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An Experimental Study of *Catechol-O-Methyltransferase* Val[158]Met Moderation of Δ-9-Tetrahydrocannabinol-Induced Effects on Psychosis and Cognition

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Observational studies have suggested that psychometric psychosis liability and a functional polymorphism in the *catechol-O-methyltransferase* (*COMT* Val[158]Met) gene moderate the psychosis-inducing effect of cannabis. To replicate and extend this finding, a double-blind, placebo-controlled cross-over design was used in which patients with a psychotic disorder ($n = 30$), relatives of patients with a psychotic disorder ($n = 12$), and healthy controls ($n = 32$) were exposed to Δ-9-tetrahydrocannabinol (Δ-9-THC, the principal component of cannabis) or placebo, followed by cognitive assessment and assessment of current psychotic experiences. Previous expression of psychometric psychosis liability was also assessed. Models of current psychotic experiences and cognition were examined with multilevel random regression analyses to assess (i) main effects of genotype and condition, (ii) interactions between condition and genotype, and (iii) three-way interactions between condition, genotype, and psychometric psychosis liability. Carriers of the Val allele were most sensitive to Δ-9-THC-induced psychotic experiences, but this was conditional on prior evidence of psychometric psychosis liability. Δ-9-THC impacted negatively on cognitive measures. Carriers of the Val allele were also more sensitive to Δ-9-THC-induced memory and attention impairments compared to carriers of the Met allele. Experimental effects of Δ-9-THC on cognition and psychosis are moderated by *COMT* Val[158]Met genotype, but the effects may in part be conditional on the additional presence of pre-existing psychosis liability. The association between cannabis and psychosis may represent higher order gene–environment and gene–gene interactions.

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INTRODUCTION

Cannabis use is associated with poor outcome of not only existing psychotic disorder, but also of the liability to develop psychotic disorder in individuals at risk. Thus, in patients, cannabis use predicts higher relapse rates and higher likelihood of a continuous illness course (Linszen *et al,* 1994). In young people with higher than average expression of psychometric psychosis liability, the likelihood to develop overt psychotic states is higher with additional exposure to cannabis use (Henquet *et al,* 2005a; Verdoux *et al,* 2003). These results suggest that at least part of the pathway of risk from psychometric psychosis liability to overt psychotic disorder may be mediated by an abnormal sensitivity to the psychotropic effects of cannabis. Evidence for abnormal sensitivity was confirmed recently in an experimental study showing that patients with schizophrenia, compared to healthy controls, were more sensitive to the cognitive effects of delta-9-tetrahydrocannabinol (Δ-9-THC; hereafter THC), the principal psychoactive component of cannabis, in particular with regard to memory (D'Souza *et al,* 2005). This finding is relevant, as schizophrenia is associated with severe cognitive deficits that are also present, to a lesser degree, in the healthy first-degree relatives of patients (Krabbendam *et al,* 2001) who, compared to the general population, have a 10 times higher risk to develop schizophrenia (Kendler and Diehl, 1993). A first clue to the genes controlling abnormal sensitivity to the psychosis-inducing effects of cannabis was provided in a recent study by Caspi and colleagues, who showed that a

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functional polymorphism in the *catechol-O-methyltransferase (COMT)* gene moderated the risk to develop adult psychotic disorder following cannabis use in adolescence (exposure rate of 26% in the sample examined; Caspi *et al,* 2005). For individuals homozygous for the *COMT* Valine (Val) allele, the relative risk to develop psychotic illness after cannabis exposure was 10.9, whereas in individuals homozygous for the *COMT* Methionine (Met) allele, this risk was only 1.1. The *COMT* gene encodes the enzyme catechol-*O*-methyltransferase, which plays an important role in the degradation of dopamine in the brain, in particular in the prefrontal cortex. The *COMT* gene, mapped to chromosome 22q11, contains a functional polymorphism (*COMT* Val[158]Met) resulting in two common variants of the enzyme (Val and Met) corresponding to high- and low-activity enzyme, respectively. Increased COMT activity may result in a combination of (i) reduced dopamine neurotransmission in the prefrontal cortex, hypothesized to result in poorer performance of frontally mediated cognitive tasks, in particular working memory and attention (Egan *et al,* 2001; Meyer-Lindenberg *et al,* 2005; Rosa *et al*, 2004) and (ii) increased levels of meso-limbic dopamine signaling hypothesized to result in increased risk for delusions and hallucinations, the core symptoms of psychosis (Akil *et al,* 2003; Bilder *et al,* 2004). The work to date therefore suggests that *COMT* genotype, psychometric psychosis liability, and being a patient with a psychotic disorder moderate the effect of cannabis on psychosis outcomes in the community (Caspi *et al,* 2005; Henquet *et al,* 2005a) and that differential sensitivity to cannabis not only involves the positive symptoms of psychosis, but also cognition, in particular memory (D'Souza *et al,* 2005). It is unlikely that the moderating effects of psychometric psychosis liability and *COMT* on the association between cannabis and expression of psychosis represent one and the same underlying mechanism, because psychometric psychosis liability is strongly associated with psychotic disorder (Hanssen *et al,* 2005) whereas recent systematic reviews show only weak evidence of an association between *COMT* genotype and psychotic disorders (Fan *et al,* 2005; Munafo *et al*, 2005). Thus, psychometric psychosis liability and *COMT* genotype likely represent different mechanisms impacting on the same final common pathway of developing psychosis after cannabis exposure. It is therefore attractive to hypothesize that the separate moderating mechanisms of psychometric psychosis liability and *COMT* genotype show synergistic effects. To the extent that psychometric psychosis liability represents a genetic influence (Jacobs *et al,* 2005), synergism between *COMT* genotype and psychometric psychosis liability may support the hypothesis of underlying gene–gene interaction rather than the hypothesis of independent assortment of the genes involved. In the current study, findings from the above work on psychometric psychosis liability, *COMT* gene polymorphism, and the effects of THC on expression of psychosis and cognition were combined to examine two hypotheses. First, it was examined to what degree the *COMT* genotype associated with increased COMT activity independently moderates the acute effects of THC on psychotic symptoms and on cognition in an experimental design in individuals with different levels of psychosis liability. Psychosis liability for this purpose was defined as (i) psychometric measures of psychosis liability and (ii) illness risk (an ordinal variable indicating progressively increasing risk from healthy controls to relatives of patients with a psychotic disorder to patients with a psychotic disorder). Second, potential interactions between *COMT* genotype and psychosis liability in moderating THC-induced effects on psychotic symptoms and cognition were examined.

