Independent Study Project

UCSD School of Medicine
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Catechol-O-methyltransferase val^{158}met (COMT) polymorphism modulates d-amphetamine effects on working memory

ABSTRACT

In order to guide amphetamine's effective use as a treatment, it would be helpful to identify biomarkers that predict its clinical effects. It has been shown in previous studies that there are inter-individual differences in amphetamine's effects on working memory. Expanding on a genetic target implicated in the literature, this experiment tested the hypothesis that the COMT val^{158}met polymorphism contributes to differences in sensitivity to the effects of d-amphetamine on working memory. 69 healthy subjects completed the MATRICS Consensus Cognitive Battery (MCCB) to test their neurocognitive performance after ingesting placebo or d-amphetamine (20 mg). Before and after order effect was corrected, amphetamine's effect on t-scores was inversely proportional to baseline scores in a significant manner on both the letter-number span (R=-0.411, p<0.0001) and the spatial span tasks (R=-0.430, p<0.0001); the lower the score on the placebo day, the more that amphetamine improved scores, and vice versa. Otherwise, there were no significant differences between genotypes or sex and MCCB t-scores. Permutation analyses could not rule out regression to the mean as a potential confound. In conclusion, this experiment was unable to support the hypothesis that COMT genotype is significantly associated with amphetamine's different effects on working memory. Other sample characteristics such as ethnicity may be complicating this relationship and can be examined in future studies.

INTRODUCTION

Dextroamphetamine is a stimulant that increases dopamine and norepinephrine availability in the brain by blocking their re-uptake through monoamine transporters (Robertson *et al*, 2009), and, at higher doses, by triggering their presynaptic release. It is used in the treatment of such disorders as Attention Deficit Hyperactivity Disorder, narcolepsy, and chronic fatigue syndrome due to its ability to increase alertness, modulate attention, and enhance cognitive performance (Mattay *et al*, 2003). Amphetamine also has positive hedonic properties that contribute to its liability for abuse.

There is significant inter-individual variability in amphetamine effects, particularly among its effects on cognition and brain function. For example, it has been shown that d-amphetamine enhances working memory (WM) among individuals with low basal WM performance, and deteriorates WM among individuals with high basal WM performance (Mattay *et al*, 2000). In addition, subjects performing WM tasks after ingesting amphetamine demonstrate heterogeneous patterns of BOLD fMRI activation in the prefrontal cortex that have been attributed to different polymorphisms of the gene for COMT.

COMT is an enzyme that metabolizes catecholamines in the central nervous system and periphery (Hariri, 2011). When methionine (nucleotide A) is substituted for valine (nucleotide G) at codon 158 (the SNP Rs4680), the enzyme becomes more sensitive to temperature and degrades, thus having decreased activity compared to the more stable valine form. With regards to dopamine, an individual carrying the Val/Val COMT genotype will have lower basal dopaminergic tone (due to higher COMT activity) than someone who carries the Met/Met COMT genotype at COMT-populated synapses (including those located in prefrontal cortex).

This study tested the hypothesis that amphetamine effects on WM in healthy individuals are dependent on the COMT val^{158}met polymorphism, and specifically, that amphetamine will predominantly enhance WM among subjects carrying the Val/Val genotype. This study used a repeatable neurocognitive battery - the MATRICS Consensus Cognitive Battery (MCCB) - to test a genetic and presumed

neurochemical explanation for inter-individual differences in the neurocognitive effects of amphetamine that may have important implications for amphetamine's therapeutic use and its abuse potential.

