Tolcapone Effects on Gating, Working Memory, and Mood Interact with the Synonymous Catechol-*O*-methyltransferase rs4818C/G Polymorphism

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Background: The functional catechol-*O*-methyltransferase (COMT) valine158methionine (val158met) polymorphism determines prepulse inhibition (PPI) levels and working memory performance and the effects of tolcapone on these functions. Here, we explored the effects of the synonymous COMT rs4818 C/G polymorphism and tolcapone on PPI and working memory.

Methods: Thirteen G/G (low prefrontal cortex [PFC] dopamine [DA]) and 12 C/C (high PFC DA) healthy male subjects entered and completed the study. Subjects participated in two weekly sessions associated with either acute oral tolcapone (200 mg) or placebo according to a balanced, crossover, double-blind design. Prepulse inhibition was assessed with 5 dB and 15 dB above background prepulses at 30-msec, 60-msec, and 120-msec intervals. Subjective mood and working memory performance (n-back and letter-number sequencing) were also assessed.

Results: Prepulse inhibition was lower and reaction time in the n-back was slower in the G/G compared with the C/C group in the placebo condition. Tolcapone increased PPI and improved performance in both working memory tasks in the G/G group only. Baseline startle was greater in the C/C group and was not affected by tolcapone. Mood profile was worse in the C/C group and tended to deteriorate with tolcapone. Status of val158met alone could not explain these results.

Conclusions: Catechol-*O*-methyltransferase haplotype analyses are essential in future research. Prepulse inhibition and working memory may both relate to PFC DA levels according to an inverted U-shaped curve function. Tolcapone could be potentially useful in the treatment of conditions with deficient sensorimotor gating and working memory such as schizophrenia and prodromal states but only in a genotype-specific manner.

Key Words: Cognition, COMT, prefrontal cortex, prepulse inhibition, synonymous COMT rs4818C/G polymorphism, tolcapone

Several lines of evidence suggest that the catechol-*O*-methyltransferase (COMT) enzyme is an important determinant of prefrontal cortex (PFC) performance during executive cognition by enhancing prefrontal physiological efficiency. The majority of the literature has focused on the functional rs4680 valine158methionine (val158met) polymorphism, where findings support that met158 allele loading, which results in optimal PFC dopamine (DA) levels, is dose dependently associated with superior performance on cognitive tests assessing executive function, as well as prefrontal physiology as assessed by neuroimaging (1,2). Nevertheless, there is abundant evidence that synonymous, silent polymorphisms may play a significant role in the modulation of the COMT expression levels in the PFC. The rs4818 C/G is a synonymous polymorphism that tags a haplotype that affects messenger RNA (mRNA) stability and therefore protein abundance and activity over an 18-fold difference (3). The C and G allele frequencies range from 52% to 58% and 42% to 48%, respectively, in Caucasians (Perlegen Human Genome Resources [http://genome.perlegen.com/] and HapMap Project [http://www.hapmap.org]). A recent meta-analysis of the COMT gene in the SZGene database (http://www.schizophreniaforum.org/res/sczgene/meta.asp?geneID=420) showed that the rs4818 is the COMT polymorphism most strongly associated with schizophrenia, while no significant effect was revealed for the rs4680 polymorphism (4). If this large difference between these two polymorphisms is not due to common publication bias (rs4818: four studies, rs4680: 30 studies), it might be secondary to the rs4818 polymorphism accounting for a greater variation of the COMT activity compared with rs4680 (3).

Prepulse inhibition (PPI) is thought to reflect sensorimotor gating, a form of central nervous system inhibition wherein irrelevant sensory information is filtered out during the early stages of processing so that attention can be focused on more salient features of the environment (5). Prepulse inhibition in rodents is modulated by activity in a well-defined cortico-striato-pallido-pontine circuitry (6,7), which has been confirmed with neuroimaging studies in human subjects (8–12). Consistent with these neuroimaging findings and the notion that sensorimotor gating is important in human cognition (13), neuropsychological studies show that higher PPI levels predict superior executive function (14–17). Deficient PPI is well documented in conditions with frontostriatal pathology and deficient executive function such as schizophrenia (18–20).

Suboptimal PFC DA levels conferred by the COMT val158 allele are associated with lower PPI in healthy men (21) and schizophrenia patients (22). Moreover, the nonstimulant COMT inhibitor tolcapone, which leads to relatively specific increases in PFC DA (2,23–25), improved PPI (26), working memory performance (26,27), and prefrontal efficiency (27) in healthy val158 homozygotes who have lower PFC DA concentrations at baseline but worsened the performance of met/met individuals who have high PFC DA concentrations and operate near or at ceiling levels at baseline. These effects taken together are consistent with the increasingly accepted model of the inverted U-shaped relation-

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ship between PFC DA signaling and PFC function (28,29). However, the possibility remains that these effects may be specific to the val158met COMT polymorphism.

In the present study, we investigated the impact of the synonymous rs4818 COMT polymorphism on PPI and working memory in healthy male subjects after administration of placebo or tolcapone. Similar to the val158met polymorphism, we predicted that tolcapone would improve PPI and working memory in G/G homozygotes (high expressed enzyme activity leading to low tonic DA PFC signaling), while it would decrease or have no effect on these functions in C/C homozygotes (low expressed enzyme activity leading to high tonic DA PFC signaling).

Methods and Materials

Subjects

We restricted the sample to men to avoid additional cognitive function and PPI variability related to gender and menstrual cycle in women (30,31), likely to be mediated partly by transcriptional regulation of COMT activity by estrogens (32,33). Twenty-eight healthy male subjects were recruited from a previous cohort that had participated in a study examining the effects of the COMT rs4818 polymorphism on cognition (34) and was independent from the cohort examining the effects of the COMT val158met polymorphism on PPI (21) and the effects of tolcapone (26). Based on availability, we selected subjects so as to form two equal homozygote groups (C/C, $n = 14$; G/G, $n = 14$). All subjects had previously undergone extensive assessments (34), but they had a new urine toxicology screening before testing. The study was approved by the Ethics Committee of the University of Crete. All participants gave written informed consent before screening.

