Catechol-O-Methyltransferase (COMT) Genotypes and Working Memory: Associations with Differing Cognitive Operations

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Background: *Catechol-O-methyltransferase (COMT) is a strong candidate gene for schizophrenia and cognitive functions disrupted in this disorder. This report examines the relation of COMT genotypes to performance on a battery of working memory tests differing in the cognitive operations to be performed on the material.*

Methods: *A large sample of 402 healthy adults were tested on four working memory tests: Spatial Delayed Response (SDR), Word Serial Position Test (WSPT), N-back, and Letter–Number Sequencing. A subsample (*n *= 246) was tested on the Wisconsin Card Sorting Test (WCST). A saliva swab was used to obtain DNA from all participants.*

Results: *Letter–Number Sequencing, which requires both storage and manipulation of information, was the only working memory test that showed expected differences among COMT genotypes, with the met/met group showing the best performance and the val/val group the poorest performance. As in previous studies, the met/met group also performed better than the val/val group on the WCST.*

Conclusions: *COMT genotypes were not associated with performance on tests measuring simple storage, maintenance of temporal order or updating of information in working memory. Genotype differences in Letter–Number Sequencing and WCST suggest that higher-order components of processing (e.g., mental manipulation) are more closely related to this gene.*

Key Words: Catechol-O-methyltransferase, COMT genotypes, dopamine, executive function, working memory

Working memory impairment is a primary cognitive deficit in schizophrenia (Goldman-Rakic 1991; Park and Holzman 1992) and a promising endophenotypic marker present in unaffected relatives of schizophrenic patients (Cannon et al 1994; Goldberg et al 1995; Park et al 1995). Weinberger et al (2001) reviewed evidence that dopamine neurotransmission in prefrontal cortex plays a critical role in both normal cognitive processing and schizophrenia. They suggested that a functional polymorphism in the gene that encodes catechol-O-methyltransferase (COMT) is a strong genetic candidate affecting prefrontal dopamine, working memory, and executive cognitive functions mediated by this region, as well as risk for schizophrenia. This hypothesis received support from studies finding that schizophrenic patients and healthy adults with the COMT *met/met* allele performed better (i.e., showed fewer perseverative errors) on a neurocognitive test of executive function, the Wisconsin Card Sorting Test (WCST), when compared with subjects with the *val/val* allele (Egan et al 2001; Malhotra et al 2002). Two additional studies, one in patients with schizophrenia (Bilder et al 2002) and one in healthy female subjects (Tsai et al 2003), found the same direction of difference in WCST performance between COMT genotypes, but the difference was not statistically significant. This likely reflects the need for larger samples to detect the relatively small effect size evident for COMT genotype differences on the WCST (Egan et al 2001; Malhotra et al 2002).

The WCST is a complex test that involves multiple cognitive processes (e.g., problem solving, set shifting, working memory, and attention). Studies have begun to provide information concerning specific components of cognitive processing that may contribute to the differences in neurocognitive performance between COMT genotypes. Bilder et al (2002) examined COMT polymorphism effects in schizophrenia patients using a large battery of neurocognitive tests. The pattern of results across tests suggested that aspects of visuospatial processing, attention, and cognitive flexibility shared most variance with the COMT genotype. Goldberg et al (2003) found evidence of a COMT genotype effect on working memory, but only on the more difficult conditions of the task. They tested schizophrenia patients, their family members, and healthy control subjects in an N-back working memory test and a continuous performance test (CPT). No effect of the COMT genotype was found for the 0-back task or CPT, but in both the 1-back and 2-back conditions, the *met/met* genotype showed greater accuracy than the *val/val* genotype, with the *met/val* group showing intermediate performance. They suggest that executive processes involved in updating and temporally ordering information in the N-back task are of key importance for understanding COMT genotype effects.

Stefanis et al (2004), on the other hand, found no association between COMT genotypes and either attention (CPT) or working memory (N-back task similar to that used by Goldberg et al 2003), although they did find an association to negative and disorganized traits on a schizotypy questionnaire. Findings with working memory tasks are therefore not consistent across studies, and further work is needed to characterize the specific aspects of working memory performance that are or are not associated with the COMT gene.

