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Thinking of ecstasy : neuropsychological aspects of ecstasy use

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Chapter 5

The effect of Ecstasy on memory is moderated by a functional polymorphism in the catechol- O-methyltransferase (COMT) gene

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Abstract

There is ample evidence for decreased verbal memory in heavy Ecstasy users. However, findings on the presence of a dose-response relation are inconsistent, possibly due to individual differences in genetic vulnerability. Catechol-O-methyltransferase (COMT) is involved in the catabolism of Ecstasy. Therefore, COMT gene polymorphisms may moderate this vulnerability. We prospectively assessed verbal memory in subjects with a high risk for future Ecstasy use, and compared 59 subjects after first Ecstasy use with 60 subjects that remained Ecstasy-naive. In addition, we tested the interaction effect of Ecstasy and the functional *val¹⁵⁸met* polymorphism on verbal memory. *Met*-allele carriers were somewhat more sensitive to the effects of Ecstasy on verbal learning than homozygous *val*-subjects. After correction for the use of other substances this effect was no longer statistically significant. The findings suggest that the COMT gene moderates the negative effect of Ecstasy on memory, but also other drug use seems to play a role.

Introduction

Neurotoxic effects of Ecstasy or +/- 3,4 methylenedioxymethamphetamine (MDMA)³, have been studied extensively. Both animal and human studies provide converging evidence for damage to the brain serotonin system^{191,200}. Because serotonin is involved in cognition and mood¹⁴⁷, it is not surprising that many studies have revealed effects of Ecstasy on these functions, with consistent evidence for the negative effects of Ecstasy on verbal memory⁸⁴. Studies on the effects of Ecstasy on visual memory, visuospatial ability and executive functions are less conclusive^{2,84,171}. Several studies also demonstrated a dose-response relationship between Ecstasy dose and cognitive parameters (e.g.^{67,88,184,193}), but others did not^{41,87,141}. A possible explanation for these inconsistent dose-relationship findings is that individuals vary in their susceptibility to the harmful effects of MDMA. Since MDMA has major effects on brain serotonin pathways, three recent studies investigated the relationship between a polymorphism of the serotonin transporter promoter region gene (5-HTTLPR) and affective and cognitive outcomes in Ecstasy users versus controls. Roiser et al (2005) found that carriers of the *g* allele of the 5-HTTLPR gene are more sensitive to Ecstasy induced emotional dysfunction than homozygous carriers of the *l* allele^{205,206}. In addition, Roiser et al (2006) found a moderating effect of the 5-HTTLPR gene on Ecstasy use and the performance on a decision-making task. However, in this study, the number of subjects carrying the *ss* allele was rather small and the finding that carriers of the *ss* allele outperformed carriers of the *ll* allele on some indices was unexpected, and therefore this result should be interpreted with great caution. Moreover, both Roiser et al (2006) and Reneman et al (2006) failed to find such a moderating effect of 5-HTTLPR on memory function¹⁹⁴. Also, in a previous study, Reneman et al (2001) did not find a correlation between cortical 5-HT transporter density and memory. Possibly, other heritable factors than the 5-HTTLPR gene could better explain individual differences in the sensitivity to Ecstasy-induced memory impairment. There are several reasons why the catechol-O-methyltransferase (COMT) gene is a potential candidate for the modulation of the effect of Ecstasy on memory. **First**, COMT is involved in the breakdown of MDMA and it is plausible that a slow degradation of MDMA increases MDMA-induced toxicity. **Second**, the COMT enzyme catalyzes the metabolism of extracellular dopamine, which seems to be involved in the process that leads to serotonin toxicity caused by MDMA^{118,165}. MDMA not only induces the release of serotonin in the synapse, but also of dopamine^{91,95,269}. The involvement of dopamine in MDMA-induced serotonin toxicity is twofold. First, the increased amounts of dopamine after Ecstasy use are transported into the serotonergic cells¹¹⁵, where dopamine is deaminated by monoamine oxidase-B (MAO-B)²³³. This oxidation results in free-radical formation and selective serotonin toxicity^{225,232}. Second, higher levels of extracellular dopamine are related to hyperthermia, which in turn creates serotonergic damage^{34,91,92,144}. Moreover, several studies have reported an attenuation of MDMA-induced serotonin toxicity after administration of pharmacological agents that suppress the MDMA-induced dopamine release^{24,118,165,225}. The COMT gene, which is involved in the breakdown of both MDMA and dopamine, contains a functional polymorphism leading to a valine (*val*) to methionine (*met*)

³ The term 'MDMA' is used when +/- 3,4 methylenedioxymethamphetamine (MDMA) is meant; 'Ecstasy' is used in case it is uncertain whether the substance is MDMA or a MDMA-derivative.

substitution, resulting in decreased enzymatic activity and slower degradation of MDMA and dopamine in carriers of the *met*-allele^{30,121,268}.