Design and Drugs

Subjects participated in two weekly sessions associated with either single, acute oral tolcapone (200 mg) or placebo (35) in identical capsules. Within each separate genotype group, subjects were allocated to sessions and treatments according to a balanced, crossover, double-blind design. All phenotypic assessments were performed by an investigator (S.G.G.) blind to genotype and drug status.

Assessment of Mood, PPI, and Working Memory

This was identical to our previous tolcapone study (26) and is described in detail in Supplement 1. Briefly, we administered the Profile of Mood States (POMS) (36) questionnaire before and after treatment to assess subjective mood. To elicit the startle response and assess PPI, we used 12 pulse-alone (40 msec, 115 dB) and 36 prepulse (20 msec, 75 dB and 85 dB) trials with three lead intervals (30, 60, and 120 msec) and 6 no-stimulus (NOSTIM) trials. Equipment descriptions, setup, and scoring criteria have been previously described in detail (37). We used a computerized version of the n-back sequential letter task (38) and the Letter Number Sequencing (LNS) task from the Wechsler Adult Intelligence Scale (39) to assess working memory.

Statistical Analysis

Demographic data were compared between the groups using one-way analysis of variance (ANOVA). Pretreatment and posttreatment changes in each POMS scale were compared using separate 2 × 2 (genotype × treatment) ANOVAs. The mean background electromyograph (EMG) activity from the six NOSTIM trials was subjected to separate 2 × 2 (genotype × treatment) ANOVAs. Startle data from the 12 pulse-alone trials were collapsed in four blocks of three trials each and the means of each block were subjected to a 2 × 2 × 4 (genotype × treatment × block) ANOVA. The maximum amplitudes of the raw EMG responses from each trial were averaged across all trials of the same type. Percentage PPI ([(Amplitude$_{\text{pulse-alone}}$ − Amplitude$_{\text{prepulse–pulse}}$)/Amplitude$_{\text{pulse-alone}}$] × 100) was analyzed with 2 × 2 × 2 × 3 (genotype × treatment × prepulse × interval) ANOVA. Performance variables from the neuropsychological tests were analyzed using ANOVAs with genotype as the between-subject and treatment, difficulty level, and order (drug then placebo or placebo then drug) as the within-subject factors. All repeated measures with more than 1 degree of freedom were Greenhouse-Geisser corrected, with the corrected p and the ε-values reported. Effect sizes (partial η^2) were also reported. Since group comparisons were planned and hypothesis-driven, we did not consider Bonferroni (or false discovery rate [FDR]) correction of the threshold of statistical significance was necessary.

Table 1. Demographic Characteristics (Mean ± SD) for the Two Genotype Groups Under the Two Treatment Conditions

	G/G ($n = 13$)	C/C ($n = 12$)	F	p
Age (Years)	24.6 ± 4.1	24.6 ± 2.9	<1	>.1
Education (Years)	16.9 ± 2.6	17.3 ± 1.9	<1	>.1
IQ	107.7 ± 8.3	110.4 ± 8.1	<1	>.1
Smokers/Nonsmokers	7/6	6/6	$x^2 = .04$	<.1

IQ, intelligence quotient.

Results

One G/G and two C/C individuals failed to complete the study and were excluded from the analyses. There were no differences in age, years of education, IQ, and smoking status between the two genotype groups (Table 1).

Subjective Mood

Figure 1 shows the pretreatment and posttreatment scores in the seven POMS items for the two genotype groups. The C/C group presented with higher fatigue [$F_{\text{group}}(1,23) = 4.89$, $p < .05$], tension [$F_{\text{group}}(1,23) = 4.7$, $p < .05$], confusion [$F_{\text{group}}(1,23) = 5.61$, $p < .05$], and mood disturbance [$F_{\text{group}}(1,23) = 3.94$, $p = .058$] than the G/G homozygotes. Separate 2 × 2 (treatment × genotype) ANOVAs of the Δscores in the POMS ratings did not reveal significant main effects of treatment, genotype, or interaction (all $p > .06$), although tolcapone tended to increase anger in the C/C with the opposite effect in the G/G group [treatment by genotype interaction: $F(1,23) = 3.23$, $p = .086$].

Basal EMG and Startle Characteristics

There were no genotype or treatment main effects or interaction on background EMG, startle onset, or peak latencies (all $p > .1$) (Table 2). The 2 × 2 × 4 (treatment × genotype × block) ANOVA of startle in pulse-alone trials showed significant main effects of block indicating habituation [$F(3,66) = 4.99$, $p < .01$, $\varepsilon = .716$, $\eta^2 = .18$] and genotype [$F(1,22) = 5.1$, $p < .05$, $\eta^2 = .187$] with the C/C group presenting with higher startle amplitude (Table 2) (all other $p > .2$). These results were not altered when smoking status was included as an additional between-subject factor or when Δanger scores were taken as the covariates. However, the effect of genotype on startle was no longer significant [$F(1,20) = 3.4$, $p = .08$, $\eta^2 = .146$] after covarying for pretreatment fatigue levels, suggesting that higher startle in the C/C genotype could be attributed to higher fatigue.

