COMT* genotype in each of the three analyses. For each age group, the number of participants from round 1 and round 2 is indicated above each bar (round 1 *n* / round 2 *n*). fMRI, functional magnetic resonance imaging; Met, Methionine; Val, Valine; VBM, voxel-based morphometry.

in the effect of *COMT* genotype with age on behavioral and neuro-imaging data associated with visuospatial WM, a cognitive ability that develops during childhood and adolescence (35).

Methods and Materials

Participants and Genetic Data

Participants in nine different age groups (6, 8, 10, 12, 14, 16, 18, 20, and 25 years) were recruited using random sampling from the population registry in Nynäshamn in Sweden (Brainchild study [36]). Informed consent was obtained from the participants and from the parents of children under 18. The study was approved by the local ethics committee of the Karolinska University Hospital, Stockholm. See Figure 1 and Table 1 for a description of included participants. Deoxyribonucleic acid was extracted either from blood or saliva. The *COMT* genotype is located on chromosome 22q11.2 and SNP rs4680 is in exon 4 of the gene (see Supplement 1 for details of the genetic analyses and excluded participants).

Assessment of Working Memory

Participants completed a large neuropsychological battery administered individually and in a quiet room. Visuospatial WM was assessed using a grid task (Dot Matrix) from the Automated Working Memory Assessment battery (37). This computerized task involves remembering the location and order of dots displayed sequentially in a four-by-four grid, for 1000 milliseconds each, with a 500-millisecond interval between dots. After training with one, two, and three dots, the test started with one dot. Each level consisted of six trials. Four correct answers were required for moving to the next level, where one more dot needed to be remembered. The test terminated when three errors were committed on one level. The score used was the total number of correct trials.

Dot Matrix Statistical Analyses

Linear mixed model analyses, which allow the inclusion of data from participants who have not attended all testing waves and adjust for intercorrelation between testing waves (38-40), were performed using the PASW 18.0 statistical package (41). A compound symmetry covariance structure was used and Dot Matrix score was treated as a repeated measure (rounds 1 and 2). We first identified how to best model changes in WM capacity with age, including the leveling of performance during mid-adolescence. We compared entering the age variable as age without transformation (linear effect), the inverse of age (age^{-1}), and the natural logarithm of age (ln[age]) (42,43). In all cases, age was transformed into *Z*-scores to evaluate effects of interest at the mean age of our sample and reduce collinearity between main effects and interactions (44). Sex and sex × age were entered as covariates. The best model was identified using Akaike's information criterion and Schwartz's information criterion (45,46).

We then tested whether including *COMT* genotype and age × *COMT* genotype as additional fixed effects improved the model. Genotype × sex and genotype × age × sex interactions were also included to test for potential sex differences in the effect of *COMT* genotype (47). Although the effect of rs4680 on dopamine degradation is additive, the effect on cognition is suggested to follow a nonlinear, inverted-U curve. To allow the detection of such effects, we evaluated both additive (0 = Met/Met, 1 = Val/Met, 2 = Val/Val) and dominance models (Val dominance: 0 = Met/Met, 1 = Val carriers; Met dominance: 0 = Met carriers, 1 = Val/Val) (similar to [9,48]).

Brain Imaging: fMRI

Magnetic resonance imaging data were collected on a 1.5 T Siemens scanner (Siemens, Erlangen, Germany) (Supplement 1). Participants performed two 5-minute sessions, each including 16 WM and 16 control trials in a pseudorandomized order. Dots were presented sequentially in a four-by-four grid. To reduce potential age or genotype differences in behavior, the task included load 2 (two dots) and 4 (four dots) trials only. After the last dot was shown, a number was presented in the grid. Participants were asked to indicate whether the number and its position in the grid matched. For example, "2?" would prompt the participant to indicate

Table 1. Distribution of the Participants and Longitudinal Measures According to *COMT* Genotype in Each of the Three Analyses

	Behavioral Analysis			fMRI Analysis			VBM Analysis		
	Participants	Total *n*	M/F	Participants	Total *n*	M/F	Participants	Total *n*	M/F
Met/Met	98 (79)	177	87/90	22 (9)	31	16/15	22 (12)	34	18/16
Met/Val	147 (115)	262	136/126	38 (19)	57	32/25	39 (21)	60	33/27
Val/Val	77 (66)	143	70/73	21 (16)	37	16/21	23 (14)	37	17/20
Total	322 (260)	582	293/289	81 (44)	125	64/61	84 (47)	131	68/63

The numbers in brackets indicate the number of subjects with repeated measures.
F, female; fMRI, functional magnetic resonance imaging; M, male; Met, Methionine; Val, Valine; VBM, voxel-based morphometry.

whether the second circle had appeared in the grid position filled by the number. In the control condition, the dots were presented in the corners of the grid and the cue ("8?") always required a "no" response.

Preprocessing and statistical analysis (36) were carried out with SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5). Contrast images comparing WM and control conditions, irrespective of load, for each participant were used in flexible factorial design second-level analyses that modeled whether the contrasts were from the same or different participants by including subject and testing round as factors. This permitted the inclusion in a single analysis of participants with and without longitudinal data. Age in months was transformed into Z-score (age$_Z$).

First, age$_Z$ and sex were entered as covariates and the main WM-control contrast was performed, correcting for multiple comparison across all voxels within the brain, with a false discovery rate (FDR) threshold of $p < .05$, to identify regions recruited during the task. Then, genotype and genotype $\times$ age$_Z$ were entered as additional covariates. Genotype was entered with an additive, Met dominant or Val dominant effect. In each model, a single F-test was performed to test whether any main effect of genotype or age $\times$ genotype interaction could be observed (FDR, $p < .05$). Contrasts were inclusively masked by the WM-control main effect previously defined. Results were plotted on a surface-based human atlas (Population-Average, Landmark- and Surface-Based atlas) (49,50) using the Caret software (http://www.nitrc.org/projects/caret/) (51). Mean parameter estimates from the first level contrasts were obtained in the significant clusters using MarsBar (52) and further analyzed using linear mixed models with PASW 18.0.