Taken together, it can be hypothesized that individuals carrying the *met*-allele, with lower COMT activity leading to a relatively slower breakdown of MDMA and relatively higher levels of extracellular dopamine, are more vulnerable to MDMA-induced neurotoxicity than individuals carrying the *val*-allele. If so, carriers of the *met*-allele will show greater cognitive impairments after Ecstasy use than *val*-carriers. In a previous report on our prospective study regarding the cognitive effects of Ecstasy use in novice low dose Ecstasy users, we found a significant effect of Ecstasy use on verbal memory, but not on any other cognitive domain²¹⁶. In the current report we investigated the moderating role of the COMT *val*⁵⁸/*met* polymorphism on the relation between Ecstasy use and verbal memory.

Experimental procedures

This study is part of the larger NeXT (Netherlands XTC Toxicity) study, investigating causality, course, and clinical relevance of Ecstasy neurotoxicity⁴⁷.

Participants

Between 2002 and 2004, 188 Ecstasy-naïve volunteers (18-35 y) who considered starting Ecstasy use in the near future were recruited by targeted site sampling at colleges and dance events, paper and website advertisements, and snowball sampling²⁶⁰. Exclusion criteria were: Ecstasy use in the past (at baseline session), a serious medical, neurological or mental illness; use of medications that may influence cognition; pregnancy; and intravenous drug use. Subjects had to abstain from using psychoactive substances for at least two weeks and from alcohol for at least one week prior to examinations. Drug use during the days before assessment was checked through urinalysis (enzyme-multiplied immunoassay for amphetamines, MDMA, opiates, benzoylecgonine (cocaine), benzodiazepines, 11-nor- Δ^9 -THCCOOH, ethanol).

The study was approved by the local ethics committee. Subjects were informed about potential negative effects of Ecstasy and all subjects gave written informed consent. Subjects were paid for participation (€ 100 or €150 per session).

Study design

At baseline examination all subjects underwent neuropsychological assessment and blood samples were taken for DNA isolation. In addition, all subjects had to complete questionnaires about their drug use sent to them by mail every three months during a follow-up period of approximately 18 months. Within three years after baseline examination, all incident Ecstasy users and an individually matched (gender, age, verbal IQ) control group of persistent Ecstasy-naïve subjects were invited for a follow-up session during which the neuropsychological assessment was repeated. The examiner was blinded to whether a subject had used Ecstasy or not.

Assessments

Dependent variable: Verbal Memory

A Dutch version of the Rey Auditory Verbal Learning Test (RAVLT)^{195,250} was used to assess verbal memory. For this test subjects must memorize a series of 15 concrete nouns in five learning trials. Immediate recall is tested after each trial. The outcome parameter is the sum of correctly reproduced words over five trials (max. 75). Delayed recall and recognition are measured after 20 minutes. Outcome parameters are the total number of correctly reproduced and recognized words (max. 15 and 30 respectively).

Independent variable: Ecstasy use

Ecstasy use was assessed at follow-up approximately 18 months after baseline using validated substance-use questionnaires²⁴⁸. First time Ecstasy use (between baseline and follow-up) was categorized in a binary variable (yes = 1, no = 0).

Effect modifier: COMT- polymorphism

Blood samples were collected from all subjects for DNA isolation. Genomic deoxyribonucleic acid (DNA) was extracted using a MagNA Pure LC system and a MagNA Pure LC DNA Isolation Kit I (Roche Diagnostics). Genotyping of the COMT *val*⁵⁸/*met* polymorphism (rs4680) was performed using single base primer extension and analyzed by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a Bruker III Daltonics Mass Spectrometer as described previously^{212,274} (details available upon request). All DNA samples were genotyped in duplicate to ensure reliability. The COMT genotype was categorized in three variables: *val/val* = 0, *val/met* = 1, *met/met* = 2.