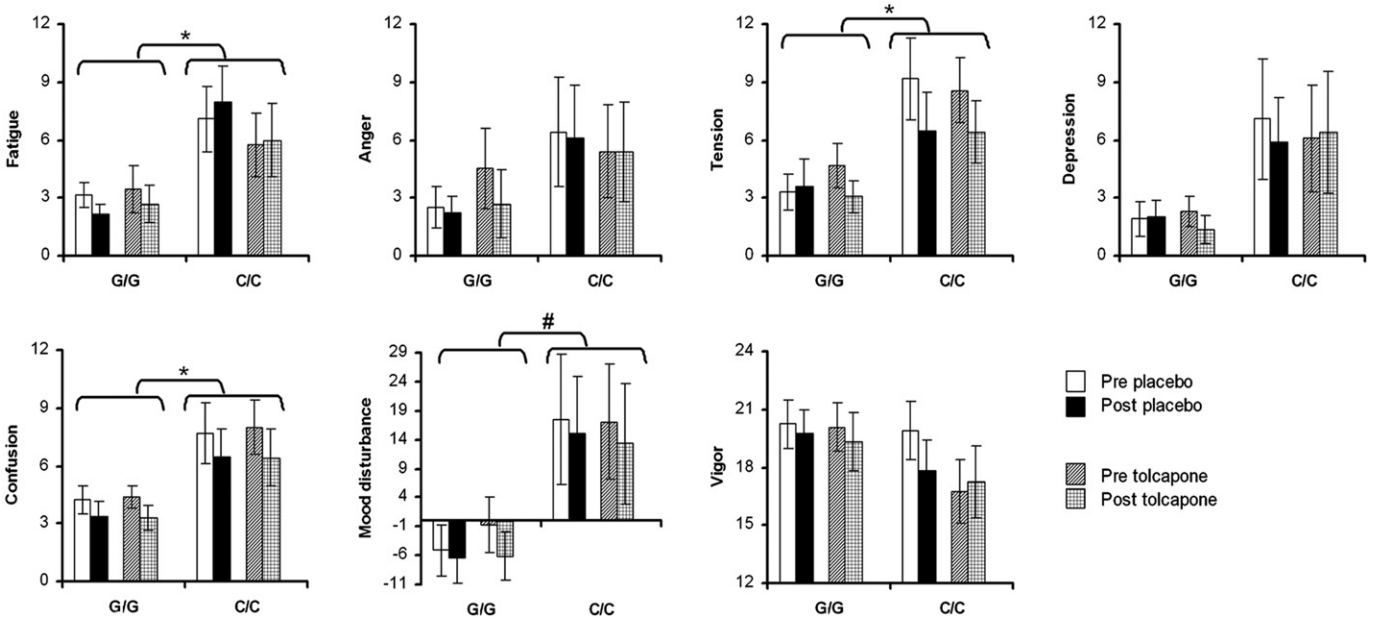

Figure 1. Pretreatment and posttreatment ratings of the Profile of Mood States items for the two genotype groups under the two treatment conditions. Bars represent SEM. Please note the difference in scale for Mood disturbance and Vigor. *$p < .05$ after a 2 × 2 × 2 (group [CC, GG] × treatment [placebo, tolcapone] × occasion [pretreatment, posttreatment]) analysis of variance comparison.

Because we had previously hypothesized that tolcapone-induced increase in baseline startle in met158 homozygotes may have been due to tolcapone-induced dysphoric mood in those subjects (26), we constructed a series of regression analyses to explore whether variance in tolcapone-induced mood changes independently contributed to variance in tolcapone-induced effects on baseline startle. For each separate genotype group, we entered the Δpulse-alone score as the dependent variable and the pure Δ effects of tolcapone (defined as the placebo-tolcapone difference of the Δ [pretreatment and posttreatment] scores) on each POMS measure in a forward regression analysis. This analysis showed that in the C/C group only, the effect of tolcapone on tension predicted significantly ($t = 2.9$, $p < .02$) 46.1% of the variance in the tolcapone-induced increase in baseline startle, while the tolcapone effects on tension, fatigue, and confusion together predicted 64% of the variance in tolcapone-induced increase in baseline startle. Table S1 in Supplement 1 shows the Pearson's correlations between pure Δscores in each POMS measure and Δpulse-alone in the C/C and G/G groups. Baseline startle in the placebo or tolcapone condition did not correlate significantly with any of the pretreatment or posttreatment POMS ratings in any genotype group or the entire sample (all $p > .1$).

Prepulse Inhibition

Figure 2 shows that, compared with placebo, tolcapone had opposite effects on percentage PPI in the two genotypes, confirmed by a significant treatment × genotype interaction [$F(1,23) = 6.9$, $p < .05$, $\eta^2 = .23$] in the overall 2 × 2 × 2 × 3 (genotype × treatment × prepulse × interval) ANOVA. There were also significant main effects of prepulse [$F(1,23) = 38.1$, $p < .001$, $\eta^2 = .624$] and interval [$F(2,46) = 40.1$, $p < .001$; $\varepsilon = .791$, $\eta^2 = .636$] (all other $p > .2$). None of the effects above was altered when smoking status was included as an additional between-subject factor or the pretreatment fatigue or the Δanger scores were taken as the covariates. Follow-up of the significant treatment × genotype interaction revealed that tolcapone significantly increased PPI in the G/G [$F_{\text{treatment}}(1,12) = 5.43$, $p < .05$, $\eta^2 = .311$] but had no effect in the C/C group [$F_{\text{treatment}}(1,11) = 1.8$, $p > .2$]. The C/C had higher % PPI in the placebo condition compared with the G/G group (Figure 2) as confirmed by a 2 × 2 × 3 (genotype × prepulse × interval) ANOVA for the placebo data only [$F_{\text{genotype}}(1,23) = 5.47$, $p < .05$, $\eta^2 = .192$]. Figure S1 in Supplement 1 shows higher percentage PPI in a CC subgroup matched for baseline startle with the GG group (see Supplement 1 for details).

Table 2. Startle Characteristics (Mean ± SD) for the Two Genotype Groups Under the Two Treatment Conditions

	G/G		C/C	
	Placebo	Tolcapone	Placebo	Tolcapone
Background EMG Activity (μV)	13.6 ± 5.5	14.0 ± 3.4	13.9 ± 5.7	12.3 ± 3.9
Mean Baseline Startle (μV)	214.4 ± 125	243.5 ± 120	402.5 ± 237[a]	409.9 ± 234[a]
Startle Onset Latency (msec)	44.4 ± 4.9	44.6 ± 4.9	41.9 ± 5.2	41.7 ± 5.3
Startle Peak Latency (msec)	61.4 ± 3.9	61.5 ± 2.9	61.9 ± 3.7	62.3 ± 3.9

EMG, electromyography.
[a]Significant difference from G/G group $p < .05$.