In a recent review of neuroimaging studies of working memory, Wager and Smith (2003) note that all working memory tasks involve temporary storage of information but that they vary with regard to the demands placed on what is done with this information. In addition to the demands created by the type of stimuli (e.g., verbal vs. spatial vs. object information), they

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defined three general features of working memory tasks that extend beyond simple retention, including maintenance of temporal order, continuous updating, and overt manipulation of information. Each of these "classes" of task demands was associated with different patterns of brain activation in the imaging studies that they reviewed.

The goal of our study was to survey the working memory performance of genotypes along these lines. Subjects were assessed using a battery of working memory tests commonly used in studies of schizophrenia but that vary with regard to task demands. Simple retention of information about spatial location over a brief time span was assessed using a Spatial Delayed Response (SDR) test (Carter et al 1996; Park and Holzman 1992). Demands on memory for temporal order of verbal information were assessed with the Word Serial Position Test (WSPT; Wexler et al 1998), which requires retention of the order of spoken words. The "online" updating of information in working memory was evaluated using a sequential-letter N-back task (adapted from Cohen et al 1997) that uses a continuous performance format. Finally, overt manipulation of information was assessed via the Letter–Number Sequencing subtest from the Wechsler Adult Intelligence Scale—III (WAIS-III; Gold et al 1997; Wechsler 1997). This task requires subjects to not only store information (spoken letters and numbers), but to reorder it according to preset rules.

A large community-based sample of healthy adults was tested on this battery, and material for genetic analyses was extracted. To relate the findings of this study to previous studies, the Wisconsin Card Sorting Test (WCST) was administered to a subsample (61.2%) of subjects.

Methods

Subjects

A total of 402 healthy adults between the ages of 18 and 55 were recruited from Internet-based advertisements and from postings at the medical center and local colleges. All subjects gave written informed consent to participate in the study. They were screened to exclude those with current or past psychiatric or neurologic disorders, as well as substance abuse. The subjects also completed self-rating scales for assessing psychopathology, including the Beck Depression Inventory (BDI-II; Beck et al 1961) and the Hopkins Symptom Checklist (SCL–90; Derogatis et al 1974). One subject was excluded because of an excessively high BDI score. They were tested on a battery of working memory tests and an estimate of general intellectual ability (WAIS-III Vocabulary subtest) as described later. A subsample of 246 healthy adults was also tested on the WCST.

Spatial Delayed Response (SDR) Test

A computerized version of this test (Lyons-Warren et al 2004) was used to provide a measure of simple retention of visuospatial information over brief time delays (Park and Holzman 1992). While the subject fixates on a central cross on a monitor screen, a dot appears for 150 msec in one of 32 possible locations at a 4.5 in radius from the central cross. During a delay period of 2, 5, 15, or 30 sec, a series of geometric shapes appears in place of the fixation cross, and the subject presses a space bar whenever a diamond shape appears. After the delay, the fixation cross returns and the subject's task is to point to the location on the computer screen where they remember seeing the dot. There were eight trials at each delay. The mean error in millimeters (distance between the recalled position and the actual target) is calculated for each delay and averaged across delays. The log of the mean error in millimeters was used as the dependent variable because there were outliers with large errors. This transformation normalized the distribution of scores and reduced outlier influence on statistical analyses.

Word Serial Position Test (WSPT)

A six-word version of the auditory WSPT (Wexler et al 1998) provided a measure of verbal working memory in a delayed-response paradigm requiring the retention of both item content and serial order. Each trial begins with six nouns spoken with 1 sec between words. After a delay of 9 sec, one of these words is repeated. The subject is instructed to remember the six words in the order presented and to indicate the serial position of the repeated word by pressing one of six keys (labeled 1 to 6) on the computer keyboard. After practice, each subject is tested in 36 trials, with a random order of the six serial positions. The outcome measures are the percent correct responses for all serial positions and for each position separately.

N-Back Task

This N-back is a continuous working memory task (adapted from Cohen et al 1997) that requires subjects to monitor a series of letters presented sequentially on a computer screen and to respond when a letter is identical to the one that immediately preceded it (1-back condition), one presented two letters back (2-back), or three letters back (3-back). As each new letter is presented, the subject is required to update his or her temporary storage of previous letters (the number depending on the condition). Sixty letters were presented in each block of items, the 2- and 3-back conditions were each presented twice (1-back performance is generally at or very near ceiling levels in nonpatient samples; repeating it provides little additional variability in scores). Stimuli were presented for 500 msec, with 2500-msec interstimulus intervals. A total of 12, 20, and 20 targets were presented for the 1-, 2-, and 3-back conditions, respectively. An overall measure of sensitivity (d') for detecting targets and avoiding nontarget responses (which controls for differences in response bias) was computed for all conditions, and for the 1-, 2-, and 3-back conditions separately.