METHODS

Subjects – Participants were recruited via local advertising and screened by phone for major medical and psychiatric illnesses, recreational drug use, schizophrenia in a first-degree relative, a history of seizures, a history of head trauma with loss of consciousness for over a minute, hearing or vision problems, lack of English fluency, or current psychotropic medications, as in previous studies (Swerdlow *et al*, 2003). The study was described to potential participants both on the phone and in person, when they came to UCSD Medical Center to sign consent forms. During the in-person screening procedure, participants were interviewed using the Structured Clinical Interview for DSM IV Disorders (SCID) (First *et al*, 1996), in order to screen for mental illness, including current depression, current mania, any history of depression or manic episode, dysthymia, psychotic/schizophrenic symptoms, or substance abuse. Potential participants also received a physical examination, including electrocardiogram, to identify any potential medical exclusion criteria (Table 1). Subjects who met inclusion criteria returned for tests on two days, separated by 1 month. The target sample size for statistical analysis was 60 healthy, 18-35 year-old, right-handed adults (M:F = 1:1), based on power requirements for a beta = 0.8 and alpha = 0.05 (Cohen, 1988). The planned sample size of 60 reflected a predicted effect size (d) of 0.38; this value is based on the ability of amphetamine to increase prepulse inhibition (PPI) of acoustic startle in normal women with low basal PPI levels (Talledo *et al*, 2008). Assumptions underlying this prediction included: 1) the Val/Val COMT genotype is assumed to be over-represented in this low-PPI subgroup (Roussos *et al*, 2008); 2) increased PPI is significantly associated with higher levels of WM (Light *et al*, 2007); and 3) allelic frequencies for Val and Met in the present study sample approximate those in the published literature (Lipsky *et al*, 2005). Subjects were compensated a total of $275 distributed over 4 sessions (pre-screen, screen, testing day 1, and testing day 2). Breakfast and lunch were provided on test days.

CATEGORY	EXCLUSION CRITERIA
AGE	<18 or >35 years old
HANDEDNESS	Left
MEDICAL	Vision or hearing problems, major medical illness (diabetes, HIV, AIDS, cancer, stroke, heart attack), history of seizures, loss of consciousness >1 minute from head injury
MEDICATIONS	Psychotropics, stimulants
PSYCHIATRIC	Depression, mania, dysthymia, psychotic/schizophrenic symptoms, schizophrenia or bipolar disorder in a first-degree relative

Table 1. Exclusion criteria.

A pre-screening protocol was initiated during the study after the discovery that the minor allele frequencies for COMT in the current sample were different from frequencies in the published literature. The pre-screen consisted of collecting saliva samples from potential participants that were screened for genotypes. Only Val/Val or Met/Met subjects were included from this point in order to focus testing on the rarer homozygotes. As a result, 9 additional subjects were included in this analysis (n=69).

Design – In a double-blind, within-subject design, each subject received a placebo or 20 mg of amphetamine (Hutchinson and Swift, 1999); he or she was tested again with the alternate treatment 28-30 days later, to ensure comparable hormonal status in women (Talledo *et al*, 2008). Experimental design of the test days can be seen in Figure 1, and generally followed the schedule used in previous studies from this laboratory (Swerdlow *et al*, 2003). On both test days, subjects ate a standardized meal, provided a urine sample to test for recreational drugs and pregnancy, and completed a hearing test (to assess the ability to detect a 1000 hZ tone at 40 dB). The subject then consumed the placebo or active pill and completed testing as shown in Figure 1. The hearing test was used to establish auditory thresholds for measures of acoustic startle and prepulse inhibition ("Intervals" 1 and 2), which are not

described here. Heart rate, blood pressure, and symptom-rating measures were measured before pill consumption and at regular intervals thereafter. Of most relevance to this report, the MATRICS Consensus Cognitive Battery (MCCB) was administered to assess performance in a number of neurocognitive domains, including speed of processing, attention/vigilance, WM, verbal learning, visual learning, reasoning and problem solving, and social cognition (Nuechterlein & Green, 2006). After study completion, all subjects received venopuncture for validation of COMT status.