Brain Imaging: Voxel-Based Morphometry

Structural T1-weighted spin echo images were acquired with a three-dimensional magnetization prepared rapid gradient echo sequence (field of view = 256 $\times$ 256 mm, 256 $\times$ 256 grid, 1 mm³ voxel size). Voxel-based morphometry (VBM) was performed using SPM5. Following segmentation of the T1-weighted images, high-dimensional normalization was performed using the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra toolbox in statistical parametric mapping (53). The modulated warped gray matter (GM) images were then smoothed with an 8 mm Gaussian kernel. The same flexible factorial analyses and statistical thresholds as those used for the fMRI data were used for the VBM GM data, with the addition of total GM volume as a covariate.

Results

Analyses combined data from all participants and both testing rounds using linear mixed models. The complicated nonindependent design did not allow the calculation of standardized effect statistics (54). Effect sizes from analyses performed on the round 1 data only are reported in Supplement 1.

Behavioral Analysis

WM Capacity Development. Behavioral data were available for 322 participants, including 260 with longitudinal data. We first compared the information criteria of three different models (with the age variable as age$_Z$, age$^{-1}_Z$, or ln[age]$_Z$) to identify the best model of WM capacity development. Linear mixed model analyses included Dot Matrix score as the dependent variable, and sex, age, and their interaction were entered as fixed effects. The best model was where WM capacity was explained by age$^{-1}_Z$, with a steeper change in WM capacity over younger ages and then flattening to an asymptote.

Influence of COMT Genotype on WM Capacity Development. We then tested whether including the main effect of *COMT* genotype and the genotype $\times$ age interaction improved this model. Genotype $\times$ sex and genotype $\times$ age $\times$ sex interactions were also included. Genotype was modeled with an additive effect, Val dominance, or Met dominance.

Only the Val dominance model led to a significantly improved fit of the data compared with the age and sex only model (likelihood-ratio test, $D = 9.77$, 4 degrees of freedom, $p = .045$). The only significant effects observed in this full model were a main effect of age$^{-1}_Z$ [$F(1,502.7) = 171.01, p < .001$] and an interaction between genotype (Met/Met vs. Val carriers) and age$^{-1}_Z$ [$F(1,500.1) = 4.40$, $p = .036$]. The Met/Met individuals switched from being poorer performers during childhood to being the better performers from mid-adolescence onward (Figure 2). According to the model estimates, the cross-over occurred at 10.2 years of age. Neither sex nor interactions between sex and other variables had a significant effect on behavior.

fMRI Analysis

In-Scanner Performance. Functional magnetic resonance imaging data were available for 81 participants, including 44 with longitudinal data. Accuracy was high overall. Participants were less accurate in the WM condition (WM: mean 90.6% $\pm$ SD 10.6; control condition: 98.4% $\pm$ 3.5) [$t(124) = 9.52, p < .001$], as well as slower to respond (correct trials only, WM: 1277 msec $\pm$ 360; control condition: 738 msec $\pm$ 215) [$t(124) = 25.11, p < .001$]. The difference between WM and control conditions decreased with age in terms of both accuracy and reaction time [main effect of age$^{-1}_Z$, respectively, $F(1,87.8) = 10.06, p = .002$, and $F(1,97.1) = 32.05, p < .001$]; however, there was no main effect of genotype or age by genotype interaction (all p's > .29). Genotype thus did not affect WM versus control condition performance. Furthermore, only correct trials were used in the brain activity analyses.

WM-Control Main Effect. Functional magnetic resonance imaging data were analyzed using a flexible factorial second-level
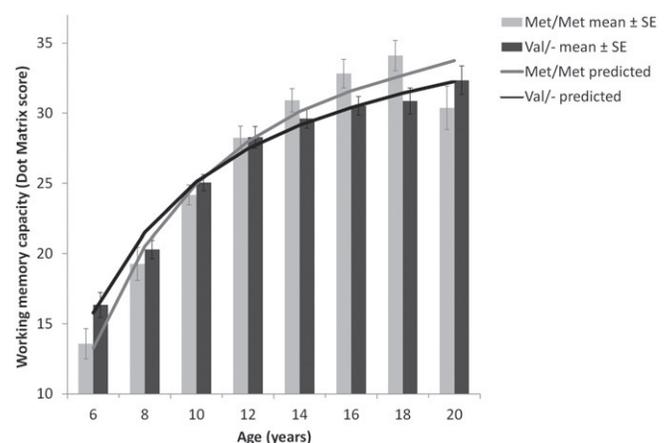