Letter–Number Sequencing

The Letter–Number Sequencing subtest from the WAIS-III (Tulsky et al 1997; Wechsler 1997) is an auditory task that requires subjects to store and reorder (recite in numeric and alphabetical order) strings of intermingled letters and numbers. The dependent measure is the total number of correct strings. The subtest loads on the working memory factor extracted from among WAIS-III subtests (Tulsky et al 1997).

WAIS-III Vocabulary

The Vocabulary subtest is the best correlate of overall IQ on the WAIS-III and was administered to obtain an estimate of general level of intellectual ability (Wechsler 1997; Tulsky et al 1997).

Wisconsin Card Sorting Test (WCST)

The WCST (Grant and Berg 1948) was administered for the purpose of comparing results for our sample to those for other studies that have used this test. We used the 128-card computerized version of the WCST (Heaton et al 1999). The main dependent measures were the number of categories, errors, perseverative errors, and failures to maintain. The WCST scores were converted to demographically adjusted standard scores (or

percentile bands for categories and fail to maintain) based on published norms (Heaton et al 1993).

Genotyping

A saliva swab was obtained from all subjects and DNA was analyzed with BuccalAmp DNA extraction kit from Epicentre (Madison, Wisconsin). A 176-bp fragment located from position 1822 to 1997 in COMT gene was amplified by polymerase chain reaction (PCR) from genomic DNA, which includes at position 1947 the G/A polymorphism coding for Val158Met. The genotypes were determined with Fluorescent Polymorphism (FP). An FP primer adjacent to position 1947 on the complementary strand (TCA
GGCATGCACACCTTGTCCTTCA) was used to distinguish G from A. Selected samples were sequenced to confirm the reliability of FP genotyping method, and the genotypes generated from both assays were consistent.

Statistical Analyses

Comparisons of demographic characteristics between the COMT genotypes were performed using one-way analysis of variance (ANOVA) or chi-square tests, as appropriate.

A principal components analysis (PCA) was initially performed on the correlation matrix of overall accuracy scores for the four working memory tests to determine whether performance across these tests could be characterized in terms of a single or multiple working memory "factors."

All raw working memory test accuracy scores, including extracted factor scores, were then adjusted for demographic influences (age, gender, ethnicity, and education) using stepwise regression. The regressor for ethnicity was a three-point scale (Caucasian, Other, African American). Demographically adjusted scores for each test were then compared among COMT genotypes using one-way ANOVA. Analyses were initially conducted in the total sample, and then within only the Caucasian subject sample. These same comparisons were conducted for the WCST standard scores.

The last set of analyses focused on the embedded difficulty level conditions within three of the working memory tests: SDR (four levels of delay), WSPT (six serial positions), and N-back (1-, 2-, and 3-back conditions). Repeated-measures analysis of variance was used to determine whether genotype effects varied by difficulty-level within each task.

Results

Characteristics of COMT Genotypes

Genetic analyses identified 67 subjects with a *met/met* genotype (16.7%), 188 with a *met/val* genotype (46.8%), and 147 with a *val/val* genotype (36.5%). There was no deviation of genotype frequencies from Hardy–Weinberg equilibrium. Table 1 gives the demographic characteristics of these genotypes. They did not differ in gender, age, or education. Most subjects in each group were native English speakers, and there was no evidence of a genotype difference in intellectual ability as measured by WAIS-III vocabulary scores. Similarly, differences among groups in self-ratings of psychopathology on the BDI-II or SCL–90 scales were extremely small and nonsignificant. There was essentially no correlation between performance on the four working memory tests and either BDI-II depression scores ($r = -.01$ to $-.06$, *ns*) or SCL–90 total scores ($r = .00$ to $-.07$, *ns*), which would be expected given the attenuated range of these scores in healthy adults. (In supplemental analyses, all working memory findings described subsequently remained the same after covarying for BDI and SCL–90 scores.) A higher percentage of subjects in the *met/met* group were Caucasian, and a smaller percentage were Asian, Hispanic, or African American when compared with the other groups. The working memory data given in Table 2 and Figure 1 were therefore demographically corrected, and analyses were also performed on the data for only Caucasian subjects. There were no significant demographic, rating scale, or estimated intelligence differences among the Caucasian only genotypes and no deviation of frequencies from the Hardy–Weinberg equilibrium.