CHARACTERISTICS	MEAN (SD)

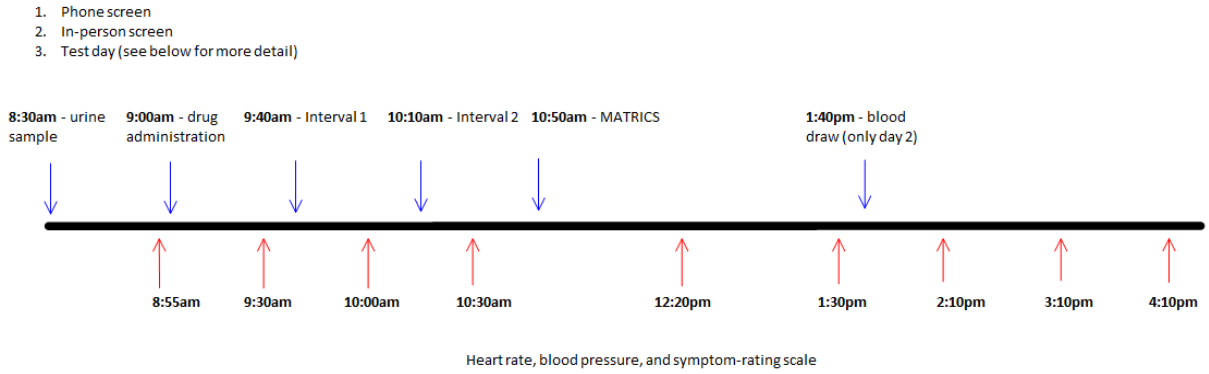

Figure 1. Timeline for both test days

Statistical analysis –To test the primary hypothesis, analysis of variance (ANOVA) and other statistics were utilized to compare amphetamine effects on MCCB performance, specifically on WM, among individuals carrying Val/Val vs. Met/Met COMT genotypes, using genotype as a between-subject factor and amphetamine dose as a within-subject factor. One concern with using repeatable tasks is that there can be a significant order effect that could independently improve day 2 scores simply due to repetition of the day 1 tasks. We countered this effect by calculating the mean difference between day 2 scores and day 1 scores, assuming that this mean order effect could be applied to every participant, and subtracting this difference from all day 2 scores. As described in prior papers from this lab,

SUBJECTS (n)	69
GENDER (n)	
Male	43
Female	26
AGE (y)	23.8 (4.7)
EDUCATION (y)	14.4 (1.5)
ETHNICITY (n)	
Asian	19
Black	3
Hispanic	21
White	23
Other	3
COMT ALLELES (n)	
Val/Met	33
Val/Val	28
Met/Met	8
ACTIVE SMOKER (n)	3

Table 2. Subject characteristics

additional analyses were done in order to explore whether a regression to the mean contributed to amphetamine effects on baseline scores (Chou *et al*, 2013). This takes place when subjects in a group (e.g. placebo) randomly achieve high or low scores on day 1 testing but then score closer to the true population mean on day 2 (amphetamine), creating a pattern that is not attributable to treatment effect alone (Barnett *et al*, 2005). Potential moderating variables that have been implicated in the literature (Swerdlow *et al*, 1993), including basal neurocognitive performance, sex, and test order, were assessed, as were physiological measures of amphetamine sensitivity.

RESULTS

With respect to demographics, our study population was primarily Asian, Hispanic, or white (Table 2). The male to female ratio was approximately 1.6:1 and the mean age was 23.8. An analysis of physiological measures demonstrated significant increases in heart rate (F=21.25, df 1,68, p<0.0001), diastolic blood pressure (F=26.43, df 1,68, p<0.0001), and systolic blood pressure (F=56.57, df 1,68, p<0.0001) after amphetamine ingestion (Figure 2). ANOVA of "drowsiness", the decrease of which was used to assess amphetamine's activity in the central nervous system, revealed a significant "drug week" by "time" interaction, with drug week and time after drug ingestion as within-subject factors (F=4.06, df 7,476, p<0.0005). Inspection of the data revealed consistent reductions in drowsiness with amphetamine beginning at the time point

prior to MCCB administration and continuing through the end of testing, which narrowly missed statistical significance (F=3.73, df 1,68, p<0.06); d=0.25). These results support that amphetamine was bioactive peripherally and centrally.