MATERIALS AND METHODS

Subjects

Thirty patients with a clinical diagnosis of psychotic disorder according to Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV) criteria, 12 first ($n = 9$) and second-degree ($n = 3$) relatives of patients who were not included in this study, and 32 healthy controls were recruited through in-patient and outpatient mental health service facilities in South-Limburg, The Netherlands, or were nonmental health users recruited from local coffee shops (cafes where cannabis is sold legally). Subjects attended a screening session during which a complete description of the study was provided and written informed consent was obtained. Subjects were told that the objective of the study was to obtain insight into the acute effects of THC on cognition and whether this was influenced by having a DSM-IV diagnosis of psychotic disorder. In addition, past and current psychiatric illness and past and current substance use were assessed using a structured interview (WHO, 1990). Subjects underwent a physical examination and were included if they were aged 18–60 years, had a body mass index between 18.5 and 25.0 kg/m^2, blood pressure not exceeding 120/80 mmHg, had been previously exposed to cannabis and used nicotine more than once a week. Exclusion criteria were: head trauma (with loss of consciousness); respiratory, cardiovascular, or neurological disease; alcohol use in excess of 5 units per day; weekly use of illicit drugs (other than cannabis); use of any drugs in the 5 days; and use of alcohol in the 2 days before the two test sessions. Pregnant women were also excluded, urine and breath analyses were performed before the testing to confirm exclusion criteria. A personal or positive family history of psychosis or use of antipsychotic medication was a further exclusion criterion for the control group, as was a personal history of psychosis or use of antipsychotic medication in the relatives. The study was carried out in accordance with the World Medical Association's declaration of Helsinki (Edinburgh modification, 2000) and was approved by the standing Medical Ethics Committee of Maastricht University Hospital.

Design and Assessment

The study design was a double-blind, placebo-controlled cross-over design. During two test sessions, separated by 1 week, blinded subjects received in randomized order either 300 μg THC/kg body weight in tobacco cigarettes in the exposure condition (Ramaekers *et al,* 2004), or 0 μg THC/kg body weight in tobacco cigarettes in the placebo condition. THC and placebo exposure took place minimally 4 h after

the last nicotine and caffeine intake and within 15 min after a standardized meal and caffeine-free beverage. THC and placebo cigarettes were prepared beforehand for each individual from stock provided by the Netherlands' Office of Medicinal Cannabis (BMC) of the Health Ministry and coded by a study coordinator who did not participate in the testing procedures. Active cigarettes were prepared from cannabis containing 13% THC and placebo cigarettes from cannabis containing a negligible percentage of THC (Laloup *et al,* 2005). Subjects smoked the cigarette as completely as possible in their customary fashion in the absence of the researcher. Testing took place for each subject separately. After 15 min, a neuropsychological, computer-assisted test battery was administered in the presence of a researcher in a research room other than the room in which the cigarette had been consumed, immediately after THC or placebo exposure in a fixed sequence, using parallel versions to avoid practice effects. Following the neuropsychological assessment, subjects were asked to complete questionnaires with the instruction to report symptoms that might have occurred in the time interval following the use of the THC/placebo cigarette. Cognition and questionnaires' scores were coded by subject number and entered in a database by a research assistant.

Psychosis Measures

In order to determine illness risk, a diagnosis was made according to Research Diagnostic Criteria (RDC) using the Operational Criteria Checklist and associated OPCRIT (McGuffin *et al,* 1991) computer program (hereafter group: an ordinal three-level variable indicating progressively increasing risk from healthy controls to relatives of patients with a psychotic disorder to patients with a psychotic disorder). The Positive and Negative Syndrome Scale (PANSS) was used to measure psychotic symptoms during the past 2 weeks. In order to determine psychometric psychosis liability, subjects completed the 40-item Community Assessment of Psychic Experiences (CAPE) during the screening session. The CAPE is a self-report instrument and captures variation in the positive and negative dimensions of nonclinical psychotic experiences as well as variation in depression. This scale was recently validated against clinical interview measures of schizotypy and psychosis-proneness (Konings *et al,* 2006). Previous work has shown that subclinical psychotic experiences measured with valid self-report questionnaires show longitudinal continuity with more severe states of psychosis, such as psychotic illness (Chapman *et al,* 1994; Hanssen *et al,* 2005) and are transmitted in families (Hanssen *et al,* 2006). Similarly, established risk factors for psychotic illness also affect the occurrence of these subclinical psychotic experiences (Krabbendam *et al,* 2005; Nuechterlein *et al,* 2002; van Os *et al,* 2002, 2000, 2001). Subclinical psychotic experiences as measured with the CAPE can therefore be considered as a proxy for an underlying liability to psychosis (Johns and van Os, 2001). For the current analyses, the total score on the positive dimension (hereafter: CAPE-trait score, modeled as a binary variable *a priori* dichotomized at the 50th percentile) was used as indicator of psychometric psychosis liability, for which purpose it was validated in a previous study (Hanssen *et al,* 2005) and for which purpose it had also been used in previous investigation (Verdoux *et al,* 2003). In order to capture transient positive psychotic symptoms during THC intoxication, the items of the CAPE-positive dimension were slightly modified to measure momentary psychotic experiences, rather than trait, during both test sessions (hereafter: CAPE-state score).