Working Memory Performance

Only performance on the Letter–Number Sequencing test showed the expected difference among genotypes [see upper portion of Table 2; $F(2,399) = 5.78$, $p = .003$]. Post hoc tests revealed that subjects in the *met/met* group performed significantly better than those in the *val/met* [$t(253) = 2.84$, $p = .005$] and *val/val* groups [$t(212) = 3.49$, $p = .001$; see Figure 1]. There was no significant difference among genotypes in performance on SDR, WSPT, or N-back. The difference among genotypes on the Letter–Number Sequencing test was also found when analyses were repeated for Caucasian subjects only [see lower portion of Table 2; $F(2,215) = 3.52$, $p = .03$]. The *met/met* group differed from the *val/met* [$t(152) = 1.96$, $p = .05$] and *val/val* [$t(109) = 2.82$, $p = .006$] groups in showing better accuracy on the Letter–Number Sequencing test, but there was no significant difference among groups on the other tests.

In the principal component analysis of accuracy scores for the four working memory tests, only one factor was sufficiently common across tests to yield an eigenvalue greater than 1 (i.e., 2.21, 55.1% variance accounted for), with all tests weighted approximately equally (loading for SDR = –.61; WSPT = .79; N-back = .78, Letter–Number = .77). The demographically corrected factor scores did not differ significantly among the COMT genotypes (Table 2). This factor, which represents common variance across the four working memory tests, was correlated with vocabulary score estimates of intellectual ability ($r = .31$ for demographically corrected factor scores). There was, however, no group difference on this factor even after covarying for vocabulary scores, which indicates that the COMT genotypes did not differ in general intellectual ability or in this composite index of working memory performance.

Difficulty-Level Analyses

Difficulty-level manipulations on the SDR test [main effect for delay: $F(3,1188) = 101.87$, $p < .001$], WSPT [main effect for list position: $F(5,1960) = 97.93$, $p < .001$], and N-back [main effect for number of items back: $F(2,792) = 132.02$, $p < .001$] all produced expected, statistically significant differences in performance. There were, however, no significant Condition × Genotype interactions for either SDR [$F(6,1188) = .79$, $p = .58$] or N-back [$F(4,792) = .42$, $p = .79$]. As can be seen in Table 2, performance was poorer at the longer delays (15 and 30 sec) in the SDR test and in the 2 and 3 back conditions in the N-back test; however, there was no significant difference in performance among genotypes for these more difficult conditions. The condition × genotype interaction for the WSPT approached significance in the full sample [$F(10,1960) = 1.77$, $p = .06$]. This marginal interaction, however, was related to the superior performance of the *met/val* group (68.5 ± 23.1% correct) relative to the *val/val* group (61.7 ± 24.7% correct) at serial position 5 ($p = .01$). This was the only significant difference in performance among groups at any serial position.

Table 1. Descriptive Statistics for Genotypes

		Met/Met	Met/Val	Val/Val	F/χ^2	p
n		67	188	147		
Gender (% female)		50.7	55.3	61.2	2.34	.31
Age (years)	Mean	29.6	29.8	30.6	.36	.67
	SD	8.5	8.3	9.6		
Education (years)	Mean	15.6	15.9	15.4	2.49	.08
	SD	2.0	2.2	2.2		
Ethnicity (%)						
Caucasian		71.6	56.4	42.9	22.11	**.005**
Asian		4.5	7.4	15.6		
Hispanic		6.0	9.6	13.6		
African American		10.4	20.2	22.4		
Other		7.5	6.4	5.4		
Percent Native English		85.1	84.0	82.3	.31	.86
WAIS-III Vocabulary	Mean	13.6	13.2	12.8	1.32	.27
	SD	2.7	3.4	3.2		
Beck Depression	Mean	7.25	5.72	5.55	2.11	.12
Inventory-II	SD	6.87	5.70	5.67		
SCL-90 General Severity	Mean	58.7	55.2	54.2	2.69	.07
Index	SD	12.4	13.3	12.8		

SCL, Hopkins Symptom Checklist; WAIS, Wechsler Adult Intelligence Scale.
Bold type indicates significant p values.