Figure 2. Working memory capacity as a function of each age year group at testing and *COMT* Valine158Methionine polymorphism. The bars are included for illustrative purposes only and represent the mean and standard error of working memory capacity, as measured by the Dot Matrix score, in each age group, collapsing across longitudinal and cross-sectional data. Participants tested at both rounds are included as two data points. Analyses were performed with age as a covariate rather than considering the age groups separately. The lines represent the mean predicted Dot Matrix score for each genotype group, as obtained from the mixed model analysis using the inverse of age as a factor and genotype effects with Valine dominance. Met, Methionine; SE, standard error; Val, Valine.
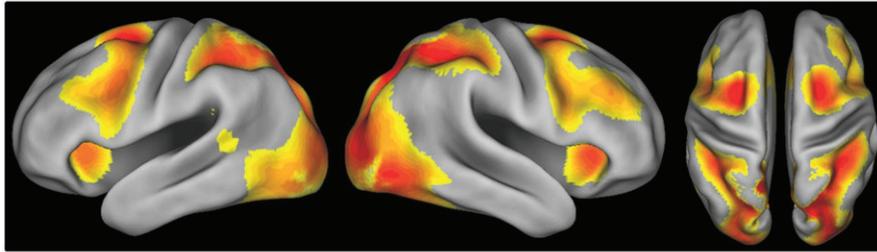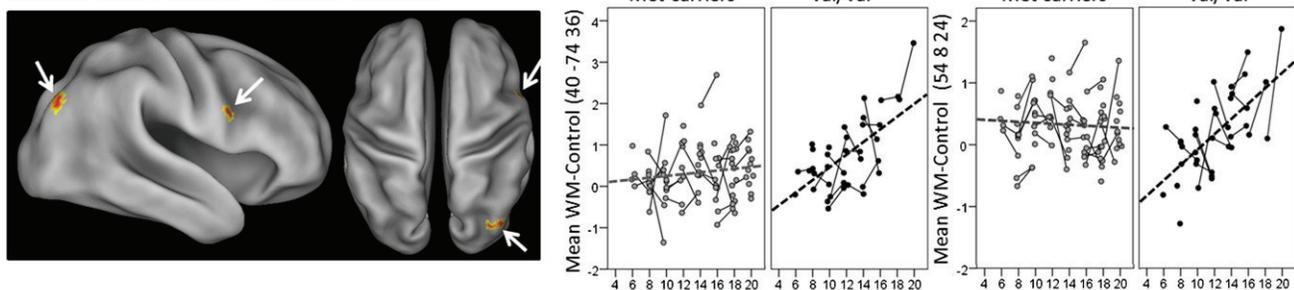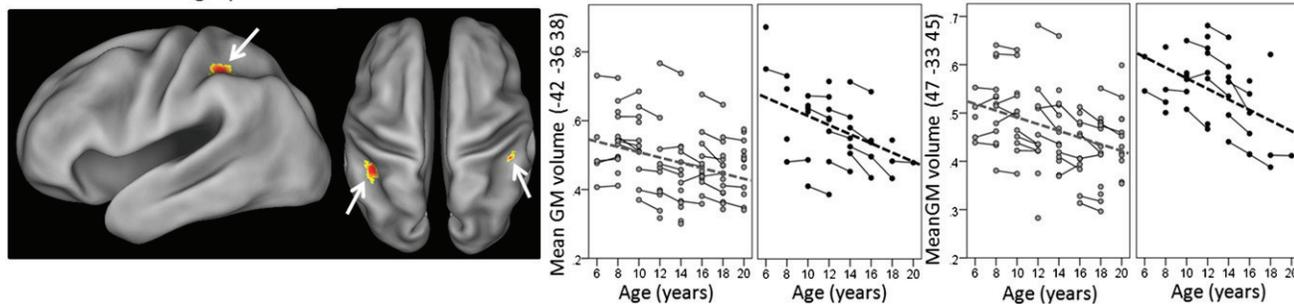
Figure 3. Functional magnetic resonance imaging (fMRI) and voxel-based morphometry results. **(A)** Render of the fMRI main effect working memory (WM)-control (false discovery rate [FDR], $p < .05$) on a surface-based human atlas (see Methods and Materials). From left to right: lateral view of the left and right hemispheres, dorsal view of the left and right hemispheres. This contrast was used as an inclusive mask for the tests of the effect of *COMT* genotype presented in **(B)** and **(C)**. **(B)** *F*-test of *COMT* genotype or genotype × age effects on the fMRI WM-control contrast (FDR, $p < .05$). Mean parameter estimates of the WM-control contrast at the first level were calculated and plotted for the parietal and frontal clusters observed in the right hemisphere. Full lines connect those participants who participated in both testing rounds. Dashed lines are the predicted fit from linear mixed models including sex, age_z, *COMT* genotype (with Methionine dominance), and genotype × age_z interaction as covariates. The age by genotype interaction was significant in both clusters. There was an additional main effect of genotype in the parietal cluster. **(C)** *F*-test of *COMT* genotype or genotype × age effects on the voxel-based morphometry gray matter data (FDR, $p < .05$). Mean gray matter volume was calculated and plotted for the two largest parietal clusters (Table 2). Full lines connect those participants who participated in both testing rounds. Dashed lines are the predicted fit from linear mixed models including sex, age_z, total gray matter volume (Z), *COMT* genotype (with Methionine dominance), and genotype × age_z interaction as covariates. The main effect of genotype but not the age × genotype interaction was significant in both clusters (Figure S1 in Supplement 1). COMT, catechol-*O*-methyltransferase; GM, gray matter; Met, Methionine; Val, Valine.

analysis of the WM-control contrast, which coded subject and testing round as factors and age_z and sex as covariates. Because of the smaller sample size of the fMRI data, only the linear effect of age was tested in statistical parametric mapping analyses. The resulting WM-control contrast image (FDR, $p < .05$) was saved and used as a mask for the subsequent analyses. A large frontoparietal network of regions was more active in the WM than the control condition (Figure 3A).

Effect of COMT Genotype on the WM-Control Contrast. The *COMT* genotype and genotype × age_z were added as additional covariates in the flexible factorial analysis. Genotype was entered as an additive effect, or with Val or Met dominance. Interactions between genotype and sex and genotype, sex, and age were included in the follow-up analyses only. In each analysis, a single *F*-test assessed whether genotype or genotype × age_z had significant ef-

fects (FDR, $p < .05$, within WM-control mask). Only the Met dominance model resulted in significant clusters located in the right inferior frontal gyrus (IFG) and in the posterior section of the right intraparietal sulcus (IPS)/angular gyrus (Figure 3B and Table 2; Figure S1 in Supplement 1).