Potential confounders

Substance-use was measured using validated questionnaires at baseline and at follow-up sessions²⁴⁸. Last year use of alcohol (units/week), tobacco (cigarettes/week), cannabis (number of joints), and amphetamines and cocaine (occasions) were measured.

At baseline, verbal intelligence was estimated for use as a covariate in the statistical analyses. For this purpose the Dutch version of the National Adult Reading Test¹⁶⁶, the Dutch Adult Reading Test (DART), was administered (DART-IQ) because it is relatively insensitive to cognitive impairment caused by neurological disorders²¹⁷.

Statistical analyses

Sociodemographics and substance use

Incident Ecstasy users versus persistent Ecstasy-naïves (between group differences)

For each COMT-genotype subgroup, unpaired t-tests were used to test whether incident Ecstasy users and persistent Ecstasy-naïves were statistically different in terms of age, verbal IQ, and time between baseline assessment and follow-up. Group differences in gender were investigated using the Chi-Square test (table 1). Substance-use (cannabis, alcohol, tobacco, cocaine, and amphetamines) at baseline and at follow-up were analyzed with non-parametric Mann-Whitney tests, because these variables were not normally distributed (table 2).

The different genotype groups (within group differences)

Univariate ANOVA was used to compare IQ, age, and time between baseline and follow-up, of the three different genotype subgroups within the group of incident Ecstasy users and within the group of persistent Ecstasy-naïves, respectively. Differences in gender were investigated using the Chi-Square test (table 1). Wilcoxon signed rank tests were used in all subgroups to assess whether substance-use changed between baseline and follow-up (table 2).

Verbal memory

Substance-use variables were not normally distributed. Therefore, log-transformed measures of substance-use were used as covariates in the following analyses. The following six groups were distinguished based on Ecstasy use and COMT polymorphism: 1: Ecstasy+*val/val*, 2: Ecstasy+*val/met*, 3: Ecstasy+*met/met*, 4: Ecstasy-*val/val*, 5: Ecstasy-*val/met*, and 6: Ecstasy-*met/met*). Differences in RAVLT immediate recall and RAVLT delayed recall between the six groups at baseline were analysed using MANCOVA, with group and gender as fixed factors and other potential confounders (age, DART-IQ, substance-use other than Ecstasy) as covariates. Because RAVLT recognition was not normally distributed, a non-parametric Kruskal-Wallis test was used, with group as independent variable and RAVLT recognition as dependent variable.

Moderation of the effect of Ecstasy use on verbal memory by COMT *val¹⁵⁸met* polymorphism

To test whether the COMT genotype moderated the effect of Ecstasy on verbal memory performance at follow-up, change scores between performance at baseline and follow-up examination were calculated for each subject and used in an ANCOVA, with future Ecstasy use, COMT genotype, and gender as fixed factors; age, DART-IQ; and the Ecstasy*COMT interaction as an effect moderator. In addition, baseline performance scores were added to the model as a covariate in order to control for differences in baseline cognitive functions between the different genotype subgroups. In a second analysis, substances other than Ecstasy (log-transformed) were added as covariates. Because RAVLT recognition was not normally distributed and showed very little variation, scores were transformed into a dichotomous variable, in which a decline was labelled 1 and no decline was labelled 0. Logistic regression analysis was performed with decline as dependent variable and use of Ecstasy, COMT genotype, Ecstasy*COMT, gender, and IQ as covariates. In a second analysis other substances than Ecstasy were added as covariates.

All analyses were performed using SPSS version 12.0.1 (SPSS Inc., Chicago, IL, USA). *P*-values < 0.05 were considered statistically significant.

Results

Sociodemographics and substance use

Of the 188 Ecstasy-naïve subjects at baseline, 158 completed the last follow-up questionnaires about drug use sent to them by mail. The other 30 volunteers were regarded as drop-outs, either because they refused to participate in follow-up or because we could not reach them anymore. Of the 158 compliant subjects, 64 declared that they

had started using Ecstasy since inclusion in the study, while the other 94 subjects declared to have remained continuously Ecstasy-naïve. Of the 64 Ecstasy users, 59 were willing to participate in the follow-up session, together with 61 persistent Ecstasy-naïve controls matched for gender, age and DART-IQ. One Ecstasy user was excluded because of a positive urine test on cocaine and one Ecstasy-naïve control was excluded because of dyslexia and attention deficit disorder diagnosed during childhood. Therefore, analyses included 58 Ecstasy users and 60 matched controls. Our sample mainly consisted of Caucasians ($n = 111$; 94.1%).