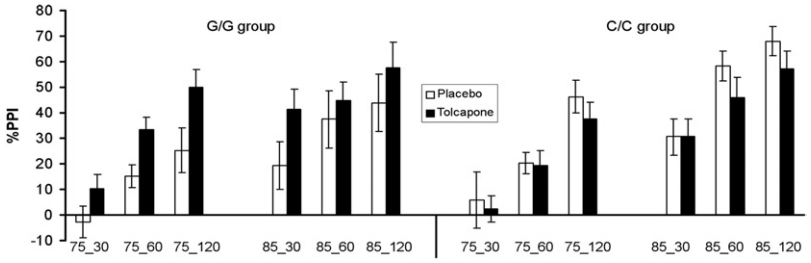

Figure 2. Percent prepulse inhibition (%PPI) for the two genotype groups under the two treatment conditions. Bars represent SEM. PPI, prepulse inhibition.

Neuropsychological Tests

N-Back Reaction Times. The overall 2 × 2 × 2 × 3 (genotype × treatment × order × difficulty) ANOVA revealed significant main effects of difficulty level [$F(2,42) = 3.6$, $p < .05$, $\varepsilon = .917$, $\eta^2 = .148$] and treatment [$F(1,21) = 10.0$, $p < .01$, $\eta^2 = .323$] and a significant treatment by genotype interaction [$F(1,21) = 4.6$, $p < .05$, $\eta^2 = .179$], which remained significant after covarying for IQ [$F(1,20) = 4.4$, $p < .05$] but not for pretreatment fatigue levels [$F(1,19) = 1.9$, $p > .1$] (all other $p > .1$). Follow-up ANOVAs confirmed a significant treatment effect in the G/G [$F(1,11) = 10.9$, $p < .01$, $\eta^2 = .498$] but not in the C/C group ($F < 1$) (Figure 3, top); in the latter, only the treatment by difficulty interaction was significant, confirming some improvement in the 3-back condition with tolcapone. There was a trend for shorter reaction times in the C/C Group ($p = .07$).

Correct Responses. We found only a significant difficulty main effect [$F(2,42) = 5.0$, $p < .05$, $\varepsilon = .734$, $\eta^2 = .193$] (all other $p > .08$). Accuracy was not different between the two genotypes in the placebo condition.

Letter Number Sequencing. The overall 2 × 2 × 2 × 7 (genotype × treatment × order × difficulty) ANOVA of the accuracy data revealed significant main effects of difficulty [$F(6,126) = 217.27$, $p < .000$, $\varepsilon = .462$, $\eta^2 = .912$] and treatment [$F(1,21) = 4.62$, $p < .05$, $\eta^2 = .180$] and significant treatment × genotype interaction [$F(1,21) = 5.43$, $p < .05$, $\eta^2 = .206$], which remained significant after covarying for IQ [$F(1,20) = 7.4$, $p < .02$] but not for pretreatment fatigue levels [$F(1.19 = 2.6$, $p > .1$] (all other $p > .1$). Follow-up of the interaction confirmed a significant treatment effect in the G/G [$F(1,11) = 6.83$, $p < .05$, $\eta^2 = .383$] but not in the C/C group ($F < 1$) (Figure 3, bottom). Accuracy in the placebo condition was not different between the two genotype groups ($F = 1.1$, $p > .3$).

Details on order effects are provided in Supplement 1. Multiple regression analyses showed that in the GG group, but

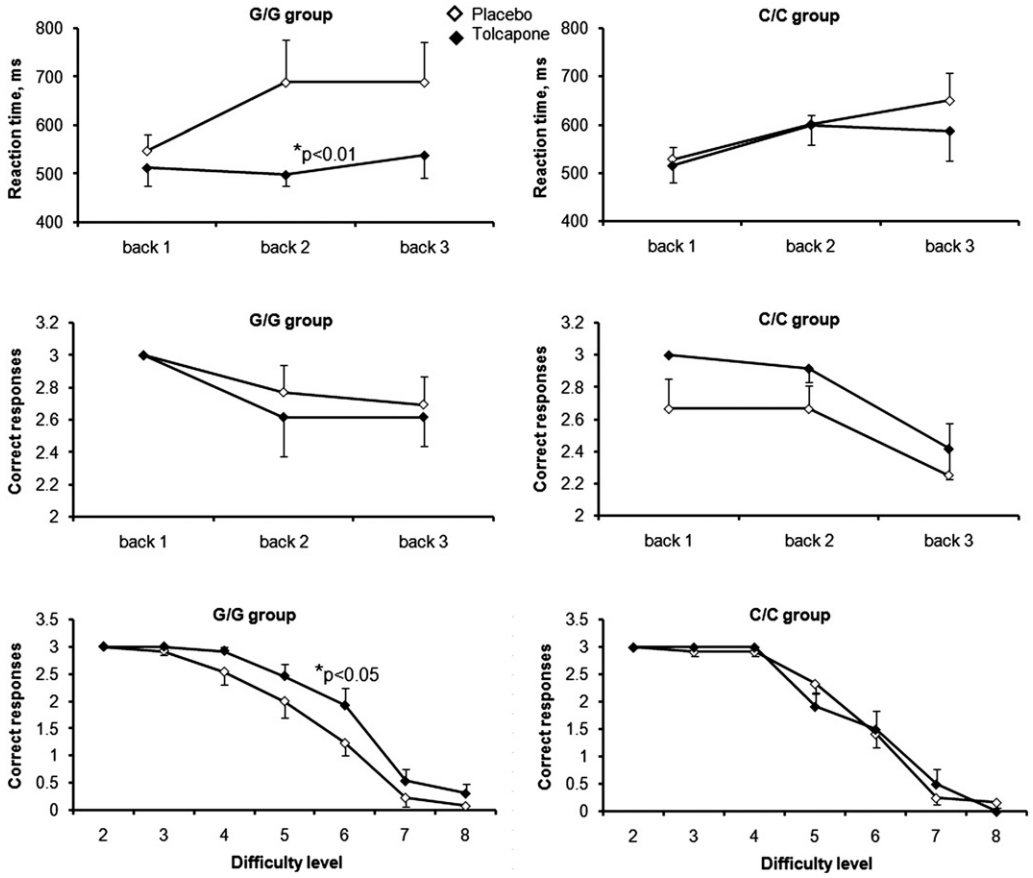

Figure 3. Reaction time (top) and correct responses (middle) for the n-back task and correct responses for the Letter Number Sequencing (bottom) for the two genotype groups under the two treatment conditions. Bars represent SEM.