Mean parameter estimates from the individual first-level WM-control contrasts were obtained for these two clusters. Linear mixed level model analyses (see Table 2 for statistics) demonstrated that the parietal cluster exhibited both a main effect of genotype, with greater activation in Val/Val participants than Met carriers, and an age by genotype interaction, with an increase in activation with age in the Val/Val participants only [Val/Val, $F(1,22.5) = 21.5$, $p < .001$; Met carriers, $p > .2$]. The frontal cluster exhibited an age by genotype interaction with a similar pattern [Val/Val, $F(1,20.3) = 25.9$, $p < .001$; Met carriers, $p > .4$] (Figure 3B). Note that for both

Table 2. *COMT* Genetic Effects on the fMRI and VBM Data

Label	SPM				Linear Mixed Model Analyses			
	x, y, z (MNI)	n	Z	F-test p_{FDR}	rs4680[a]	rs4680 × age$_z$[a]	rs4680 × sex[b]	rs4680 × age$_z$ × sex[b]
fMRI								
Posterior Section IPS/Angular Gyrus	40 −74 36	77	5.05	.007	$F(1,69.2) = 18.15$[c]	$F(1,89.8) = 11.96$[d]	$F(1,67.3) = 3.55$[e]	ns
Inferior Frontal Gyrus	54 8 24	17	4.19	.023	ns	$F(1,83.0) = 22.84$[f]	ns	$F(1,79.3) = 3.10$[e]
VBM: Gray Matter Volumes								
Lateral bank IPS	−42 −36 38	276	4.67	.023	$F(1,82.2) = 18.94$[c]	$F(1,64.9) = 2.77$[e]	$F(1,80.4) = 4.39$[h]	ns
Lateral bank IPS	47 −33 45	64	4.30	.023	$F(1,81.2) = 18.64$[c]	ns	ns	ns
Lateral bank IPS	42 −43 35	6	3.93	.028	$F(1,82.6) = 15.66$[d]	ns	$F(1,80.6) = 7.44$[g]	ns

Clusters were identified from F-tests of effects of *COMT* genotype or age$_z$ × *COMT* genotype. Contrasts were performed at an FDR-corrected threshold of $p < .05$ and inclusively masked by the fMRI main effect of WM-control (FDR, $p < .05$). The right section of the table reports the statistical results obtained in follow-up linear mixed model analyses.

FDR, false discovery rate; fMRI, functional magnetic resonance imaging; GM, gray matter; IPS, intraparietal sulcus; MNI, Montreal Neurological Institute; n, cluster size; ns, nonsignificant; p_{FDR}, FDR corrected p value; SPM, statistical parametric mapping; VBM, voxel-based morphometry; WM, working memory; Z, Z-score.

[a]Fixed effects comprised rs4680, age$_z$, sex, and rs4680 × age$_z$ (and total GM volume$_z$ for VBM analyses).
[b]Fixed effects comprised, in addition to [a], rs4680 × sex, age$_z$ × sex, and rs4680 × age$_z$ × sex.
[c]$p < .0001$.
[d]$p < .001$.
[e]$p \leq .10$.
[f]$p < .00001$.
[g]$p < .01$.
[h]$p < .05$.

clusters the genotype effects were significant when using age$^{-1}_z$, but the information criteria indicated that the models including age$_z$ were a better fit of the data. Figure 4 relates our findings to the suggestion that PFC functioning and WM performance follow an inverted-U curve function of dopamine levels.

The main effect of genotype and age × genotype interaction remained significant (p's ≤ .001) when including genotype × sex, age × sex, and genotype × age × sex interactions in the model. In the IPS, the main effect of genotype tended to be greater in male subjects, while in the IFG the interaction between age and genotype tended to be greater in male subjects (Table 2).

Further analyses (Figures S2 and S3 in Supplement 1) indicated that activations in the IFG and IPS clusters were more strongly correlated in the Val/Val group and that these participants also showed a positive correlation between IFG activation and Dot Matrix performance.

Voxel-Based Morphometry

Structural magnetic resonance imaging data were available for 84 participants, including 47 with longitudinal data. The effects of rs4680 on GM volumes were analyzed in the same way as for the fMRI data. Total GM volume$_z$ was included as an additional covariate to permit the study of regional rather than global effects. However, similar results were obtained when total GM volume was not included.

Again, only the Met dominance model resulted in significant clusters, located in the lateral bank of the IPS bilaterally (Figure 3C and Table 2; Figure S1 in Supplement 1). Individual mean GM volumes were calculated for each cluster. Linear mixed level model analyses indicated that all three clusters showed a main effect of genotype, with greater GM volumes in Val/Val participants than Met carriers. There was a weak trend in the largest cluster for a genotype by age interaction, with a steeper decrease in GM with age in the Val/Val individuals than Met carriers. The main effects of genotype in the three clusters remained significant (all p's ≤ .0001) when including genotype × sex, age × sex, and genotype × age × sex interactions in the model. Significant interactions between sex

and genotype were observed bilaterally (Table 2). In both cases, the effect of genotype was greater in males than females.

Discussion

This longitudinal and cross-sectional study of 6- to 20-year-olds combined a large sample and full age range of participants to investigate developmental changes in the effect of a functional polymorphism of the *COMT* gene on cognition, brain structure, and brain function. Previous studies of children and adolescents observed inconsistent *COMT* genotype effects and were restricted by either a small age range (21) or a small sample size (22,23). Our results show that the adult pattern of the effects of the *COMT* Val158Met polymorphism on brain and behavior during the performance of a visuospatial WM task emerges during adolescence. The Met/Met individuals showed a steeper increase in performance with age than Val carriers, while Val/Val individuals exhibited an increase in activation in the right IFG and right IPS/angular gyrus with age. Additional main effects of genotype were observed in the parietal cortex for both fMRI and gray matter data, with larger activation and GM volumes in Val/Val participants than Met carriers.