Wisconsin Card Sort Test Performance

The percentage of each COMT genotype in the subsample tested on the WCST (17.5% *met/met*, 47.2% *met/val*, 35.4% *val/val*) is comparable to that in our complete sample.

In the WCST subsample including all ethnicities, there were no significant differences among groups in standard scores for categories or perseverative errors. Between group differences in standardized error scores were, however, in the expected direction and approached significance in the overall ANOVA [$F(2,243) = 2.43$, $p = .09$]. Pairwise comparisons indicated that the *met/met* group (103.0 ± 14.3) performed significantly better than *val/val* group (96.8 ± 16.5; $p = .03$). If the failure to maintain score is collapsed into those falling into the highest standardized percentile band (≥16th percentile) and those below (<16th percentile), the *met/met* (97.7% in highest percentile group), *met/val* (85.2%), and *val/val* (81.6%) groups were in the expected order, and the overall group effect was significant ($\chi^2 = 6.36$, $df = 2$, $p = .04$). The high percentage of good performers in each group indicates that there were likely ceiling effects in the WCST data for these healthy adults; nonetheless, group differences were found.

A similar pattern of findings was obtained for the Caucasian only subjects ($n = 132$). There were 24.2% *met/met*, 50.0% *met/val*, and 25.7% *val/val,* which did not deviate from the Hardy–Weinberg equilibrium. Group differences on the standardized WCST error score were found in pairwise comparisons (*met/met*: 105.6 ± 12.8 vs. *val/val*: 101.8 ± 11.9, $p = .04$), but the overall difference among groups in the ANOVA fell short of significance [$F(2,129) = 2.15$, $p = .12$]. Percentages of those in the highest failure to maintain grouping were similar to those when all ethnicities were included (*met/met*: 96.9%, *met/val*: 86.4%, *val/val*: 85.3%), but did not reach significance ($\chi^2 = 2.87$, $df = 2$, $p = .24$).

Discussion

The Letter–Number Sequencing test showed the predicted relationship between the COMT genotypes and performance, with the *met/met* group outperforming the *met/val* and *val/val* groups. The other working memory tests and the global working memory factor, derived from a principal components analysis of performance on the four working memory tests, were not related to the COMT genotypes. Expected genotype differences were not enhanced by or found on the most difficult conditions of the SDR, WSPT, or N-back tasks, which indicates that the difficulty level was not critical. The lack of genotype differences on the majority of these commonly used working memory tests suggests that the COMT gene is not related to the temporary storage of information, retaining its temporal order, or continuously updating that information. What, then, is the distinguishing feature that may be responsible for genotype differences on the Letter–Number Sequencing test? After encoding of letters and numbers into working memory, this test requires participants to reorder this information according to numeric and alphabetical rules. This additional "mental manipulation" of information in working memory may be critical to COMT genotype differences. It does not appear to be a surrogate for general intelligence because there were no differences among genotypes in our estimate of general intellectual ability (WAIS-III Vocabulary). Other studies also have not found a difference in IQ between COMT genotypes (e.g., Egan et al 2001).

Our findings regarding WCST performance for COMT genotypes are not only consistent with prior studies (Egan et al 2001; Malhotra et al 2002) but suggest that the previously observed WCST differences among COMT genotypes are not simply due to the working memory demands of the test. Although the WCST places demands on working memory, it also requires evaluation of rules and manipulation of information. A substantial majority of the subjects in all COMT groups completed the WCST to criterion (in terms of categories achieved), but those in the *met-met* group did so more efficiently, with fewer errors and a lower likelihood of losing set. The findings of our study suggest that differences in performance among the COMT genotypes on the WCST are *not* likely to be due to attention or maintenance of information in working memory because these genotypes did not differ on several working memory tasks demanding these cognitive functions. Higher order executive processes appear to

Table 2. Demographically Adjusted Scores for Total Sample and for Caucasian Subjects Only