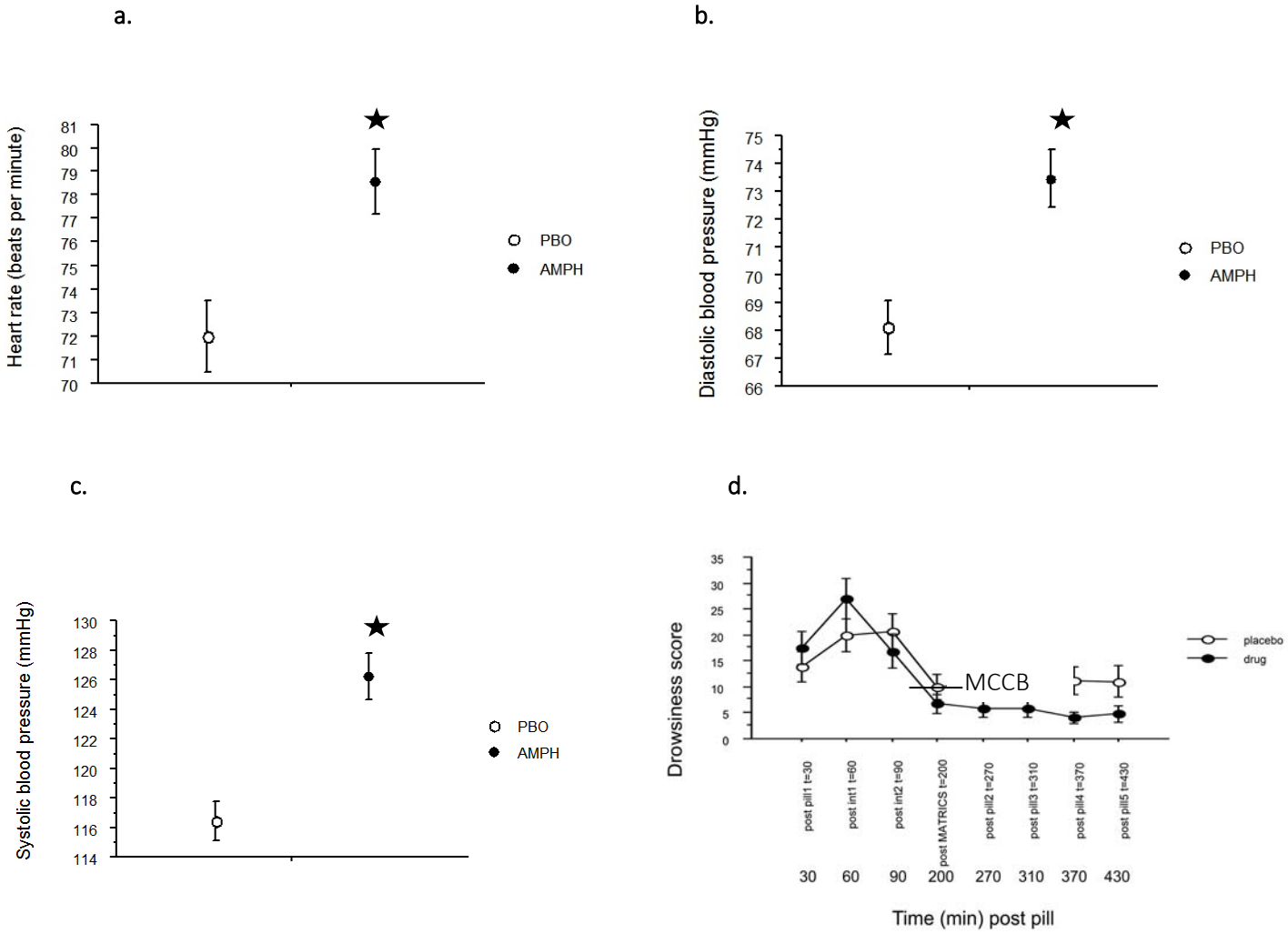

Figure 2: Effect of drug condition (placebo = PBO, amphetamine = AMPH) during MCCB performance (t=200min post pill) on heart rate **(a)**, diastolic blood pressure **(b)**, and systolic blood pressure **(c)**, with all changes being significant and demonstrating amphetamine bioactivity during WM tasks (*=p<0.0001). Effect of drug condition on self-reported drowsiness **(d)** throughout the entire test day, with notation of time point at which MCCB performance was assessed.

Mean t-scores for the placebo condition for both the letter-number span (LNS) task and the Weschler Memory Scale – III spatial span (SS) task were approximately 50. A statistically significant negative correlation was demonstrated between baseline (placebo) t-score for either WM task and the overall "amphetamine effect", calculated as taking the difference of placebo t-score from amphetamine t-

score (*R*=-0.411, *p*<0.0001 for LNS and *R*=-0.430, *p*<0.0001 for SS). In other words, subjects who tended to have lower baseline t-scores also tended to show improvement in their scores when amphetamine was administered, and vice