Table 3. Correlation Matrix Between Placebo-Tolcapone Difference (Δ) Scores of Both Working Memory Tasks and %PPI at Six Trial Types, as Well as ΔStartle Amplitude in Pulse-Alone Trials, for the G/G Genotype Group

	75–30	75–60	75–120	85–30	85–60	85–120	Pulse-Alone
LNS	.224	.515[a]	.306	.637[b]	.575[b]	.371	.044
n-Back RT	−.050	−.252	−.262	−.148	−.196	−.147	.037

Values represent Pearson's correlation coefficients.

LNS, Letter Number Sequencing (total correct strings); n-back RT, n-back total reaction time; PPI, prepulse inhibition.

[a] $p < .008$.

[b] $p < .003$ [p correction for accumulation of alpha error (.05:7) = .00714].

not the CC group, tolcapone-induced PPI changes significantly predicted tolcapone-induced performance improvements in the LNS but not in the n-back (Supplement 1). Table 3 shows the Pearson's correlations between placebo-tolcapone difference (**Δ**) scores of both working memory tasks and percentage PPI at six trial types, as well as Δstartle amplitude in pulse-alone trials for the G/G genotype group.

Effects of rs4818 Versus rs4680

To investigate whether the effects of rs4818 were independent of rs4680 polymorphism, we determined the val158met status of our subjects. All GG individuals were val158 homozygotes, while from the CC subjects, five were met158 homozygotes, six were val/met, and one CC subject was val/val. To examine if the rs4680 status adds a differential effect in the C/C rs4818 group, we conducted a statistical comparison between C-met/met individuals and C-val carriers. Figure S2 in Supplement 1 shows that PPI in the placebo condition was greater in the C/val compared with the C/met group (left) [$F_{group}(1,10) = 33.9$, $p < .001$, $\eta^2 = .790$], while tolcapone-induced PPI reduction (right) was more profound in the C/val group [$F_{group}(1,10) = 11.5$, $p < .008$, $\eta^2 = .560$]. The C/val group also presented with greater startle increase and deterioration in mood (anger and tension items) and LNS performance after tolcapone, although none of these effects reached significance ($.1 < p < .14$).

Discussion

This is the first study to show that the rs4818 COMT polymorphism has an effect on human PPI and working memory and is an important determinant of tolcapone effects on these functions. More specifically, we found that G/G subjects, who presumably have suboptimal PFC DA levels, have lower PPI than C/C homozygotes in the placebo condition. Catechol-*O*-methyltransferase antagonism by tolcapone significantly increased PPI in the former and tended to have the opposite effect in the latter group. Genotype and tolcapone effects were evident on PPI at both prepulses at short (30 msec) through to longer (120 msec) intervals. These results are in perfect homology to previous findings with the val158met polymorphism (21,26). Catechol-*O*-methyltransferase inhibition by tolcapone also improved performance in both working memory tests in G/G but not in C/C homozygotes, in perfect symmetry with its effects on PPI. The regressions showed that tolcapone-induced improvements on working memory and PPI in G/G homozygotes correlated considerably, as they had previously in val/val subjects (26).

In the placebo condition, the C/C subjects were faster in the n-back compared with the G/G homozygotes for similar accuracy of responses. Although this effect was weak, it is toward the expected direction, extending the influence of this polymorphism from problem solving and decision making (34) to working memory. These results highlight the functional implication that synonymous polymorphisms might have in the complex process of human cognition and confirm the importance of high PFC DA levels on working memory (26,27,40,41). Accuracy does not appear to be as sensitive as reaction time or PPI to the effects of genotypes impacting PFC DA levels (rs4818 [present study] or val158met [26,27]), despite the well-documented effects of the latter in prefrontal efficiency (40,41). This apparent insensitivity of the accuracy measure agrees with previous reports (26,27) and may be due to an built-in ceiling effect on performance, since we used well-functioning healthy volunteers (but see discussion below).

After the functional val158met, the synonymous rs4818 COMT polymorphism reported here is the second COMT polymorphism determining PPI levels and working memory performance, as well as tolcapone's effects on these functions. Although the rs4818 is a synonymous polymorphism, it tags a functional haplotype that also includes the rs4680; Nackley *et al.* (3) suggested that the G versus C allele of the rs4818 tag a greater variability in the COMT activity compared with that of the val versus met changes of the rs4680. We were able to present large PPI differences between the C/met versus C/val groups, supporting the model as proposed by Nackley *et al.* (3). However, this should be considered preliminary evidence, as small numbers prohibit a comprehensive haplotype analysis. It is thus crucial for future studies to examine the effect of haplotypes instead of separately examining the effects of the rs4680 and rs4818 polymorphisms. Nevertheless, a rather consistent story is beginning to emerge with the following being its major points. High PFC DA levels are associated with higher PPI (this study, [21,26]) and better working memory (26,27,40,41). Under conditions of COMT inhibition, PPI and working memory of subjects with suboptimal PFC DA improve, while in subjects with high PFC DA they tend to deteriorate (this study, [26,27]). The robust treatment by genotype effect observed in both PPI and working memory is consistent with relatively lower PFC DA signaling in G/G (and val/val) subjects and presumed benefit of enhanced PFC DA in this context (40–44). The above support an inverted U-shaped relationship between PFC DA and working memory on the one hand and PFC DA and PPI on the other. The latter may underlie the finding that baseline PPI levels are important for the effect of dopaminergic drugs, as has been previously highlighted by different research groups (17,37,45,46). The mechanism for working memory, a PFC-mediated function, has been postulated to be improved signal-to-noise ratio within the PFC (41), while for PPI, a phenomenon mediated by subcortical mesolimbic structures, the mechanism is not yet elucidated but it could involve downstream effects of high tonic PFC DA levels on phasic DA release in the nucleus accumbens, which is known to mediate PPI ([47,48]; see [26] for a full discussion). Nevertheless, PPI and working memory improvements seem to go hand in hand, possibly due to partially overlapping circuits, one common