Differences in genotype effects on the behavioral and neuroimaging data, such as the Val versus Met dominance effects, may have been driven by a range of factors. For example, the fMRI visuospatial WM task included only relatively low WM loads and a control task, while the behavioral score reflected the performance achieved up to the participants' maximum WM load level. In addition, behavior may reflect more complex and global dopamine effects on brain activity and structure in a wide network of brain regions.

Behavioral results of the Dot Matrix task indicated that Met/Met homozygotes switched from being the underperformers to follow the adult pattern of better WM performance at around the age of 10 to 12 years old, typically considered as the start of puberty. Barnett *et al.* (21) reported a greater effect of *COMT* genotype on verbal IQ in 8-year-old boys who were pubertal at age 9 compared with prepubertal boys or girls. This pattern was not observed on other measures, including a counting span measure of WM. The present study
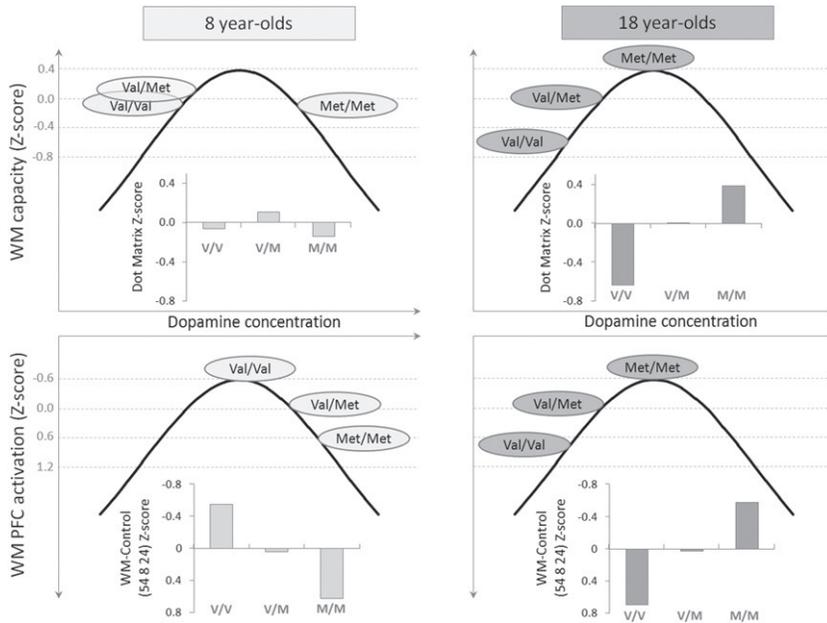
Figure 4. *COMT* genotype, working memory (WM) capacity, and WM activity and their relation to a hypothetical basal dopamine concentration. Brain activity is from the right lateral prefrontal cortex (PFC). Eight- and 18-year-old age groups were chosen to illustrate the changes in the influence of *COMT* genotype on WM function during development. On the top row, WM capacity Z-scores were calculated using the Dot Matrix scores of the 8- and 18-year-olds separately and are plotted in the inset bar charts for each *COMT* genotype. The three *COMT* genotypes were accordingly located on the hypothetical inverted-U curve of prefrontal functioning as a function of dopamine concentration proposed from adult and animal data (e.g., [25,27]). Here, an arbitrary Gaussian curve was used. On the bottom row, similar steps were applied to the WM-control activation in the right lateral PFC. Note that greater brain activation is associated with worse performance (27), and thus the y axis is inverted to highlight the similarities between the behavioral and imaging results. Eight-year-olds showed a detrimental effect of the Methionine (Met) allele, while 18-year-olds showed the typical adult pattern of a beneficial effect of the Met allele, with higher WM capacity and lower brain activity in the frontal cortex. This pattern of change in genotype effect with age can be regarded as a shift during development of the position of the *COMT* Valine158Met genotypes on the inverted-U curve of PFC function relative to dopamine levels in the direction of lower basal dopamine levels. Met, Methionine; PFC, prefrontal cortex; Val, Valine.

did not evaluate pubertal state. However, WM capacity showed no significant effect of sex or interaction between sex and age or gene. We therefore interpret the observed interaction of *COMT* genotype by age as reflecting a changing dopamine system during development, independent of possible additional interactions with hormonal effects. Note, however, that substance use was not recorded in this study and is a potential confound of the age effect observed here (55). In Sweden, daily or almost daily smoking increases during adolescence from < 1% at age 11 to 12 years to 12% at age 18 to 20 years (56,57), and the *COMT* Val allele has been shown to be a small risk factor (odds ratio 1.12) for smoking (58). Smoking is associated with lower WM in adults, while the evidence is mixed in younger samples (59). The increasingly poorer WM performance in Val carriers compared with Met/Met individuals may thus partly reflect increased smoking during adolescence, particularly in Val carriers. However, smoking rate is low and the associations between the Val allele and smoking and between smoking and WM are weak and therefore unlikely to explain the entire effect observed here.

The effects of genotype and genotype × age interactions also remained significant in the neuroimaging data when interactions with sex were included in the models. In the IPS, both brain activity (trend in the right IPS) and GM volumes (bilateral IPS) exhibited an interaction between sex and genotype, with greater genotype effects in males than females. In addition, a greater interaction effect on IFG activation tended to be observed in males. There is widespread but weak evidence of an interaction between sex and rs4680 on behavior, brain structure, and the incidence of psychiatric disorders (47). Estrogens have been implicated to explain these sex differences, as they are thought to downregulate COMT activity (47). The current study included only small age groups when split for sex. This limitation and the lack of pubertal stage information prevented us from investigating sex differences during development in more detail.