Genotypes were equally distributed over Ecstasy users and persistent Ecstasy-naïve controls: *val/val* 19% vs 17%, *val/met* 47% vs 50%, and *met/met* 34% vs 33% ($\chi^2_2 = .17$; $P = .92$) and did not deviate from those expected according to the Hardy-Weinberg equilibrium.

Table 1. *Demographics and characteristics of ecstasy users versus persistent ecstasy naïves.*

	Ecstasy users n=58			Ecstasy naïves n=60		
	Val/val n=11	Val/met n=27	Met/met n=20	Val/val n=10	Val/met n=30	Met/met n=20
Gender, no.	3M, 8F	13M, 14F	9M, 11F	4M, 6F	13M, 17F	8M, 12F
Age, mean(SD), median, range	23.2(3), 23.1, 18-28 ^a	20.7(2.5), 19.7, 18-29 ^a	22.4(3.5), 21.3, 18-29	21.3(1.8), 21.1, 19-25	21.5(2.3), 21.1, 18-29	21.5(1.8), 21.5, 19-25
DART-IQ, mean(SD), median, range	100.5(5.4), 99, 95-113	102.3(10.2), 102, 85-126	106.4(8.3), 106, 93-122	101.1(11.1), 101.5, 85-121	106.3(10.8), 104.5, 89-126	106.4(8.6), 106, 88-122
Weeks between baseline and follow-up, mean(SD), median, range	50.6(32.7), 60, 7-123 ^b	44.8(22.9), 57, 4-74 ^b	51.5(29.0), 56.5, 4-128 ^b	85.7(35.1), 70.9, 54-161 ^b	79.5(28.9), 69, 52-162 ^b	86.6(37.6), 81.5, 50-161 ^b

^a *val/val* vs *val/met* future ecstasy users $p < 0.05$ (UNIANOVA)

^b future ecstasy users vs persistent ecstasy naïves $p < 0.05$ (unpaired t-test)

Tables 1 and 2 show that genotype subgroups in Ecstasy users and persistent Ecstasy-naïve controls did not significantly differ in age, gender, DART-IQ, alcohol, cocaine and amphetamine use at baseline, and alcohol, tobacco and amphetamine use at follow-up. Due to the fact that ecstasy-naïve subjects could only be matched after the novice Ecstasy users were known, in all genetic subgroups, the number of weeks between baseline and follow-up was longer in the controls than in the Ecstasy users. However, within the group of Ecstasy users and persistent Ecstasy-naïves respectively, time between baseline and follow-up did not differ between the three genotypes. In addition, in the Ecstasy using group *val/val* subjects were 2.5 years older than *val/met* subjects. At baseline, there were some significant differences between the Ecstasy using versus the persistent Ecstasy-naïve *val/met* carriers regarding tobacco and cannabis use. At follow-up, there was a significant difference between the Ecstasy using versus the persistent Ecstasy-naïve *val/met* and *met/met* carriers regarding cannabis and cocaine use.

Within the group of Ecstasy users, use of substances other than Ecstasy did not differ significantly between baseline and follow-up. Within the group of persistent Ecstasy-naïves, *val/val* homozygotes used significantly less cannabis at follow-up compared to baseline ($\zeta = -2.24$; $P = .03$). *Val/met* persistent Ecstasy-naïves used more cigarettes ($\zeta = -2.16$; $P = .03$) and less alcohol ($\zeta = -2.09$; $P = .04$) at follow-up compared to

Table 2. Substance use characteristics of ecstasy users versus persistent ecstasy naives. Expressed as mean (SD), median, range.