link being improvement in PFC DA levels. All the above support previous claims that the PFC influences PPI levels and by inference the early stages of attentional processing (15,16), However, the possibility for reciprocity also remains, i.e., that efficient preattentive perceptual processing, as reflected in efficient gating (improved PPI) may facilitate attentional allocation to sensory inputs upstream in the PFC and by extension may optimize PFC function, e.g., working memory. This latter possibility resonates with the classical view that deficient gating may cause sensory overload and fragmentation of higher cognitive functions (49). Regardless of mechanisms involved, these findings have therapeutic implications in conditions with deficient gating and working memory, such as schizophrenia, especially in patients with high COMT activity (e.g., homozygotes of the G-val haplotype).

Our findings cannot be attributed to differences in gender, age, education, IQ, smoking status, or basal EMG activity, since the groups did not differ in this respect. We also tested and ruled out potential confounding effects from mood on the day of testing and baseline startle. In agreement with previous reports (26,27), tolcapone had no effect activation and mood, suggesting that its beneficial effects on PPI and cognition would be expected to occur in the absence of psychostimulant effects and abuse potential.

Tolcapone increased startle reactivity arithmetically but not significantly in both genotype groups. This is in contrast to our previous study where tolcapone had significantly increased startle in the C/C homologous, met158 homozygotes (26). It is possible that a ceiling effect may underlie this discrepancy, as in the present study startle was considerably greater in the high PFC DA C/C subjects in the placebo condition. Startle increases during negative mood states (50) and high PFC DA met158 homozygotes have reduced resilience to negative mood (51–53). It is therefore possible that high startle in the C/C group may reflect higher negative mood, especially in view of their worse POMS profile and greater fatigue, which accounted for the genotype difference in startle as evidenced by the analysis of covariance (ANCOVA). It appears that compared with G/G subjects, C/C individuals were generally more dysphoric and less enthusiastic before the experimental session and tolcapone worsened their mood, as evidenced by the nearly significant drug by group interaction in the anger item of the POMS. In the absence of significant tolcapone effects on mood or startle, it is all the more important that in the C/C but not the low PFC DA G/G subjects, tolcapone-induced increase in startle correlated highly with the (also nonsignificant) tolcapone-induced increases in negative mood as evidenced by the regressions. We had not found such an obvious genotype effect on startle reactivity and mood in our previous study with met158 subjects (26), which suggests that either this effect is not very robust or, more interestingly, that C/C homozygotes may be more prone to these effects as they have greater PFC DA levels than met158 subjects (3). It is relevant here that while high PFC DA C/C homozygotes have superior problem-solving abilities compared with G/G homozygotes, they perform worse in emotional decision-making tasks where mood state becomes important (34). It seems that a reason for the high frequency in the general population of the cognitively disadvantaged G and val alleles of the rs4818 and val158met COMT polymorphisms, respectively, is that the G/G and val/val homozygotes (who presumably have suboptimal PFC DA levels) compensate with better integration of emotional stimuli; this may assist them with more flexible decision making, emotional resilience against negative and dysphoric mood (54), and possibly greater adaptability in social settings.

Another interesting aspect of these results is that genotype differences in mood/motivation on the day of testing may have affected engagement, and thus performance, in working memory tasks. Greater fatigue in C/C subjects would be expected to reduce their putatively superior working memory task performance in the placebo condition, thus masking a theoretically expected genotype main effect in this condition, at least in the less sensitive accuracy measure. It is interesting that compared with G/G individuals, C/C subjects in the placebo condition presented with (arithmetically) lower accuracy in the n-back. Moreover, improved 3-back reaction time with tolcapone in the C/C group (evidenced by significant treatment by difficulty interaction) could be construed as impulsive responding, as it was not accompanied by accuracy improvements. It is also interesting that regressions (not shown) revealed that 34% and 26% of the variance in the n-back and LNS, respectively, in the placebo condition in the C/C group were predicted by POMS vigor and mood disturbance scores. The n-back might be more prone to such motivational influences compared with the more engaging LNS, which requires not only to store information but also to reorder it according to preset rules. The possibility that intolerance of negative mood in high PFC DA subjects (51–53) could reduce motivation and thus interfere with performance could explain why in some studies high PFC DA met158 homozygotes were, opposite to prediction, no better or even worse in the n-back (55) or in the antisaccade task (56), which also requires engagement. Passive or unattended PPI would be relatively immune to such motivational influences, and in this context, PPI may be a unique tool for the study of COMT effects on task engagement, as it can be elicited under both passive unattended (no task engagement required) and active controlled conditions (task engagement required). Future studies should test these issues with a priori hypotheses.