Cognitive Measures

The cognitive battery was based on previous research, to include tasks that are most sensitive to differential COMT activity and psychosis liability, and consisted of tests on verbal and nonverbal learning and memory (Bates *et al,* 2003; D'Souza *et al,* 2005; de Frias *et al,* 2004; Egan *et al,* 2001), sustained and selective attention (Chen and Faraone, 2000; Eisenberg *et al,* 1999), and psychomotor speed (Krabbendam *et al,* 2001). All tests were administered by computer using E-prime for Windows and presented on a 15-inch monitor. Overall intellectual functioning was measured by three subtests of the Groningen Intelligence Test (Luteijn and van der Ploeg, 1983).

Verbal and visual memory. The standardized Dutch version of the Visual Verbal Learning Test (Rey, 1964) was used to evaluate memory storage and retrieval of verbal information (15 monosyllabic nonrelated words) from episodic memory (immediate recall and delayed recall and recognition after 20 min). Nonverbal learning was tested using the Abstract Visual Pattern Learning with delayed recognition after 20 min (Sahakian *et al,* 1988).

Attention. Sustained attention was measured with a continuous performance test using an AX-version (CPT). Responses were expressed as correct detections, reaction time of correct detections, and false alarms (Nestor *et al,* 1991). The Stroop Color-Word test was used to tap selective attention and inhibition (Houx *et al,* 1991; Stroop, 1935). The time needed to complete Card III (color names printed in inconsistent ink colors) relative to Cards I (color names) and II (colored patches) was transformed into a Stroop interference score as a measure of selective attention. The Digit Symbol Substitution Test (DSST; Wechsler, 1981) was computerized in an adapted 3-min version and total correct scores were used as a measure of attention and psychomotor speed and coding.

Genotyping

DNA was extracted from the buccal mucosa by means of a cotton swab by using the BuccalAmp DNA Extraction Kit. *COMT* Val[158]Met genotypes were assayed by polymerase chain reaction, enzymatic digestion with *Nla*III followed by acrylamide gel electrophoresis as described by Daniels *et al* (1996).

Analyses

As all subjects had two measurements (placebo and THC), compromising statistical independence of the observations, multilevel random regression analyses were conducted

using the XTREG routine in STATA, examining the effects of condition (placebo *vs* THC) and genotype (0 = Met/Met; 1 = Val/Met; 2 = Val/Val) on psychotic symptoms and cognition.

Given the fact that the previously reported moderating influence of *COMT* genotype on cannabis-induced psychosis was dose–response (Caspi *et al,* 2005), genotype × condition interactions were tested with a continuous variable indicating the degree of Val loading (0 = 0 Val alleles, 1 = 1 Val allele, 2 = 2 Val alleles). In addition, the genotype × condition interaction was fitted with genotype as dummy variables with Met/Met as the reference category, allowing estimation of THC effect sizes for each genotype separately by calculating the appropriate linear combinations using the STATA LINCOM routine.

In order to test independence of, and possible synergy between, any moderating influence of *COMT* genotype and psychosis liability on THC-induced effects on the outcomes examined, the three-way interaction representing condition × genotype × psychosis liability was fitted, followed by calculation of stratified effect sizes using the LINCOM routine, as described above. As the two measures of psychosis liability (CAPE-trait and group) were very strongly associated ($\chi^2 = 20.5$, df = 2, $p < 0.001$), there was no CAPE-trait contrast between controls and relatives ($\chi^2 = 0.61$, df = 1, $p = 0.43$), and results with the two measures of psychosis liability were similar, only three-way interactions with the dichotomous CAPE-trait are shown. The group with high CAPE-trait thus included nine controls, two relatives, and 25 patients.

All analyses were *a priori* adjusted for age, sex, age of onset of cannabis use, frequency of use during the period of most heavy use, lifetime use of cocaine and/or stimulants, CAPE-trait, group (controls/relatives/patients), and use of antipsychotic medication. The analyses of main effects of condition on cognitive outcome measures were additionally adjusted for genotype. Main effects and interaction were assessed by the Wald test. All tests were two-tailed and a p-value < 0.05 was considered to be statistically significant. In addition, the Simes correction for multiple testing, which represents, in the case of correlated outcomes, an improvement over the Bonferroni procedure (Simes, 1986) was applied within the hypotheses of main effect of condition, two-way interactions, and three-way interactions. Cannabis effects stratified by genotype were derived directly from the two-way interactions as described above and therefore were not eligible for additional Simes' corrections. According to the Simes procedure, observed p-values greater than Simes' p-values are considered statistically nonsignificant. Simes p-values are shown for p-values below 0.05 (Simes, 1986).

The study was powered on the hypothesized two-way interaction effects. Power of the two-way interaction was based on published effect size of the THC × *COMT* gene interaction (Caspi *et al,* 2005) and calculated by a simulation adapted from a STATA routine developed for this purpose (www.stata.com/support/faqs/stat/power.html) in which the multilevel structure was taken into account. The analyses indicated that the sample size of this study yielded a power of 97% to detect significant two-way interactions. Given the fact that the power calculation was conservative, as effect sizes in the current experimental study are likely larger than the effect sizes of the observational study the power simulation was based on (Caspi *et al,* 2005), exploratory three-way interactions were also fitted.