Our findings of rs4680 effects on GM volumes are consistent with previous evidence for an effect of this polymorphism on both adolescent and adult GM volumes (14,16) and cortical thickness (15,18). However, the locations of the observed effects differ between studies, which may be due to differences in methodology and age of the participants. The SNP rs4680 is a functional variant and could be thought to affect brain function but not brain structure. However, the pruning of synapses and axons during development is affected by synaptic functioning (60,61). Thus, during maturation, genotype effects on brain function could gradually impact on brain structure and GM volumes in particular. The weaker genotype by age interaction observed in the VBM compared with the fMRI and behavioral data may be because these processes occur over a protracted period and are not as flexible and rapid as functional changes in brain activity.

It is unclear yet what the greater parietal GM volumes observed in Val/Val individuals across ages precisely reflect and why the Val carriers are those with greater volumes. Further investigation using a combination of cortical thickness, cortical surface area, and volume data may provide more insight on the underlying developmental changes in structure (62). A possible interpretation of the results of this study relates them to the altered trajectories of brain structure maturation reported in developmental psychiatric disorders (63). For example, delayed peaks in cortical thickness in attention-deficit/hyperactivity disorder (64), a disorder associated with visuospatial WM impairments (65), lead to increased cortical thickness in attention-deficit/hyperactivity disorder during late childhood and early adolescence. In the current study, Val/Val participants had greater gray matter volumes in the parietal cortex and showed poorer WM capacity from mid-adolescence onward.

The effects of *COMT* genotype we observed were not limited to the PFC. In line with these results, dopamine D1 receptor availability (34) and correlation between D1 receptor availability and *COMT* genotype (66) are similar throughout the cortex, and dopamine D1 receptor density and change in WM capacity after training are correlated in both prefrontal and parietal cortex (67). Recent multimodal studies combining positron emission tomography and fMRI data to study the dopamine system in adults have repeatedly identified parietal and lateral PFC regions similar to the clusters observed in the present study and provide independent evidence that

these regions are sensitive to basal dopamine levels and dopamine levels in interconnected subcortical regions (68–70).

In both the behavioral and fMRI data sets, we observed a gradual emergence from age 12 of the adult pattern of *COMT* genotype effects. Prefrontal cortex functioning and WM performance have been suggested to follow an inverted-U curve function of dopamine levels (24), with adult rs4680 Met/Met individuals near the apex of this curve (2). The pattern observed in children (Figure 4) resembles the reduction of lateral PFC activation and improvement in WM performance observed in Val/Val adults administered amphetamine, which increases basal dopamine levels (27). Thus, our findings are compatible with a decrease in basal dopamine levels during adolescence (23), providing indirect evidence for the development of the dopamine system in humans.

Increased prefrontal activity could reflect top-down boosting of WM capacity in the parietal cortex (71). The Val/Val individuals showed an increase in prefrontal and parietal activation during adolescence, a greater correlation between the activation in these two clusters than Met carriers, and a correlation between greater WM activity in the right IFG and better WM capacity outside the scanner. Together, these results suggest that a top-down excitation from frontal to parietal cortex may be gradually implemented during adolescence in Val/Val individuals to compensate for deficient amounts of dopamine levels and parietal functioning.

Given that the onset of many psychiatric conditions occurs during adolescence (72) and that genotype variations have been associated with the incidence of developmental psychiatric disorders, elucidating the role of genetics in determining brain function during childhood and adolescence is critical to our understanding of the development of these disorders. The findings presented here show that the full developmental picture should be considered when trying to understand the impact of genetic polymorphisms on the mature cognition of healthy adult or psychiatric populations.

Supplementary material cited in this article is available online.