versa (Figure 3). A closer look at a median split of subjects based on their baseline t-score performance for either WM task revealed that subjects on the lower half of baseline t-scores had significant improvements in t-scores after amphetamine ingestion compared to subjects on the higher half of baseline t-scores, who experienced a significant worsening in t-scores after amphetamine ingestion (*F*=7.193, *df* 1,68, *p*<0.01 for LNS and *F*=7.673, *df* 1,68, *p*<0.01 for SS). However, COMT genotype was not associated with any patterns of amphetamine-induced changes in either WM task t-scores (Figure 4).

a.

b.

c.

d.

Figure 3: Significant difference between amphetamine's effect on subjects with placebo t-scores below the median vs. above the median for both WM tasks (a,b). Significant negative correlation between amphetamine's effect and baseline t-score across all subjects (c,d).

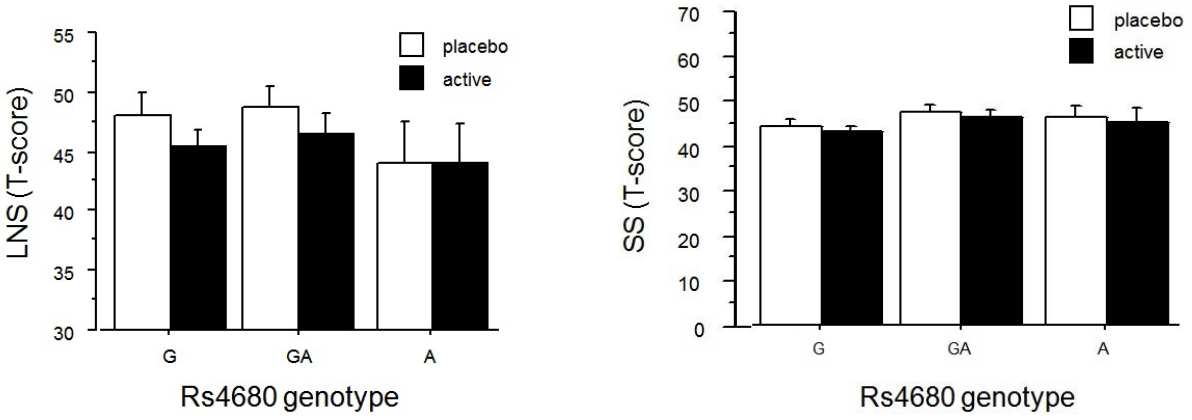

Figure 4: Effect of drug (placebo vs. 20 mg amphetamine) on WM tasks by COMT genotype (G=Val/Val, GA=Val/Met, A=Met/Met). There was no significant association between genotype and amphetamine-induced t-score changes.