RESULTS

Of the 30 patients RDC lifetime diagnoses were: schizophrenia ($n = 11$), schizo-affective ($n = 11$), and psychosis not otherwise specified ($n = 8$). Within the patient group, 22 subjects were using antipsychotic medication at the time of testing, eight were medication-free or used medication other than antipsychotic medication. Within the relatives group, three subjects had a lifetime diagnosis of bipolar disorder and one of major depression. Mean score on the PANSS was 48.3 (SD = 11.5) in the patient group, 36.0 (SD = 4.0) in the relatives group, and 33.0 (SD = 3.0) in the controls. Mean level of general intellectual functioning (IQ) was 104.7 (SD = 13.0) in the patient group, 104.4 (9.8) in the relatives group, and 103.0 (SD = 12.1) in the control group. Mean age was 27.4 years (SD = 8.7, range = 18–56). The majority were male ($n = 56$; 76%). All subjects had used cannabis in the past 12 months; frequency of use during this period was greater than once a day in 51% of the subjects. Forty-six percent had started the use of cannabis before the age of 16 years and frequency of use during the period of most heavy use was greater than once a day in 67% of the sample. Average alcohol consumption during the past 12 months was 11 units per week.

Genotype and Clinical Measures

The *COMT* genotype distribution in the whole sample was 26% (Met/Met), 27% (Val/Val), and 47% (Val/Met) and in Hardy–Weinberg equilibrium in all the groups (patients, relatives, and controls). There were no significant differences in genotype distribution between the sexes ($\chi^2 = 2.22$, df = 2, $p = 0.33$), and the three groups of patients, relatives, and controls ($\chi^2 = 4.45$, df = 4, $p = 0.35$). Similarly, genotype did not predict cannabis use before age 16 years ($\chi^2 = 3.21$, df = 2, $p = 0.20$), frequency of cannabis use in the past 12 months ($\beta = 0.08$, 95% CI = −0.46, 0.62, $p = 0.77$), frequency of use during the period of lifetime most heavy use ($\beta = 0.25$, 95% CI = −0.11, 0.62, $p = 0.17$), psychometric psychosis liability ($\beta = -0.01$, 95% CI = −0.17, 0.15, $p = 0.87$), total PANSS scores ($\beta = -0.17$, 95% CI = −3.51, 3.16, $p = 0.92$), IQ ($\beta = -0.95$, 95% CI = −4.92, 3.01, $p = 0.63$), alcohol use in the past 12 months ($\beta = -0.18$, 95% CI = −4.22, 3.85, $p = 0.93$), or use of antipsychotic medication ($\beta = -0.03$, 95% CI = −0.13, 0.07, $p = 0.49$). Groups did not differ in age of onset of cannabis use ($\chi^2 = 1.24$, df = 2, $p = 0.54$), frequency of use during the period of most heavy use ($\beta = -0.02$, 95% CI = −0.32, 0.29, $p = 0.90$), or in their alcohol use ($\beta = -2.18$, 95% CI = −5.38, 1.03, $p = 0.18$). There were significant group differences in age ($\beta = 3.41$, 95% CI = 1.97, 4.85, $p < 0.001$) and in sex ($\chi^2 = 7.26$, df = 2, $p = 0.026$). Cognitive measures and CAPE-state assessed in the placebo condition were associated with neither genotype nor with CAPE-trait (Table 1).

Table 1 Association Between Genotype and Outcome Measures and Psychometric Psychosis Liability (Cape-Trait Scores) and Outcome Measures Assessed in the Placebo Condition

Outcome measure	Genotype[a]	CAPE-trait score[b]
VVLT immediate free recall	$F=0.33$, df = 2, 59; $p=0.72$	$\beta=3.14$, 95% CI = −2.99, 9.29; $p=0.31$
VVLT delayed free recall	$F=1.64$, df = 2, 59; $p=0.20$	$\beta=0.73$, 95% CI = −0.95, 2.40; $p=0.39$
VVLT delayed recognition	$F=3.02$, df = 2, 57; $p=0.060$	$\beta=1.06$, 95% CI = −0.21, 2.33; $p=0.10$
Avipalet delayed recognition	$F=1.35$, df = 2, 58; $p=0.27$	$\beta=0.47$, 95% CI = −0.74, 1.68; $p=0.44$
CPT correct detections	$F=2.27$, df = 2, 58; $p=0.11$	$\beta=1.31$, 95% CI = −1.11, 3.74; $p=0.28$
CPT correct detections mean reaction times	$F=1.19$, df = 2, 58; $p=0.31$	$\beta=-7.10$, 95% CI = −37.41, 23.20; $p=0.64$
CPT false alarms	$F=1.23$, df = 2, 58; $p=0.30$	$\beta=0.20$, 95% CI = −0.71, 1.10; $p=0.67$
Stroop interference score	$F=0.50$, df = 2, 58; $p=0.61$	$\beta=0.03$, 95% CI = −0.32, 0.39; $p=0.85$
DSST total correct score	$F=0.68$, df = 2, 58; $p=0.51$	$\beta=4.83$, 95% CI = −2.03, 11.68; $p=0.16$
Cape-state score per group		
Controls	$F=0.14$, df = 2, 22; $p=0.87$	$\beta=0.02$, 95% CI = −0.04, 0.08; $p=0.56$
Relatives	$F=1.92$, df = 2, 6; $p=0.34$	$\beta=-0.03$, 95% CI = −0.15, 0.09; $p=0.44$
Patients	$F=1.25$, df = 2, 18; $p=0.31$	$\beta=0.21$, 95% CI = −0.21, 0.62; $p=0.30$

[a]F-value testing genotype differences adjusted for age, sex, age of onset of cannabis use, frequency of cannabis use, use of cocaine and/or stimulants, group, CAPE-trait score, and use of antipsychotic medication.
[b]Regression coefficient indicates change in outcome score associated with psychosis liability *vs* no psychosis liability, adjusted for age, sex, age of onset of cannabis use, frequency of cannabis use, use of cocaine and/or stimulants, group, genotype, and use of antipsychotic medication.