1. Craddock N, Owen MJ, O'Donovan MC (2006): The catechol-*O*-methyl transferase (COMT) gene as a candidate for psychiatric phenotypes: Evidence and lessons. *Mol Psychiatry* 11:446–458.
2. Meyer-Lindenberg A, Weinberger DR (2006): Intermediate phenotypes and genetic mechanisms of psychiatric disorders. *Nat Rev Neurosci* 7:818–827.
3. Howes OD, Kapur S (2009): The dopamine hypothesis of schizophrenia: Version III--the final common pathway. *Schizophr Bull* 35:549–562.
4. Gothelf D, Hoeft F, Ueno T, Sugiura L, Lee AD, Thompson P, Reiss AL (2011): Developmental changes in multivariate neuroanatomical patterns that predict risk for psychosis in 22q11.2 deletion syndrome. *J Psychiatr Res* 45:322–331.
5. Vorstman JA, Morcus ME, Duijff SN, Klaassen PW, Heineman-de Boer JA, Beemer FA, *et al.* (2006): The 22q11.2 deletion in children: High rate of autistic disorders and early onset of psychotic symptoms. *J Am Acad Child Adolesc Psychiatry* 45:1104–1113.
6. 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.
7. Männistö PT, Kaakkola S (1999): Catechol-*O*-methyltransferase (COMT): Biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol Rev* 51:593–628.
8. Weinshilboum RM (2006): Pharmacogenomics: Catechol-*O*-methyltransferase to thiopurine S-methyltransferase. *Cell Mol Neurobiol* 26:539–561.
9. Barnett JH, Jones PB, Robbins TW, Müller U (2007): Effects of the catechol-*O*-methyltransferase Val158Met polymorphism on executive function: A meta-analysis of the Wisconsin Card Sort Test in schizophrenia and healthy controls. *Mol Psychiatry* 12:502–509.
10. Barnett JH, Scoriels L, Munafò MR (2008): Meta-analysis of the cognitive effects of the catechol-*O*-methyltransferase gene Val158/108Met polymorphism. *Biol Psychiatry* 64:137–144.
11. Flint J, Munafò MR (2007): The endophenotype concept in psychiatric genetics. *Psychol Med* 37:163–180.
12. Dickinson D, Elvevåg B (2009): Genes, cognition and brain through a COMT lens. *Neuroscience* 164:72–87.
13. Mier D, Kirsch P, Meyer-Lindenberg A (2010): Neural substrates of pleiotropic action of genetic variation in COMT: A meta-analysis. *Mol Psychiatry* 15:918–927.
14. Cerasa A, Gioia MC, Labate A, Liguori M, Lanza P, Quattrone A, *et al.* (2008): Impact of catechol-*O*-methyltransferase Val(108/158) Met genotype on hippocampal and prefrontal gray matter volume. *Neuroreport* 19:405–408.
15. Cerasa A, Cherubini A, Quattrone A, Gioia MC, Tarantino P, Annesi G, *et al.* (2010): Met158 variant of the catechol-*O*-methyltransferase genotype is associated with thicker cortex in adult brain. *Neuroscience* 167:809–814.
16. Honea R, Verchinski BA, Pezawas L, Kolachana BS, Callicott JH, Mattay VS, *et al.* (2009): Impact of interacting functional variants in COMT on regional gray matter volume in human brain. *Neuroimage* 45:44–51.
17. Mechelli A, Tognin S, McGuire PK, Prata D, Sartori G, Fusar-Poli P, *et al.* (2009): Genetic vulnerability to affective psychopathology in childhood: A combined voxel-based morphometry and functional magnetic resonance imaging study. *Biol Psychiatry* 66:231–237.
18. Shaw P, Wallace GL, Addington A, Evans A, Rapoport J, Giedd JN, *et al.* (2009): Effects of the Val158Met catechol-*O*-methyltransferase polymorphism on cortical structure in children and adolescents. *Mol Psychiatry* 14:348–349.
19. Thomason ME, Dougherty RF, Colich NL, Perry LM, Rykhlevskaia EI, Louro HM, *et al.* (2010): COMT genotype affects prefrontal white matter pathways in children and adolescents. *Neuroimage* 53:926–934.
20. Thomason ME, Waugh CE, Glover GH, Gotlib IH (2009): COMT genotype and resting brain perfusion in children. *Neuroimage* 48:217–222.
21. Barnett JH, Heron J, Ring SM, Golding J, Goldman D, Xu K, *et al.* (2007): Gender-specific effects of the catechol-*O*-methyltransferase Val108/158Met polymorphism on cognitive function in children. *Am J Psychiatry* 164:142–149.
22. Diamond A, Briand L, Fossella J, Gehlbach L (2004): Genetic and neurochemical modulation of prefrontal cognitive functions in children. *Am J Psychiatry* 161:125–132.
23. Wahlstrom D, White T, Hooper CJ, Vrshek-Schallhorn S, Oetting WS, Brott MJ, *et al.* (2007): Variations in the catechol *O*-methyltransferase polymorphism and prefrontally guided behaviors in adolescents. *Biol Psychiatry* 61:626–632.
24. Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AF (2007): Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat Neurosci* 10:376–384.
25. Arnsten AF (1997): Catecholamine regulation of the prefrontal cortex. *J Psychopharmacol (Oxford)* 11:151–162.
26. Giakoumaki SG, Roussos P, Bitsios P (2008): Improvement of prepulse inhibition and executive function by the COMT inhibitor tolcapone depends on COMT Val158Met polymorphism. *Neuropsychopharmacology* 33:3058–3068.
27. 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.

28. Goldman-Rakic PS, Brown RM (1982): Postnatal development of mono-amine content and synthesis in the cerebral cortex of rhesus monkeys. *Brain Res* 256:339–349.

29. Goldman-Rakic PS, Brown RM (1981): Regional changes of monoamines in cerebral cortex and subcortical structures of aging rhesus monkeys. *Neuroscience* 6:177–187.

30. Lambe EK, Krimer LS, Goldman-Rakic PS (2000): Differential postnatal development of catecholamine and serotonin inputs to identified neurons in prefrontal cortex of rhesus monkey. *J Neurosci* 20:8780–8787.

31. Rosenberg DR, Lewis DA (1994): Changes in the dopaminergic innervation of monkey prefrontal cortex during late postnatal development: A tyrosine hydroxylase immunohistochemical study. *Biol Psychiatry* 36:272–277.

32. Rosenberg DR, Lewis DA (1995): Postnatal maturation of the dopaminergic innervation of monkey prefrontal and motor cortices: A tyrosine hydroxylase immunohistochemical analysis. *J Comp Neurol* 358:383–400.

33. Weickert CS, Webster MJ, Gondipalli P, Rothmond D, Fatula RJ, Herman MM, *et al.* (2007): Postnatal alterations in dopaminergic markers in the human prefrontal cortex. *Neuroscience* 144:1109–1119.

34. Jucaite A, Forssberg H, Karlsson P, Halldin C, Farde L (2010): Age-related reduction in dopamine D1 receptors in the human brain: From late childhood to adulthood, a positron emission tomography study. *Neuroscience* 167:104–110.

35. Luna B, Padmanabhan A, O'Hearn K (2010): What has fMRI told us about the development of cognitive control through adolescence? *Brain Cogn* 72:101–113.

36. Söderqvist S, McNab F, Peyrard-Janvid M, Matsson H, Humphreys K, Kere J, Klingberg T, *et al.* (2010): The SNAP25 gene is linked to working memory capacity and maturation of the posterior cingulate cortex during childhood. *Biol Psychiatry* 68:1120–1125.

37. Alloway TP (2007): *Automated Working Memory Assessment Manual*. Oxford, UK: Harcourt.

38. McCulloch CE, Searle SR (2001): *Generalized, Linear, and Mixed Models*. New York: Wiley-Interscience.

39. Molenberghs G, Verbeke G (2001): A review on linear mixed models for longitudinal data, possibly subject to dropout. *Stat Model* 1:235–269.

40. 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.

41. SPSS, Inc. (2005): *Linear Mixed-Effects Modeling in SPSS: an Introduction to the MIXED Procedure. SPSS Technical Report*. Chicago: SPSS, Inc.