	Ecstasy users n=58			Ecstasy naives n=60		
	Val/val n=11	Val/met n=27	Met/met n=20	Val/val n=10	Val/met n=30	Met/met n=20
Number of ecstasy pills	2.2(3.0), 1, 1-11	3.6(5.0), 2, 0.5-20	3.3(6.4), 1.5, 0.5-30	0	0	0
Weeks since last ecstasy use	14.8(13.9), 9.6, 1.9-43.9	11.9(11.7), 9, 2.7-59	9.9(11.6), 6.6, 1.6-55.3	0	0	0
Baseline:						
Alcohol	5.1(3.1), 5.7, 0.5-10.0	9.7(9.1), 6.4, 0.5-31.4	9.1(7.4), 7.1, 0.3-24	13.2(16.0), 7.5, 3.2-55	9.8(7.3), 8, 0.3-28.6 ^d	8.2(6.5), 6.1, 0-22.5
Tobacco	15.1(33.4), 0, 0-100	43.3(52.8), 20, 0-160 ^e	31.6(45.8), 6, 0-140	67.0(95.9), 5, 0-280	17.8(40.1), 0, 0-130 ^d	18.0(38.8), 0, 0-120
Cannabis (joints)	18.3(21.1), 15.3, 0-54.8	69.4(36.8), 15.8, 0-635 ^e	37.9(56.3), 16.9, 0-198	18.5(17.9), 15.8, 0-48 ^e	16.2(27.1), 9, 0-133 ^e	18.2(26.0), 15.5, 0-90.5
Cocaine (occasions)	0	0.9(2.2), 0, 0-6	1.2(2.5), 0, 0-6	0	0.4(1.5), 0, 0-6	0.3(1.3), 0, 0-6
Amphetamine (occasions)	0	0	0.3(1.3), 0, 0-6	0	0	0
Follow-up:						
Alcohol	6.3(4.7), 4.0, 1.7-15.5	9.8(9.9), 7.5, 0.5-35.7	9.0(5.9), 8.4, 1.4-21.4	11.4(15.5), 8.2, 0.3-53.5	8.6(5.6), 8.9, 0.3-21.4 ^d	6.2(4.8), 5, 0-21
Tobacco	36.5(63.5), 2.0, 0-180	38.0(74.3), 6, 0-350	26.1(41.0), 3.0, 0-120	53.0(71.8), 10, 0-200	24.4(45.4), 0, 0-140 ^d	10.2(19.6), 0, 0-60
Cannabis	31.8(45.5), 6.5, 0-133	56.0(156.8), 15.8, 0-830 ^e	62.4(93.0), 26.8, 0-354 ^b	8.6(11.7), 3.2, 0-35 ^e	26.2(65.9), 2.3, 0-315 ^e	18.0(32.0), 2.9, 0-133 ^b
Cocaine	0	1.6(3.2), 0, 0-12 ^e	2.1(2.9), 0, 0-6 ^b	1.2(2.5), 0, 0-6	0.2(1.1), 0, 0-6 ^e	0 ^f
Amphetamine	0	0.2(1.2), 0, 0-6	8.1(34.8), 0, 0-156	0	0	0

^a val/met future Ecstasy users vs val/met controls $p < 0.05$ (Mann-Whitney)^b met/met future Ecstasy users vs met/met controls $p < 0.05$ (Mann-Whitney)^c baseline vs follow-up val/val controls $p < 0.05$ (related, Wilcoxon)^d baseline vs follow-up val/val controls $p < 0.05$ (related, Wilcoxon)

Table 3 *Verbal memory performance of Ecstasy users versus persistent Ecstasy-naives*

RAVLT ^a	Ecstasy users <i>n</i> =58			Ecstasy naives <i>n</i> =60		
	Val/val <i>n</i> =11	Val/met <i>n</i> =27	Met/met <i>n</i> =20	Val/val <i>n</i> =10	Val/met <i>n</i> =30	Met/met <i>n</i> =20
<i>Baseline:</i>						
Immediate recall	61.09 (7.30)	57.89 (6.20)	58.55 (5.72)	55.70 (4.35)	59.30 (4.74)	56.50 (7.44)
Delayed Recall	14.09 (1.45)	13.48 (1.85)	13.95 (1.15)	13.80 (1.03)	13.70 (1.56)	12.95 (1.76)
Recognition	30.00 (0.00)	29.96 (0.2)	29.90 (0.31)	30.00 (0.00)	29.90 (0.40)	29.80 (0.41)
<i>Follow-up:</i>						
Immediate recall	63.27 (4.41)	57.33 (7.08)	60.60 (5.66)	58.10 (8.13)	62.27 (4.75)	62.55 (5.90)
Delayed recall	14.09 (1.22)	12.67 (2.43)	13.55 (1.43)	13.50 (2.12)	14.27 (0.69)	14.20 (1.28)
Recognition	29.73 (0.47)	29.52 (0.98)	29.80 (0.52)	29.90 (0.32)	29.97 (0.18)	29.90 (0.31)

^a Rey Auditory Verbal Learning Task: number of words; mean (SD)

baseline. Drug use of *met/met* persistent Ecstasy-naives did not differ between baseline and follow-up.