Symptoms

THC was not associated with a significant increase in positive symptoms ($\beta=0.04$, 95% CI = 0.02, 0.09, $p=0.19$), and no significant condition × genotype interaction was observed on the psychotic symptom outcome ($\chi^2=1.19$, df = 1, $p=0.27$). However, a significant three-way condition × genotype × Cape-trait interaction was observed (Table 2), indicating that genetic moderation of THC-induced expression of psychosis varied as a function of pre-existing psychosis liability. Thus, there was a significant condition × genotype interaction in the high CAPE-trait group (Table 2), indicating significant differences in the effect of cannabis on psychotic symptoms according to genotype, with the largest effect size in individuals with the Val/Val genotype (Table 2).

Memory

THC impaired verbal memory performance on immediate and delayed free recall ($\beta=-2.53$, 95% CI = −4.56, −0.50, $p=0.015<$ Simes' $p=0.025$; $\beta=-0.70$, 95% CI = −1.27, −0.13, $p=0.016<$ Simes' $p=0.030$, respectively) as well as on delayed recognition ($\beta=-1.63$, 95% CI = −2.60, −0.67, $p=0.001<$ Simes' $p=0.010$). Subjects with the Val/Val genotype were significantly impaired on the delayed recognition task after THC exposure and the two-way condition × genotype interaction, although falling short of the Simes criterion, suggested that the effect in the Val/Val genotype was greater than that in the Val/Met and the Met/Met genotypes (Table 3). No significant or suggestive three-way interactions for delayed recognition with Cape-trait were present ($\chi^2=0.11$, df = 1, $p=0.74$). Condition × genotype interactions were neither large nor significant for immediate free recall or delayed free recall, and similarly no significant three-way condition × genotype × Cape-trait interactions (immediate free recall $\chi^2=1.03$, df = 1, $p=0.31$; delayed free recall $\chi^2=0.02$, df = 1, $p=0.88$) were apparent. Visual memory (delayed recognition) was also significantly impaired after THC exposure ($\beta=0.79$, 95% CI = −1.41, −0.17, $p=0.013<$ Simes' $p=0.020$) and although the condition × genotype interaction was less precise than with the verbal task, the effect of THC on visual memory was large and significant only in the Val/Val subjects (Table 3). There was no evidence for a three-way condition × genotype × Cape-trait interaction ($\chi^2=0.00$, df = 1, $p=0.95$).

Attention

THC had a significant main adverse effect on sustained attention, as reflected in increased rates of false alarms on the Continuous Performance Task ($\beta=0.75$, 95% CI = 0.20, 1.29, $p=0.008<$ Simes' $p=0.015$), but not a main effect of decrease in correct detections ($\beta=-0.80$, 95% CI = −1.86, 0.27, $p=0.14$), or reaction times for correct detections ($\beta=0.52$, 95% CI = −7.10, 8.12, $p=0.90$). THC did have a main negative effect on selective attention and inhibition (Stroop interference score: $\beta=0.07$, 95% CI = 0.00, 0.14, $p=0.046>$ Simes' $p=0.035$), as well as on attention and psychomotor speed (DSST total correct score decreased after THC exposure ($\beta=-3.65$, 95% CI = −5.52, −1.78, $p<0.001<$ Simes' $p=0.005$)). Genotypes differed in their sensitivity to the attentional effects of THC (Table 4), as indicated by a significant two-way interaction in the model of CPT reaction time. Thus, reaction time for correct detections increased (ie deteriorated although statistically nonsignificant), after THC exposure in the Val/Val subjects, whereas reaction time decreased after THC exposure in the Met/Met subjects. The condition × genotype × Cape-trait

Table 2 Positive Symptoms (Cape-State); Stratified by Cape-Trait Scores (Low, High)

	Met/Met ($n=19$)		Val/Met ($n=35$)		Val/Val ($n=20$)			
Outcome measure	**Placebo scores (SD)**[a]	**Cannabis scores (SD)**[a]	**Placebo scores (SD)**[a]	**Cannabis scores (SD)**[a]	**Placebo scores (SD)**[a]	**Cannabis scores (SD)**[a]	**Two-way interaction**[b]	**Three-way interaction**[c]
1. Low CAPE-trait[d] ($n=37$)	0.07 (0.09)	0.16 (0.36)	0.02 (0.05)	0.02 (0.05)	0.04 (0.05)	0.02 (0.04)	$\chi^2=1.49$, df = 1; $p=0.22$	$\chi^2=9.00$, df = 1; $p=0.003$ ($<$ Simes' $p=0.005$)[e]
2. High CAPE-trait[f] ($n=37$)	0.29 (0.44)	0.19 (0.23)	0.09 (0.12)	0.16 (0.19)	0.25 (0.46)	0.44 (0.67)	$\chi^2=8.86$, df = 1; $p=0.003$	
β[g](95% CI; p)	−0.10 (−0.27, 0.06; $p=0.22$)		0.06 (−0.06, 0.18; $p=0.33$)		0.20 (0.03, 0.37; $p=0.021$)		($<$ Simes' $p=0.004$)[e]	