Tests	Met/Met			Met/Val			Val/Val			*F*	*p* Value
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*		
					Total Sample						
SDR[a]	1.087	.121	66	1.096	.134	186	1.093	.129	147	.12	.89
2 sec	1.002	.165		1.000	.162		.994	.144			
5 sec	1.026	.158		1.048	.169		1.056	.176			
15 sec	1.083	.156		1.115	.164		1.105	.153			
30 sec	1.157	.165		1.152	.171		1.151	.164			
WSPT[b]	69.5	14.7	64	68.3	15.4	186	67.1	15.1	145	.62	.54
1st	79.1	19.0		79.7	19.8		75.1	25.5			
2nd	66.0	24.4		64.3	25.2		66.1	23.6			
3rd	64.1	27.4		57.7	27.2		59.1	26.9			
4th	58.4	28.9		53.4	28.4		54.5	27.3			
5th	62.8	26.4		68.5	23.1		61.7	24.7			
6th	86.0	19.4		87.0	19.7		85.6	19.9			
N-back[c]	3.33	.69	64	3.37	.64	188	3.39	.74	147	.18	.84
1-back	3.72	.16		3.69	.20		3.68	.26			
2-back	3.65	.66		3.68	.49		3.65	.60			
3-back	3.15	.65		3.11	.73		3.17	.79			
Letter–Number[d]	13.91	2.62	67	12.71	3.08	188	12.48	2.86	147	5.78	**.003**
Factor Score	.153	.794	67	−.015	.858	188	−.047	.854	147	1.35	.26
					Caucasian Only						
SDR[a]	1.101	.127	48	1.091	.137	105	1.099	.120	63	.13	.88
2 sec	1.008	.179		.975	.154		.986	.142			
5 sec	1.029	.136		1.026	.176		1.055	.181			
15 sec	1.092	.171		1.118	.165		1.113	.139			
30 sec	1.181	.169		1.148	.161		1.154	.141			
WSPT[b]	69.8	14.7	45	69.1	14.3	104	66.3	16.2	62	.93	.40
1st	80.6	18.6		81.8	18.1		72.1	26.7			
2nd	67.8	23.8		64.6	24.3		67.8	23.5			
3rd	61.2	25.5		60.8	24.5		56.1	27.1			
4th	59.1	27.8		53.4	27.7		55.6	28.7			
5th	61.0	26.7		67.4	21.6		60.9	23.7			
6th	88.0	17.8		86.4	16.4		85.0	17.8			
N-back[c]	3.30	.69	46	3.45	.66	106	3.30	.69	63	1.36	.26
1-back	3.70	.18		3.69	.14		3.69	.21			
2-back	3.61	.71		3.70	.45		3.63	.53			
3-back	3.15	.61		3.21	.68		3.06	.69			
Letter–Number[d]	13.92	2.76	48	12.84	3.33	106	12.36	2.98	63	3.52	**.03**
Factor Score	.118	.843	48	.068	.882	106	−.128	.848	63	1.39	.25

Bold type indicates significant *p* values.
[a]Log error (mm) on the Spatial Delayed Response Test (SDR), total and all delay levels.
[b]Percent correct responses on the Word Serial Position Test (WSPT), total and all serial positions.
[c]d′ index of accuracy on the n-back test, total and each number-back condition.
[d]Number of correct sequences on the Letter–Number Test.

play a greater role in COMT genotype effects on both the WCST and Letter–Number Sequencing.

This study used a larger sample size of healthy adults than prior studies. Large sample size is important because of the small-to-medium effect size typically seen for neurocognitive measures in the COMT genotypes (Bilder et al 2002; Goldberg et al 2003; Malhotra et al 2002). The difference in performance between the *met/met* and *val/val* genotypes on the Letter–Number Sequencing test was of medium effect size (mean difference/SD = .52) for the full sample. In contrast, the other working memory tests had extremely small effect sizes (ranging from .05 for the SDR test to .16 for the WSPT), which would make it difficult to find significant differences between genotypes even with large samples, and such small effects may not be particularly meaningful. The effect sizes for the working memory data of only the Caucasian subjects were essentially the same as for the full sample. The difference in errors on the WCST between the *met/met* and *val/val* genotypes had a small-to-medium effect size (.40) comparable to prior studies in healthy adults (Malhotra et al 2002) and schizophrenia patients (Bilder et al 2002).