All further analyses were corrected for gender, age and IQ. First we performed the analyses without correction for cannabis, cocaine, amphetamine, alcohol and cigarette use; subsequently the analyses were repeated with correction for other substances than Ecstasy.

Verbal memory

Table 3 shows the verbal memory raw test scores for all groups. MANCOVA (Pillai's trace statistics) with subgroup (1: Ecstasy+*val/val*, 2: Ecstasy+*val/met*, 3: Ecstasy+*met/met*, 4: no Ecstasy+*val/val*, 5: no Ecstasy+*val/met*, 6: no Ecstasy+*met/met*), and gender as fixed factors, and with age and DART-IQ as covariates did not reveal any significant differences in RAVLT immediate and delayed recall at baseline ($F_{10,218} = 1.32$; $P = .22$). Adding substance use other than Ecstasy as covariates showed the same result ($F_{10,208} = 1.34$; $P = .21$). Kruskal-Wallis tests showed that baseline RAVLT recognition scores did not differ significantly between the groups ($\chi^2_5 = 6.67$; $P = 0.25$).

Univariate ANCOVA with change scores of test performance as dependent variable, with correction for DART-IQ, age, and gender, demonstrated an interaction effect of Ecstasy*COMT on RAVLT immediate recall ($F_{2,108} = 3.14$; $P < .05$). After adding the use of substances other than Ecstasy as covariates in the analysis, this interaction effect was no longer significant ($F_{2,103} = 2.37$; $P = .099$), although the change scores of *val/met* heterozygotes and *met/met* homozygotes showed the same trend, whereas a reverse tendency was shown for the change scores of *val/val* homozygotes (see figure 1). The interaction effect of Ecstasy*COMT on RAVLT delayed recall, with correcting for DART-IQ, age, and gender, was almost significant ($F_{2,108} = 3.06$; $P = .051$). After correcting for substances other than Ecstasy this effect was no longer significant ($F_{2,103} = 1.59$; $P = .209$), although a trend in the same direction was seen as in RAVLT immediate recall (figure 2). No interaction effect was found for Ecstasy*COMT on RAVLT recognition decline (OR = 2.02; Wald(1) = 0.60; $P = 0.44$). The addition of other substances than Ecstasy did not change the results (OR = 2.04; Wald(1) = 0.53; $P = .47$) (figure 3).

Because at follow-up, there was a significant difference between the Ecstasy using versus the persistent Ecstasy-naïve *val/met* and *met/met* carriers regarding cannabis and cocaine use, analyses were repeated after excluding cocaine users and cannabis users (>10joints last year). Univariate ANCOVA, with correction for DART-IQ, age, and gender, still showed a significant Ecstasy*COMT interaction effect on RAVLT immediate recall ($F_{2,60} = 3.68; P = .032$). Even after correction for other substances than Ecstasy, the interaction factor remained significant ($F_{2,60} = 3.63; P = .034$). The Ecstasy*COMT interaction effect was not significant for RAVLT delayed recall ($F_{2,60} = 0.59; P = .56$; with correction for other substances: $F_{2,60} = 0.53; P = .70$) or RAVLT recognition (OR = 1.35; Wald(1) = 0.06; $P = .80$; with correction for other substances: OR = 1.28; Wald(1) = 0.04; $P = .84$).

Figure 1.