[a]Raw score and standard deviation of the outcome variable (CAPE-state).
[b]Condition × genotype interaction adjusted for age, sex, age of onset of cannabis use, frequency of cannabis use, use of stimulants and/or cocaine, group and use of antipsychotic medication.
[c]Condition × genotype × CAPE-trait interaction adjusted for age, sex, age of onset of cannabis use, frequency of cannabis use, use of stimulants and/or cocaine, group, and use of antipsychotic medication.
[d]$\leqslant$50th percentile CAPE-trait score (low psychometric psychosis liability).
[e]Simes' significance level, finding remains statistically significant if the observed p-value is lower than Simes' adjusted p-level.
[f]$>$50th percentile CAPE-trait score (high psychometric psychosis liability).
[g]Regression coefficient indicates change in CAPE-state scores associated with THC *vs* placebo condition analyses adjusted for age, sex, age of onset of cannabis use, frequency of cannabis use, use of stimulants and/or cocaine, group, and use of antipsychotic medication.

Table 3 Verbal Memory (VVLT: Immediate, and Delayed Free Recall, Delayed Recognition), Visual Memory (Avipalet: Delayed Recognition)

	Met/Met ($n=19$)		Val/Met ($n=35$)		Val/Val ($n=20$)		
Outcome measure	**Placebo scores (SD)**[a]	**Cannabis scores (SD)**[a]	**Placebo scores (SD)**[a]	**Cannabis scores (SD)**[a]	**Placebo scores (SD)**[a]	**Cannabis scores (SD)**[a]	**2-way interaction**[b]
1. VVLT immediate recall	43.84 (11.68)	40.32 (13.15)	47.43 (10.30)	45.14 (11.75)	43.65 (13.33)	40.15 (14.49)	$\chi^2=0.01$, df = 1; $p=0.93$
2. VVLT delayed recall	9.05 (3.06)	8.42 (4.02)	10.74 (3.21)	9.74 (3.47)	9.55 (3.46)	9.15 (3.70)	$\chi^2=0.16$, df = 1; $p=0.69$
3. VVLT recognition	27.06 (3.26)	26.22 (4.95)	28.65 (1.65)	27.65 (2.45)	27.60 (2.84)	24.25 (6.88)	$\chi^2=3.89$, df = 1; $p=0.048$
β[d] (95% CI; p)	−0.83 (−2.64, 0.97; $p=0.37$)		−1.03 (−2.41, 0.34; $p=0.14$)		−3.37 (−5.13, −1.61; $p<0.001$)		($>$ Simes' $p=0.012$)[c]
4. Avipalet delayed recognition	10.79 (2.51)	10.84 (2.52)	11.74 (2.23)	10.89 (2.53)	11.35 (1.81)	9.95 (2.66)	$\chi^2=2.96$, df = 1; $p=0.085$
β[d] (95% CI; p)	0.05 (−1.11, 1.21; $p=0.93$)		−0.95 (−1.85, 0.05; $p=0.039$)		−1.41 (−2.59, −0.23; $p=0.019$)		

[a]Raw score and standard deviation of the outcome variable.
[b]Condition × genotype interaction adjusted for age, sex, age of onset of cannabis use, frequency of cannabis use, use of stimulants and/or cocaine, CAPE-trait score, group and use of antipsychotic medication.
[c]Simes' significance level, finding remains statistically significant if the observed p-value is lower than Simes' adjusted p-level.
[d]Regression coefficient indicates change in cognition scores associated with THC *vs* placebo condition analyses adjusted for age, sex, age of onset of cannabis use, frequency of cannabis use, use of stimulants and/or cocaine, CAPE-trait score, group and use of antipsychotic medication.

Table 4 Sustained Attentions (CPT), Selective Attention and Inhibition (Stroop) and Attention and Psychomotor Speed (DSST)

Outcome measure	Met/Met ($n=19$)		Val/Met ($n=35$)		Val/Val ($n=20$)		2-way interaction[b]
	Placebo scores (SD)[a]	Cannabis scores (SD)[a]	Placebo scores (SD)[a]	Cannabis scores (SD)[a]	Placebo scores (SD)[a]	Cannabis scores (SD)[a]	
1. CPT correct detections	42.94 (6.80)	42.22 (4.53)	46.00 (2.41)	45.09 (3.30)	44.90 (3.92)	43.80 (6.43)	$\chi^2=0.04$, df = 1; $p=0.85$
2. CPT mean reaction times[c]	447.16 (57.60)	426.53 (46.03)	422.06 (52.89)	426.273 (54.53)	433.91 (54.34)	446.20 (54.75)	$\chi^2=8.01$, df = 1; $p=0.005$
β^c (95% CI; p)	−17.35 (−31.54, −3.20; $p=0.017$)		3.77 (−6.77, 14.32; $p=0.48$)		11.37 (−2.12, 24.86; $p=0.099$)		(< Simes' $p=0.008$)[d]
3. CPT false alarms	1.22 (3.04)	2.00 (2.91)	0.54 (0.89)	1.09 (1.66)	0.45 (0.89)	1.45 (2.37)	$\chi^2=0.07$, df = 1; $p=0.80$
4. Stroop interference	0.56 (0.28)	0.66 (0.30)	0.65 (0.29)	0.65 (0.27)	0.77 (1.04)	0.66 (0.32)	$\chi^2=0.09$, df = 1; $p=0.77$
5. DSST total correct	69.95 (17.69)	64.89 (15.94)	72.20 (12.78)	67.97 (13.20)	63.74 (16.26)	62.89 (15.32)	$\chi^2=2.97$, df = 1; $p=0.085$
β^e (95% CI; p)	−5.05 (−8.52, −1.58; $p=0.004$)		−4.53 (−7.20, −1.86; $p=0.001$)		−0.61 (−4.17, 2.95; $p=0.74$)		