We did not find an effect of COMT genotype on performance in the N-back test, which is at odds with the findings of Goldberg et al (2003). This may stem from differences in the N-back tests in these studies. In the sequential-letter N-back task used in our study, the subject views a series of letters and responds with a button press when the letter on the screen matches the letter 1-back, 2-back, or 3-back in the sequence (Cohen et al 1997; Carter et al 1998). It therefore involves maintenance and updating of the letters and their order (in more difficult conditions) in working memory. In the N-back task used in Goldberg et al (2003), a series of numbers from 1 to 4 is displayed on the screen, and the subject is required to press one of four buttons corre-

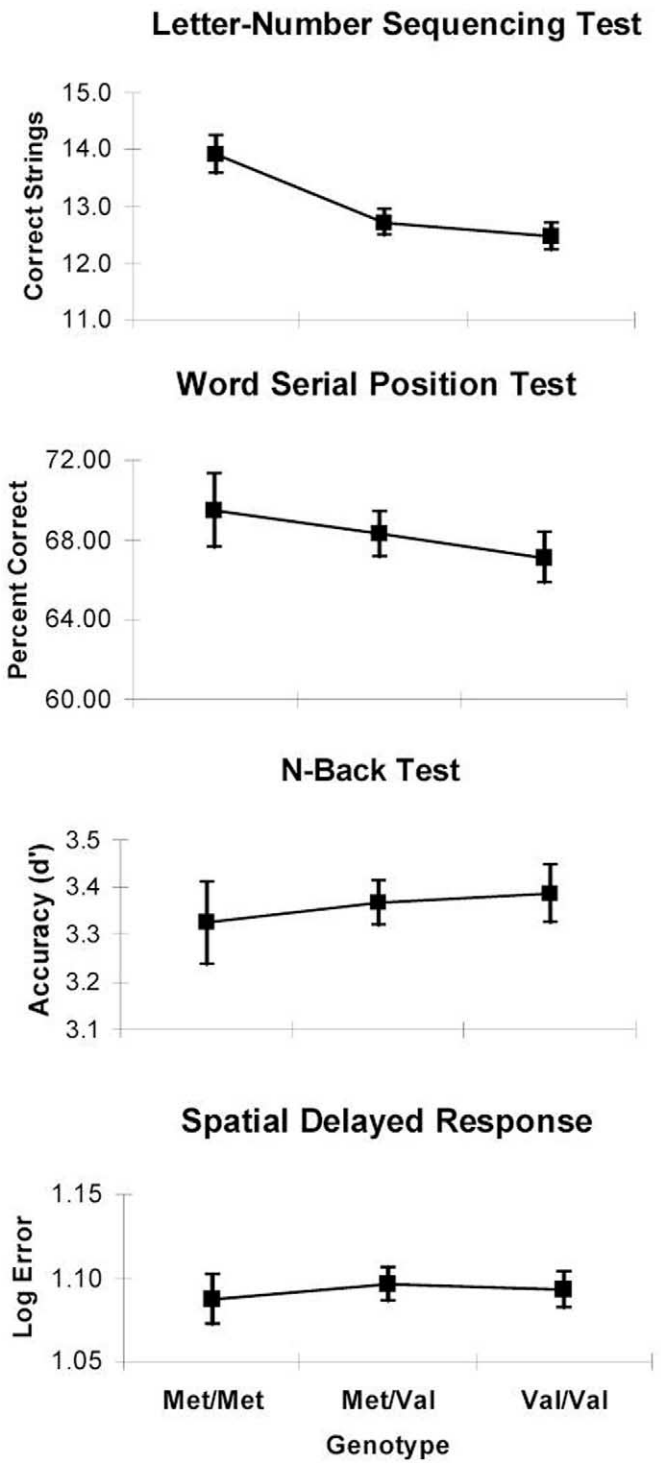

Figure 1. Demographically-corrected mean scores (error bars = standard errors) on working memory tests for catechol-O-methyltransferase genotypes.

sponding to the number 1-back or 2-back in the sequence, regardless of the number currently on the screen. This requires not only maintenance, updating and ordering of the numbers, but also inhibition of response to the number currently on the screen, and selection from among four alternative responses. It therefore has greater potential for stimulus-response conflict and may place greater demands on executive control processes. This is consistent with the findings of Fossella et al (2002) who tested healthy adults on the Attention Network Test, which provides separate measures of the alerting, orienting, and executive control aspects of attention. Only executive attention, involving processes for resolving response conflict in the face of competing stimuli, was weakly associated with COMT genotype.