42. Kail RV, Ferrer E (2007): Processing speed in childhood and adolescence: Longitudinal models for examining developmental change. *Child Dev* 78:1760–1770.

43. Luna B, Garver KE, Urban TA, Lazar NA, Sweeney JA (2004): Maturation of cognitive processes from late childhood to adulthood. *Child Dev* 75:1357–1372.

44. Aiken LS, West SG (1991): *Multiple Regression: Testing and Interpreting Interactions*. Newbury Park, CA: Sage.

45. Akaike H (1974): A new look at the statistical model identification. *IEEE Trans Automat Contr* 19:716–723.

46. Schwartz G (1978): Estimating the dimension of a model. *Ann Stat* 6:461–464.

47. Harrison PJ, Turnbridge EM (2008): Catechol-*O*-methyltransferase (COMT): A gene contributing to sex differences in brain function, and to sexual dimorphism in the predisposition to psychiatric disorders. *Neuropsychopharmacology* 33:3037–3045.

48. Hersrud SL, Stoltenberg SF (2009): Epistatic interaction between COMT and DAT1 genes on eating behavior: A pilot study. *Eat Behav* 10:131–133.

49. Van Essen DC (2005): A Population-Average, Landmark- and Surface-based (PALS) atlas of human cerebral cortex. *Neuroimage* 28:635–662.

50. Van Essen DC, Dierker DL (2007): Surface-based and probabilistic atlases of primate cerebral cortex. *Neuron* 56:209–225.

51. Van Essen DC, Drury HA, Dickson J, Harwell J, Hanlon D, Anderson CH, *et al.* (2001): An integrated software suite for surface-based analyses of cerebral cortex. *J Am Med Inform Assoc* 8:443–459.

52. Brett M, Valabregue R, Poline J-B (2002): Region of interest analysis using an SPM toolbox [abstract]. Presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2–6, Sendai, Japan.

53. Ashburner J (2007): A fast diffeomorphic image registration algorithm. *Neuroimage* 38:95–113.

54. Nakagawa S, Cuthill IC (2007): Effect size, confidence interval and statistical significance: A practical guide for biologists. *Biol Rev Camb Philos Soc* 82:591–605.

55. Saraceno L, Munafó M, Heron J, Craddock N, van den Bree MB (2009): Genetic and non-genetic influences on the development of co-occurring alcohol problem use and internalizing symptomatology in adolescence: A review. *Addiction* 104:1100–1121.

56. Hvitfeldt T, Gripe I (2009): *Rapport 118 Skolelevers Drogvanor 2009*. Stockholm: Centralförbundet för Alkohol-Och Narkotikaupplysning.

57. Galanti MR, Rosendahl I, Wickholm S (2008): The development of tobacco use in adolescence among "snus starters" and "cigarette starters": An analysis of the Swedish "BROMS" cohort. *Nicotine Tob Res* 10:315–323.

58. Tammimäki AE, Männistö PT (2010): Are genetic variants of COMT associated with addiction? *Pharmacogenet Genomics* 20:717–741.

59. Durazzo TC, Meyerhoff DJ, Nixon SJ (2010): Chronic cigarette smoking: Implications for neurocognition and brain neurobiology. *Int J Environ Res Public Health* 7:3760–3791.

60. Webb SJ, Monk CS, Nelson CA (2001): Mechanisms of postnatal neurobiological development: Implications for human development. *Dev Neuropsychol* 19:147–171.

61. Low LK, Cheng HJ (2006): Axon pruning: An essential step underlying the developmental plasticity of neuronal connections. *Philos Trans R Soc Lond B Biol Sci* 361:1531–1544.

62. Winkler AM, Kochunov P, Blangero J, Almasy L, Zilles K, Fox PT, *et al.* (2010): Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. *Neuroimage* 53:1135–1146.

63. Giedd JN, Rapoport JL (2010): Structural MRI of pediatric brain development: What have we learned and where are we going? *Neuron* 67:728–734.

64. Shaw P, Eckstrand K, Sharp W, Blumenthal J, Lerch JP, Greenstein D, *et al.* (2007): Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *Proc Natl Acad Sci U S A* 104:19649–19654.

65. Westerberg H, Hirvikoski T, Forssberg H, Klingberg T (2004): Visuo-spatial working memory span: A sensitive measure of cognitive deficits in children with ADHD. *Child Neuropsychol* 10:155–161.

66. 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.

67. McNab F, Varrone A, Farde L, Jucaite A, Bystritsky P, Forssberg H, Klingberg T (2009): Changes in cortical dopamine D1 receptor binding associated with cognitive training. *Science* 323:800–802.

68. Fischer H, Nyberg L, Karlsson S, Karlsson P, Brehmer Y, Rieckmann A, *et al.* (2010): Simulating neurocognitive aging: Effects of a dopaminergic antagonist on brain activity during working memory. *Biol Psychiatry* 67:575–580.

69. Landau SM, Lal R, O'Neil JP, Baker S, Jagust WJ (2009): Striatal dopamine and working memory. *Cereb Cortex* 19:445–454.

70. Nyberg L, Andersson M, Forsgren L, Jakobsson-Mo S, Larsson A, Marklund P, *et al.* (2009): Striatal dopamine D2 binding is related to frontal BOLD response during updating of long-term memory representations. *Neuroimage* 46:1194–1199.

71. Edin F, Klingberg T, Johansson P, McNab F, Tegnér J, Compte A, *et al.* (2009): Mechanism for top-down control of working memory capacity. *Proc Natl Acad Sci U S A* 106:6802–6807.

72. Paus T, Keshavan M, Giedd JN (2008): Why do many psychiatric disorders emerge during adolescence? *Nat Rev Neurosci* 9:947–957.