[a]Raw score and standard deviation of the outcome variable.
[b]Condition × genotype interaction adjusted for age, sex, age of onset of cannabis use, frequency of cannabis use during period of most heavy use, use of stimulants and/or cocaine, CAPE-trait score liability, group and use of antipsychotic medication.
[c]Mean reaction times of CPT correct detections.
[d]Simes' significance level, finding remains statistically significant if the observed p-value is lower than Simes' adjusted p-level.
[e]Regression coefficient indicates change in cognition scores associated with Δ9-THC *vs* placebo condition analyses adjusted for age, sex, age of onset of cannabis use, frequency of cannabis use, use of stimulants and/or cocaine, CAPE-trait score group and use of antipsychotic medication.

interaction in the model of reaction time was neither significant nor suggestive ($\chi^2=0.80$, df = 1, $p=0.37$). The condition × genotype interaction was not significant for the number of false alarms (Table 4), but there was a suggestive condition × genotype × Cape-trait interaction for the number of false alarms (condition × genotype × Cape-trait: $\chi^2=6.06$, df = 1, $p=0.014>$ Simes' $p=0.009$), suggesting positive condition × genotype interaction in individuals with high CAPE-trait scores ($\chi^2=3.76$, df = 1, $p=0.052>$ Simes' $p=0.015$) but not with low CAPE-trait scores ($\chi^2=2.45$, df = 1, $p=0.12$). For correct detection scores, and for Stroop interference score, no large or significant condition × genotype interactions were observed (Table 4) and no condition × genotype × Cape-trait interactions were apparent for correct detection scores ($\chi^2=0.98$, df = 1, $p=0.32$) or Stroop interference score ($\chi^2=1.27$, df = 1, $p=0.26$). The effect of THC on attention and psychomotor speed was large and significant only in the Met/Met and the Val/Met subjects, but the condition × genotype interaction was not significant (Table 4). Similarly, no three-way interaction was observed ($\chi^2=0.75$, df = 1, $p=0.39$).

DISCUSSION

This is the first study, to our knowledge, to investigate gene × environment interactions in the cannabis–psychosis relationship in an experimental design. The results can be summarized as follows. First, THC impacted on cognition and psychosis outcomes, but there was evidence that this was conditional on other variables. Second, the Val[158]Met functional polymorphism in the *COMT* gene moderated sensitivity to the effects of THC on psychotic symptoms. Third, the differential sensitivity to THC associated with *COMT* genotype was in part conditional on additional evidence of psychosis liability, as Val carriers with evidence of psychometric psychosis liability experienced more THC-induced transient psychotic symptoms compared to Val carriers without these additional measures of liability. For cognitive measures, the conditionality on additional psychometric psychosis liability was less evident.

Methodological Issues

Power could approximately be estimated only for the two-way interactions based on one previous observational study. Type II error cannot be ruled out as some interactions may have smaller effect sizes than others. The conservatively calculated power of 97% for the sample size used was considered large enough to include exploratory three-way interactions as well that, if positive, need to be interpreted with caution until replicated in subsequent work. Future experimental studies can be powered more accurately. After correction for multiple testing with the Simes method, the two-way interaction in the model of verbal memory became nonsignificant. The correction for multiple testing however, may be too conservative, not only because correlated outcome measures were tested by directional hypothesis, but also because multiple significant interactions were found that all were in the same, hypothesized direction. As the condition × genotype interaction on memory outcome

in addition replicates earlier findings, this outcome likely reflects a true finding and lowering the alpha in this case may lead to a false rejection of the alternative, interaction hypothesis. Our diagnostic group was heterogeneous, including schizo-affective disorder and psychosis not otherwise specified apart from schizophrenia. The rationale for this relatively broad inclusion was that the effects of cannabis on psychotic symptoms and cognition, as well the interaction with prior psychometric psychosis liability, have been demonstrated both in populations of patients with a psychotic disorder and in nonpatient populations (D'Souza *et al,* 2005; Henquet *et al,* 2005a; Verdoux *et al,* 2003), suggesting relative nonspecificity across diagnostic groups and validating our approach of showing quantitative differences in sensitivity to cannabis between groups. Four relatives had diagnoses of affective disorder. This, however, is unlikely to have biased the results as only one of these four relatives was classified as high on the CAPE-trait measure and none used antipsychotic medication. The finding that groups did not differ in their overall level of intellectual function is not in agreement with other work showing lower IQ in patients with schizophrenia. There is some work, however, to suggest better cognition in psychotic patients with comorbid substance use compared to nonusing patients (Sevy *et al,* 2001). The relative high level of IQ in the patients in our sample may nevertheless raise potential problems of generalizability of the current results to psychotic patients in general.

The fact that all subjects were regular nicotine users may further limit generalizability of the findings, and although no group or genotype differences were observed in nicotine use, replication of these findings using tobacco-free methods to administer THC are necessary. A further limitation is that blood levels were not monitored following THC exposure, as it cannot be excluded that there is differential peripheral metabolism associated with *COMT* genotype (eg carriers of the Val allele may have lower levels of peripheral THC metabolism). Further research with a uniform drug administration procedure and direct measures of peripheral levels of THC (in blood or saliva samples; Ramaekers *et al,* 2006) is warranted.