This is the first study to show that the synonymous rs4818 COMT polymorphism has an effect on human PPI, working memory, and probably mood and determines the effects of tolcapone on these functions. Importantly, these effects may be additional to those of the rs4680. The study adds evidence to the consistently emerging story of COMT activity affecting gating, cognition, and mood. Because of small samples, one should always be cautious for type I, or in fact, given our large effect sizes, type II error. Therefore, caution is required until these results can be confirmed in larger populations examining the effect of COMT haplotypes affecting enzyme expression on PPI, cognition, and mood. It would also be important to examine the interaction of COMT haplotypes with tolcapone effects on PPI, cognition, and mood in psychotic patients and high-risk subjects. Given the importance of gating/cognitive deficits in the etiopathogenesis and course of psychotic and prodromal (57) or high schizotaxia states (58), our studies suggest promising, genotype-specific therapeutic implications.

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Supplementary material cited in this article is available online.

1. Harrison PJ, Weinberger DR (2005): Schizophrenia genes, gene expression, and neuropathology: On the matter of their convergence. *Mol Psychiatry* 10:40–68.
2. Tunbridge EM, Harrison PJ, Weinberger DR (2006): Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biol Psychiatry* 60:141–151.
3. Nackley AG, Shabalina SA, Tchivileva IE, Satterfield K, Korchynskyi O, Makarov SS, *et al.* (2006): Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* 314:1930–1933.
4. Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ, *et al.* (2008): Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: The SzGene database. *Nat Genet* 40:827–834.
5. Braff D, Stone C, Callaway E, Geyer M, Glick I, Bali L (1978): Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology* 15:339–343.
6. Swerdlow NR, Geyer MA, Braff DL (2001): Neural circuit regulation of prepulse inhibition of startle in the rat: Current knowledge and future challenges. *Psychopharmacology (Berl)* 156:194–215.
7. Swerdlow N, Caine S, Braff D, Geyer M (1992): Neural substrates of sensorimotor gating of the startle reflex: A review of recent findings and their implications. *J Psychopharmacol* 6:176–190.
8. Kumari V, Gray JA, Geyer MA, Ffytche D, Soni W, Mitterschiffthaler MT, *et al.* (2003): Neural correlates of tactile prepulse inhibition: A functional MRI study in normal and schizophrenic subjects. *Psychiatry Res* 122:99–113.
9. Kumari V, Das M, Zachariah E, Ettinger U, Sharma T (2005): Reduced prepulse inhibition in unaffected siblings of schizophrenia patients. *Psychophysiology* 42:588–594.
10. Kumari V, Antonova E, Geyer MA, Ffytche D, Williams SC, Sharma T (2007): A fMRI investigation of startle gating deficits in schizophrenia patients treated with typical or atypical antipsychotics. *Int J Neuropsychopharmacol* 10:463–477.
11. Postma P, Gray JA, Sharma T, Geyer M, Mehrotra R, Das M, *et al.* (2006): A behavioural and functional neuroimaging investigation into the effects of nicotine on sensorimotor gating in healthy subjects and persons with schizophrenia. *Psychopharmacology (Berl)* 184:589–599.
12. Campbell LE, Hughes M, Budd TW, Cooper G, Fulham WR, Karayanidis F, *et al.* (2007): Primary and secondary neural networks of auditory prepulse inhibition: A functional magnetic resonance imaging study of sensorimotor gating of the human acoustic startle response. *Eur J Neurosci* 26:2327–2333.
13. Geyer MA, Swerdlow NR, Mansbach RS, Braff DL (1990): Startle response models of sensorimotor gating and habituation deficits in schizophrenia. *Brain Res Bull* 25:485–498.
14. Bitsios P, Giakoumaki SG (2005): Relationship of prepulse inhibition of the startle reflex to attentional and executive mechanisms in man. *Int J Psychophysiol* 55:229–241.
15. Bitsios P, Giakoumaki SG, Theou K, Frangou S (2006): Increased prepulse inhibition of the acoustic startle response is associated with better strategy formation and execution times in healthy males. *Neuropsychologia* 44:2494–2499.
16. Giakoumaki SG, Bitsios P, Frangou S (2006): The level of prepulse inhibition in healthy individuals may index cortical modulation of early information processing. *Brain Res* 1078:168–170.
17. Csomor PA, Stadler RR, Feldon J, Yee BK, Geyer MA, Vollenweider FX (2008): Haloperidol differentially modulates prepulse inhibition and p50 suppression in healthy humans stratified for low and high gating levels. *Neuropsychopharmacology* 33:497–512.
18. Kumari V, Fannon D, Sumich AL, Sharma T (2007): Startle gating in antipsychotic-naive first episode schizophrenia patients: One ear is better than two. *Psychiatry Res* 151:21–28.
19. Braff DL, Geyer MA, Light GA, Sprock J, Perry W, Cadenhead KS, *et al.* (2001): Impact of prepulse characteristics on the detection of sensorimotor gating deficits in schizophrenia. *Schizophr Res* 49:171–178.
20. Swerdlow NR, Light GA, Cadenhead KS, Sprock J, Hsieh MH, Braff DL (2006): Startle gating deficits in a large cohort of patients with schizophrenia: Relationship to medications, symptoms, neurocognition, and level of function. *Arch Gen Psychiatry* 63:1325–1335.
21. Roussos P, Giakoumaki SG, Rogdaki M, Pavlakis S, Frangou S, Bitsios P (2008): Prepulse inhibition of the startle reflex depends on the catechol-O-methyltransferase Val158Met gene polymorphism. *Psychol Med* 38:1651–1658.
22. Quednow BB, Wagner M, Mossner R, Maier W, Kuhn KU (2008): Sensorimotor gating of schizophrenia patients depends on catechol O-methyltransferase Val158Met polymorphism. *Schizophr Bull*.
23. Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, *et al.* (1998): Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc Natl Acad Sci U S A* 95:9991–9996.
24. Huotari M, Gogos JA, Karayiorgou M, Koponen O, Forsberg M, Raasmaja A, *et al.* (2002): Brain catecholamine metabolism in catechol-O-methyltransferase (COMT)-deficient mice. *Eur J Neurosci* 15:246–256.
25. 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.
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. Apud JA, Mattay V, Chen J, Kolachana BS, Callicott JH, Rasetti R, *et al.* (2007): Tolcapone improves cognition and cortical information processing in normal human subjects. *Neuropsychopharmacology* 32:1011–1020.
28. Goldman-Rakic PS (1998): The cortical dopamine system: Role in memory and cognition. *Adv Pharmacol* 42:707–711.
29. Williams GV, Goldman-Rakic PS (1995): Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature* 376:572–575.
30. Swerdlow NR, Auerbach P, Monroe SM, Hartston H, Geyer MA, Braff DL (1993): Men are more inhibited than women by weak prepulses. *Biol Psychiatry* 34:253–260.
31. Swerdlow NR, Hartman PL, Auerbach PP (1997): Changes in sensorimotor inhibition across the menstrual cycle: Implications for neuropsychiatric disorders. *Biol Psychiatry* 41:452–460.
32. Harrison PJ, Tunbridge 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.
33. Montag C, Hartmann P, Merz M, Burk C, Reuter M (2008): D2 receptor density and prepulse inhibition in humans: Negative findings from a molecular genetic approach. *Behav Brain Res* 187:428–432.
34. Roussos P, Giakoumaki SG, Pavlakis S, Bitsios P (2008): Planning, decision-making and the COMT rs4818 polymorphism in healthy males. *Neuropsychologia* 46:757–763.
35. Hardman J, Limbird L, Gilman AG (2001): *The Pharmacological Basis of Therapeutics*. New York: McGraw-Hill.
36. McNair D, Lorr M (1981): *ITS Manual for the Profile of Mood States*. San Diego: Multi-health System, Inc.
37. Bitsios P, Giakoumaki SG, Frangou S (2005): The effects of dopamine agonists on prepulse inhibition in healthy men depend on baseline PPI values. *Psychopharmacology (Berl)* 182:144–152.
38. Frangou S, Kington J, Raymont V, Shergill SS (2008): Examining ventral and dorsal prefrontal function in bipolar disorder: A functional magnetic resonance imaging study. *Eur Psychiatry* 23:300–308.
39. Wechsler D (1997): *Wechsler Adult Intelligence Scale-Third Revision (WAISIII)*. San Antonio, TX: Psychological Corporation.
40. 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.
41. Winterer G, Egan MF, Kolachana BS, Goldberg TE, Coppola R, Weinberger DR (2006): Prefrontal electrophysiologic "noise" and catechol-O-methyltransferase genotype in schizophrenia. *Biol Psychiatry* 60:578–584.
42. Weinberger DR, Egan MF, Bertolino A, Callicott JH, Mattay VS, Lipska BK, *et al.* (2001): Prefrontal neurons and the genetics of schizophrenia. *Biol Psychiatry* 50:825–844.
43. 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.
44. Winterer G, Goldman D (2003): Genetics of human prefrontal function. *Brain Res Brain Res Rev* 43:134–163.
45. Swerdlow NR, Stephany N, Wasserman LC, Talledo J, Shoemaker J, Auerbach PP (2003): Amphetamine effects on prepulse inhibition across-spe-