There was a significant practice effect in which raw day 2 t-scores for the LNS task improved from day 1 independent of drug condition (F=10.009, df 1,68, p<0.01). As described previously in the methods section, a correction was applied to the raw data in order to counter this order effect. As with the adjusted data, though, there was no association between amphetamine-induced changes of raw t-scores with COMT genotype. Sex differences with respect to dopamine release and neurocognition have been

described in the literature also (Riccardi *et al*, 2011). In the present study, however, adding sex as a between-subject factor did not demonstrate any significant association with changes due to amphetamine nor was there a significant interaction with COMT genotype (*F's*<2).

DISCUSSION

The hypothesis that changes in WM in response to amphetamine are mediated by COMT genotype is not supported by these current data. While the data suggested that amphetamine was bioactive after ingestion, that there was no sample-specific test-taking aberrancy as seen from mean t-scores for the WM tasks, and that dopamine's effect on WM may demonstrate an inverted-U shape pattern as previously reported (Williams & Goldman-Rakic, 1995), no significant relationship was found between COMT genotype and amphetamine-induced changes in WM scores. Additionally, there is not enough evidence after permutation analyses to be able to exclude regression to the mean as a contributor to the baseline score-dependent amphetamine effects; other analyses with data from this study revealed associations of amphetamine sensitivity with measures of age and Eysenck Personality Questionaire that were unrelated to extreme values of baseline MCCB t-scores and thus cannot reflect a regression to the mean (Chou *et al*, 2013). As such, these data do not agree with results previously demonstrated by Mattay *et al* (2003) that COMT genotype was associated with differential amphetamine effects on cognitive performance.

In thinking about differences between the study of Mattay *et al* (2003) and the present study, most exclusion criteria were similar (e.g. past psychiatric history or substance abuse). Exceptions to this were the exclusion of smokers and subjects with IQs < 90 in the study of Mattay *et al* (2003); however, the majority of subjects in the current study were UCSD students that most likely would not fall under these criteria anyway. With respect to the method of removing a mean order effect equally from all subjects' day 2 scores, there could have been some overcorrection by removing some changes in scores that were actually attributable to an amphetamine effect. However, it was observed that there were still

no significant differences between genotypes when raw data were analyzed without an order correction. Furthermore, Mattay *et al* used the Wisconsin Card Sorting Task (WCST) and N-back task as measures of WM, while the present study used the MCCB LNS and SS tasks as measures of WM. While there are few studies that compare the sensitivity of MCCB tasks to different COMT genotypes compared to the WCST and the N-back task, the MCCB has repeatedly demonstrated its sensitivity in detecting WM deficits in schizophrenia populations (Keefe *et al*, 2011).

In summary, this project reinforced the idea that WM as measured by the MCCB is sensitive to a specific range of dopaminergic activity that can be manipulated by drugs such as amphetamine. However, the study was unable to demonstrate that this sensitivity is significantly associated with COMT genotype, as was previously reported. While several factors could account for these discrepant findings, one interesting possibility follows from emerging data suggesting that the Met allele is associated with lower WM in certain populations, a result that is the opposite of that found in the Mattay *et al* (2003) study and other literature (Wang *et al*, 2013). Specifically, it was demonstrated in Han Chinese college students that the Val allele was associated with larger hippocampal volume and better WM as measured by a two-back WM task compared to the Met allele, a result opposite to that reported in Caucasian samples. This difference in COMT effects suggests that its impact on brain substrates of cognition may depend on the presence of other modifying genes, and gene x gene interactions. Conceivably, disparate findings across studies might reflect differences in the representation of ethnic groups. A more complete analysis of COMT effects on neurocognition and drug sensitivity would thus need to consider a potential role of population ancestry and gene x gene interactions.

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