THC, *COMT* Val[158]Met Genotype, and Cognition

The apparent inconsistency that the Val allele did not affect THC sensitivity for all cognitive functions may reflect low power for the two-way interaction, but may also be interpreted in the light of the tonic-phasic dopamine theory as suggested by Bilder *et al* (2004) and Grace (1991). Higher activity of COMT in the prefrontal cortex, associated with the Val allele, may produce a selective decrease in tonic dopamine subcortically, thus initiating an activation of phasic dopamine transmission. The Met allele, associated with low-activity COMT, is thought to decrease phasic and increase tonic dopamine transmission subcortically (Bilder *et al,* 2004). Phasic dopamine may be important for flexibility of neuronal activation, whereas tonic dopamine may play a critical role in cognitive stability. As a result, the Val and Met alleles may be associated with differential patterns of cognitive functioning that may translate to differential effects in the context of exposure to dopamine agonist drugs. In the current study, for example, a directionally opposite effect of THC on reaction time for correct detections on the CPT was observed in Met/Met and Val/Val subjects. Other neuropsychological tests with differential sensitivity to cortical dopamine resulting from differences in COMT activity may similarly yield alternative results. Any main effect of *COMT* genotype was taken into account in the model of the THC $\times$ *COMT* genotype interaction and therefore would have done nothing to obscure or exaggerate the interaction findings reported in this paper. Mattay *et al* (2003) reported *COMT* Val/Met genotype variations in amphetamine response, showing that amphetamine improved working memory in Val/Val subjects, but had no effect on the prefrontal efficiency in subjects with the Met/Met genotype. The authors invoked different levels of baseline dopamine transmission to explain these findings, suggesting that Met/Met subjects already may have had superior baseline prefrontal function. Thus, although precise explanations remain speculative at this stage, there is some rationale to suggest that differential mediating effects of *COMT* Val/Met genotype variations on cognition may also induce differential moderating effects in an experimental paradigm of THC on cognition. It is of interest that moderating effects of *COMT* Val/Met genotype were most apparent using a verbal memory task, which is thought to be more closely related to the hippocampal than frontal function.

THC, *COMT* Val[158]Met Genotype, and Psychosis

D'Souza *et al* (2005) demonstrated greater sensitivity to THC effects on psychosis outcomes in patients with schizophrenia compared to healthy controls, whereas Verdoux *et al* (2003) and Henquet *et al* (2005a) found that higher levels of psychometric psychosis liability moderated the effect of cannabis in terms of 'switching on' psychotic symptoms. In the current study, we did not replicate differences in THC sensitivity between patients and controls. However, rather than examining case–control differences, the design of the current study allowed for testing differential THC sensitivity at the level of psychometric psychosis liability, similar to earlier work (Henquet *et al,* 2005a; Verdoux *et al,* 2003). In agreement with these earlier studies, evidence for differential THC sensitivity was found, restricted to subjects with the Val/Val genotype. Although this three-way interaction needs to be interpreted with caution as explained above, the hypothesis of higher-order interactions is nevertheless necessary to explain the epidemiological observation that only a small minority of those exposed to cannabis develop a severe psychosis outcome (Henquet *et al,* 2005b). The liability to schizophrenia may in part consist of a developmentally mediated limitation in the capacity to modulate stress-related increased activity of meso-limbic dopamine neurons (Lieberman *et al,* 1997). *COMT* genotypes may be relevant in this regard, as higher activation of phasic dopamine transmission, associated with the Val allele, is thought to be important in responding to experientially 'salient' stimuli, a mechanism that, in excess, may contribute to the formation of psychotic symptoms (Kapur, 2003). A reciprocal interaction between the endocannabinoid and dopamine system as suggested by Giuffrida *et al* (2004) may well explain how THC further reinforces this process. THC increases

meso-limbic dopamine signaling in the acute phase (Voruganti *et al,* 2001). Similarly, exogenous cannabinoids like THC increase dopamine synthesis in rodents (Bloom, 1982; Maitre *et al,* 1970) and activation of dopamine D2 receptors triggers release of the endocannabinoid anandamide in the rat striatum (Giuffrida *et al,* 1999). In addition, a study of endocannabinoid levels in cerebrospinal fluid found higher levels of anandamide in patients with schizophrenia compared to healthy controls (Giuffrida *et al,* 2004; Leweke *et al,* 1999). Prolonged exposure to cannabinoids in rodents resulted in dysregulation of the endocannabinoid system by desensitization of the CB1 receptor (Sim-Selley, 2003) and reduction of dopamine metabolism in the prefrontal cortex (Jentsch *et al,* 1998). A close link between the endocannabinoid and dopamine system may thus explain why in individuals at increased genetic risk to dysregulation of these systems, THC may precipitate and exacerbate dopamine regulation more readily than in individuals with no evidence of psychosis liability (Howes *et al,* 2004) or in individuals with the Met/Met genotype.

Cannabis as a Cause of Psychosis

These results add to the evidence from previous studies that cannabis is a component cause in the development and course of psychotic illness, and that it co-depends on other factors, such as genetic liability to dopaminergic dysregulation, in impacting on psychosis risk. The current study confirms the role of the *COMT* Val[158]Met polymorphism as a moderating factor in THC-induced psychosis. There was no evidence for gene–environment correlation to explain this association, as *COMT* was associated with neither psychometric psychosis liability and cognitive functioning nor with frequency of cannabis use. Gene × environment interactions are more likely to explain these synergistic effects between THC and genetic liability. In addition, the moderating effect of psychometric psychosis liability on the THC × *COMT* interaction is suggestive of higher order interactions involving not only gene × environment, but also gene × gene interactions in the cannabis–psychosis relationship. More experimental and observational study on the effects of the *COMT* Val[158]Met polymorphism on THC response, as well as on the effects of other susceptibility genes, will provide a fuller understanding of the specific role cannabis plays not only in the emergence, but also in the persistence and the course of psychotic illness.

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