The findings for the Letter–Number Sequencing test and WCST, as well as those for the N-Back task of Goldberg et al (2003) and the Attention Network Test (Fossella et al 2002), are consistent with the conclusion that cognitive tasks requiring maintenance *plus* reordering of information, response inhibition, or switching of response sets differentiate COMT genotypes. This is also supported by findings for children in which the *met/met* genotype performed better than the *val/val* genotype on a dots-mixed task, which required children to remember two rules and inhibit responses on the same side as the stimulus, but not in a pointing task, which required working memory but not response inhibition (Diamond et al 2004). Diamond et al suggested that tasks that require working memory and response inhibition are particularly sensitive to dopamine level in prefrontal cortex. This is consistent with the hypothesis that the high-activity *val* allele, which leads to increased dopamine catabolism, is associated with deficits in executive and working memory functions that rely on prefrontal cortex (Egan et al 2001). More recently, Bilder et al (2004) advanced a hypothesis based on the tonic–phasic dopamine theory. They postulate that the *met* allele increases tonic dopamine transmission and thereby increases the stability of the network mediating working memory but decreases phasic dopamine, which may make it difficult to switch or update the active working memory network. Although this hypothesis would account for the reduced likelihood of the *met/met* genotype losing set during the WCST, it is not clear how it would account for the superior performance of this group on the Letter–Number Sequencing test or the lack of genotype differences on the SDR test, which involves primarily maintenance of working memory. Our study was not designed to test the hypothesis of Bilder et al, however, and it would be difficult to separate the contributions of stability and flexibility of processing in the Letter–Number Sequencing test. Support for their hypothesis was obtained by Nolan et al (2004), who found evidence of enhanced cognitive stability but poorer flexibility in the *met/met* genotype. In a competing programs task, the *met/met* group showed greater accuracy than *val/val* group for maintenance of imitation responses, which requires cognitive stability, but not for reversal responses, which require switching rules. Additional research is clearly needed to further refine the cognitive subcomponents associated with COMT genotypes.

There are limitations in our study that weaken conclusions concerning the role of specific subcomponent processes of working memory. The COMT genotype effects may have been attenuated by the very high ability level of the sample. The sample was young, relatively well educated, and had an estimated intelligence level that was a standard deviation better than the population average. The working memory tests in this study were selected to be representative of the tests used in recent studies of schizophrenia. Subject aggregate performance scores on all of these tests were normally distributed, but they reached near-ceiling performance on some of the easiest conditions of these tasks, meaning that overall variance in task performance was attributable to a smaller proportion of the trials on any test. Moreover, the Letter–Number Sequencing test may be better able to detect differences among very intelligent subjects; it was

specifically incorporated in the WAIS-III as part of an effort to make the test more sensitive to differences among the most intelligent examinees (Tulsky et al 1997). The four working memory tests in this study were not matched for psychometric characteristics, although differences in psychometric properties of the working memory tests were not likely to have been a problem. First, aggregate performance scores and scores on all but the easiest conditions of these tests were normally distributed, with variation both above and below the mean (i.e., no ceiling or floor effects). Second, genotype differences could not be attributed to difficulty-level manipulations on tests that were not related to COMT (i.e., SDR, N-back, or WSPT).

If the COMT genotype is not related to storage of information in working memory, retaining its temporal order, or continuously updating it, other genes must play a role in these cognitive abilities. One of the aims of testing a large sample of healthy adults in this study was to identify individuals who are generally better or worse in working memory performance. Other candidate genes, for example, those identified in molecular-biological studies of working memory in mice, could then be compared between these groups. Genes expressed in frontal cortex and that influence dopamine levels may be prime candidates because of their potential association to both working memory and schizophrenia (Weinberger et al 2001). It is noteworthy that a review of neuroimaging studies by Wager and Smith (2003) found that manipulation demands in working memory were associated with activation in more ventral than dorsal prefrontal areas (Brodmann areas 10, 46, and 47, primarily in right hemisphere). In contrast, activation in dorsolateral prefrontal cortex (Brodmann areas 6, 8, and 9) showed greater specialization for working memory tests involving continuous updating or temporal order, and dorsolateral prefrontal areas have been implicated in the functional abnormalities in schizophrenia. Although COMT genotype effects may be related to a specific cognitive deficit in schizophrenia, it is likely that candidate genes associated with other aspects of working memory and brain areas implicated in schizophrenia's deficits will be found. Strategies to identify such genes in nonpatient populations may prove fruitful for understanding schizophrenia.

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