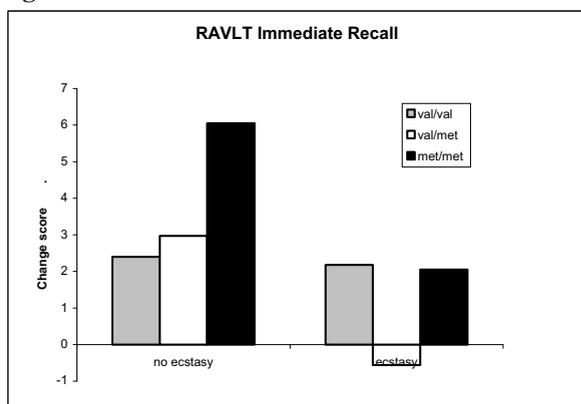


Figure 1. Moderation of the Effect of Ecstasy by the COMT gene on RAVLT Immediate Recall
Change scores present the change in number of words recalled on RAVLT immediate recall between baseline and follow-up.

Figure 2.

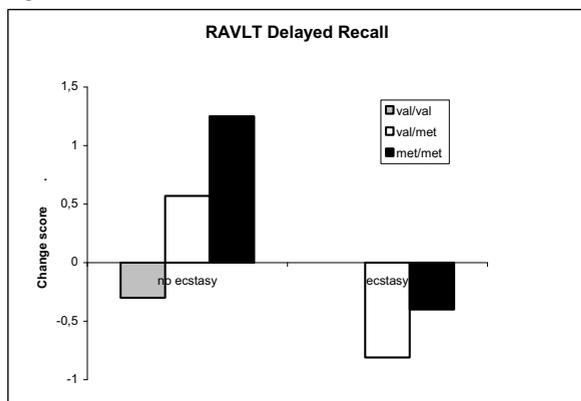


Figure 2. Moderation of the Effect of Ecstasy by the COMT gene on RAVLT Delayed Recall
Change scores present the change in number of words recalled on RAVLT delayed recall between baseline and follow-up.

Figure 3.

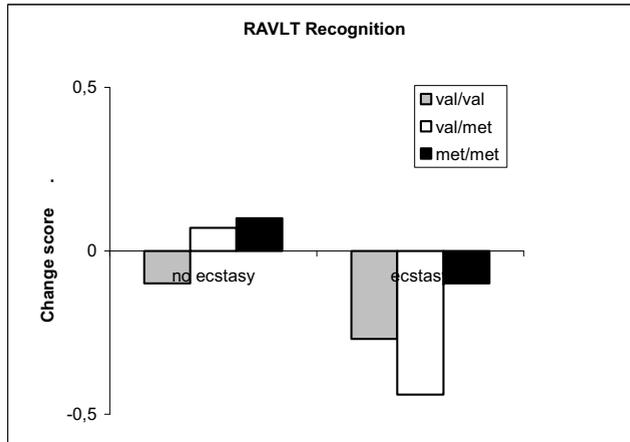


Figure 3. Moderation of the Effect of Ecstasy by the COMT gene on RAVLT Recognition
Change scores present the change in number of words recognized on RAVLT Recognition between baseline and follow-up.

Discussion

This is the first study investigating the moderating influence of the COMT-*val*⁵⁸/*met* polymorphism on the effect of Ecstasy on verbal memory. A significant interaction effect was found between Ecstasy use and the COMT gene on verbal learning, whereas the interaction between Ecstasy use and the COMT gene on verbal delayed recall was only marginally significant ($p=0.51$), and the interaction between Ecstasy use and the COMT gene on verbal recognition was not significant. After correcting for substance use other than Ecstasy the interaction effect was no longer significant. However, correcting for other substances may have led to an underestimation of the effect of Ecstasy, due to an overcorrection for the potential effects of other drugs correlated with the use of Ecstasy. Moreover, interaction terms generally have lower statistical power than tests for main effects, and therefore the application of the conventional significance level of $\alpha=0.05$ in combination with the relatively small genetic subgroups may have led to Type II errors (Fleiss, 1986). Our findings show that novice Ecstasy users carrying the *met*-allele showed a smaller retest (learning) effect on a verbal memory task than Ecstasy-naive *met*-allele carriers, whereas in *val*-carriers there was no difference in the retest effect between novice Ecstasy users and persistent Ecstasy-naives. It must be noted that the Ecstasy-using *met*-carriers used more cannabis and cocaine than control *met*-carriers, hence it could be argued that other drug use rather than a COMT*Ecstasy interaction affects verbal learning. However, repeating the analyses after excluding all cocaine users and all regular cannabis users (>10 joints last year), there was still a significant interaction effect between Ecstasy and the COMT-gene on verbal learning, even after the correction for other substances than Ecstasy. Moreover, further exploratory analyses revealed no effect of other drugs or interaction effects of other drugs with the COMT-gene on verbal memory (data not shown). We therefore conclude that carriers of the *met*-allele are particularly susceptible to sustained negative effects on verbal learning after Ecstasy use.