cies: Replication and parametric extension. *Neuropsychopharmacology* 28:640–650.
46. Talledo JA, Sutherland Owens AN, Schortinghuis T, Swerdlow NR (2009): Amphetamine effects on startle gating in normal women and female rats. *Psychopharmacology (Berl)* 204:165–175.
47. Grace AA (1993): Cortical regulation of subcortical dopamine systems and its possible relevance to schizophrenia. *J Neural Transm* 91:111–134.
48. Bilder RM, Volavka J, Lachman HM, Grace AA (2004): The catechol-O-methyltransferase polymorphism: Relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. *Neuropsychopharmacology* 29:1943–1961.
49. Braff DL, Geyer MA (1990): Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch Gen Psychiatry* 47:181–188.
50. Lang PJ, Davis M (2006): Emotion, motivation, and the brain: Reflex foundations in animal and human research. *Prog Brain Res* 156:3–29.
51. Smolka MN, Schumann G, Wrase J, Grusser SM, Flor H, Mann K, *et al.* (2005): Catechol-O-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *J Neurosci* 25:836–842.
52. Drabant EM, Hariri AR, Meyer-Lindenberg A, Munoz KE, Mattay VS, Kolachana BS, *et al.* (2006): Catechol O-methyltransferase val158met genotype and neural mechanisms related to affective arousal and regulation. *Arch Gen Psychiatry* 63:1396–1406.
53. Weiss EM, Stadelmann E, Kohler CG, Brensinger CM, Nolan KA, Oberacher H, *et al.* (2007): Differential effect of catechol-O-methyltransferase Val158Met genotype on emotional recognition abilities in healthy men and women. *J Int Neuropsychol Soc* 13:881–887.
54. Enoch MA, Xu K, Ferro E, Harris CR, Goldman D (2003): Genetic origins of anxiety in women: A role for a functional catechol-O-methyltransferase polymorphism. *Psychiatr Genet* 13:33–41.
55. Ho BC, Wassink TH, O'Leary DS, Sheffield VC, Andreasen NC (2005): Catechol-O-methyltransferase Val158Met gene polymorphism in schizophrenia: Working memory, frontal lobe MRI morphology and frontal cerebral blood flow. *Mol Psychiatry* 10:229, 287–298.
56. Haraldsson HM, Ettinger U, Magnusdottir BB, Sigmundsson T, Sigurdsson E, Ingason A, *et al.* (2008): Catechol-O-methyltransferase Val158Met polymorphism and antisaccade eye movements in schizophrenia [published online ahead of print June 17]. *Schizophr Bull*.
57. Simon AE, Cattapan-Ludewig K, Zmilacher S, Arbach D, Gruber K, Dvorsky DN, *et al.* (2007): Cognitive functioning in the schizophrenia prodrome. *Schizophr Bull* 33:761–771.
58. Stone WS, Faraone SV, Seidman LJ, Green AI, Wojcik JD, Tsuang MT (2001): Concurrent validation of schizotaxia: A pilot study. *Biol Psychiatry* 50:434–440.