The role of other drug use is unclear, but the other substances may add to the interaction effect between Ecstasy and the COMT-gene. In addition, no clear interaction effect between Ecstasy and the COMT-gene was found on verbal long term memory (delayed recall and recognition). To our knowledge, the relationship between polydrug use, the COMT gene, and cognition, has not yet been investigated.

The suggestion that *met*-allele carriers, with relatively higher levels of extracellular dopamine than *val*-allele carriers^{30,121,268}, are more sensitive to the negative effects of Ecstasy than *val*-allele carriers, is in line with previous findings regarding the role of dopamine in Ecstasy-induced neurotoxicity^{118,165,232}. Also in a previous study on the modulating role of the COMT-gene on the effects of amphetamine on cognition, only *met*-carriers appeared to be disadvantaged¹³⁶.

The explanation for these findings is probably to be found in the role of COMT in the breakdown of MDMA and in the key role of dopamine in Ecstasy-induced neurotoxicity. We presume that individuals with low COMT-activity suffer longer from the toxic effects of MDMA than individuals with high COMT-activity, due to a slower breakdown of MDMA. It is tempting to assume that dopamine is essential for serotonin toxicity after Ecstasy use. If so, brain areas that are especially rich in extracellular dopamine, COMT and serotonin should be most vulnerable to the effects of Ecstasy. A brain region in which both dopamine and serotonin are highly prevalent is the prefrontal cortex (PFC)¹⁸³. COMT especially affects the dopamine flux in the PFC and possibly also in the hippocampus^{135,242}. This may explain why we found an interaction effect of Ecstasy by COMT on immediate verbal learning, but not on verbal recognition, since immediate learning is associated with prefrontal brain areas^{21,213}. In line with this theory, it is understandable that most studies on the effects of Ecstasy report verbal memory deficits. Also, if both *val/met*- and *met/met*-carriers are susceptible to the negative effects of Ecstasy on verbal memory, it is not surprising that most studies demonstrate verbal memory decrement in Ecstasy users even without taking the moderating effect of the COMT-polymorphism into account, knowing that in the general population about 50% of the Caucasians are *val/met* heterozygotes, and 18-27% are *met/met* homozygotes¹⁶⁹.

This study has several limitations. First, our study was exploratory, and the subgroups (especially the *val/val* groups) were relatively small. Therefore, although predicted, our findings may be due to chance fluctuations in our data set and should be replicated. A second limitation is that we only studied the role of the COMT polymorphism, while the hepatic enzyme CYP2D6 is also responsible for the breakdown of MDMA. Some *in vitro* studies pointed to individual differences in CYP2D6 activity as a possible factor in individual differences in the sensitivity to MDMA-induced acute and/or sustained neurotoxicity^{103,187,241}. Future *in vivo* studies are needed to further explore the role of both CYP2D6 and COMT polymorphisms in MDMA-induced neurotoxicity simultaneously. Third, we did not investigate a dose-response relationship because the dosages that were used in this study were very low (average 3 pills, median 1.5; see also²¹⁶). Fourth, there was no control on the purity and amount of MDMA in the Ecstasy tablets used by the subjects. However, results from pill-testing in the Netherlands confirm that in 2003 and 2004, 95% of the tablets sold as Ecstasy do contain MDMA as the major component^{54,55,164}. Finally, we did not investigate environmental circumstances in which the drugs were used, like ambient heat and dehydration. Interaction effects between those factors and neurocognitive damage have been reported²³⁹.

In conclusion, this study gives support for a moderating role of the COMT-*val*⁵⁸*met* polymorphism on the effect of Ecstasy on verbal learning. *Met*-allele carriers seem to be somewhat more susceptible to the effects of Ecstasy on verbal learning than *val*-allele carriers. Conclusions must be considered with caution until the results of this study are replicated.

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Contributors

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Conflict of interest

There is no conflict of interest for any of the contributing authors.

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