

COMT *val/58met* and 5-HTTLPR Genetic Polymorphisms Moderate Executive Control in Cannabis Users

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The adverse effects of cannabis use on executive functions are still controversial, fostering the need for novel biomarkers able to unveil individual differences in the cognitive impact of cannabis consumption. Two common genetic polymorphisms have been linked to the neuroadaptive impact of Δ^9 -tetrahydrocannabinol (THC) exposure and to executive functions in animals: the catechol-O-methyltransferase (COMT) gene *val/58met* polymorphism and the *SLC6A4* gene 5-HTTLPR polymorphism. We aimed to test if these polymorphisms moderate the harmful effects of cannabis use on executive function in young cannabis users. We recruited 144 participants: 86 cannabis users and 58 non-drug user controls. Both groups were genotyped and matched for genetic makeup, sex, age, education, and IQ. We used a computerized neuropsychological battery to assess different aspects of executive functions: sustained attention (CANTAB Rapid Visual Information Processing Test, RVIP), working memory (N-back), monitoring/shifting (CANTAB ID/ED set shifting), planning (CANTAB Stockings of Cambridge, SOC), and decision-making (Iowa Gambling Task, IGT). We used general linear model-based analyses to test performance differences between cannabis users and controls as a function of genotypes. We found that: (i) daily cannabis use is not associated with executive function deficits; and (ii) COMT *val/58met* and 5-HTTLPR polymorphisms moderate the link between cannabis use and executive performance. Cannabis users carrying the COMT *val/val* genotype exhibited lower accuracy of sustained attention, associated with a more strict response bias, than *val/val* non-users. Cannabis users carrying the COMT *val* allele also committed more monitoring/shifting errors than cannabis users carrying the *met/met* genotype. Finally, cannabis users carrying the 5-HTTLPR *s/s* genotype had worse IGT performance than *s/s* non-users. COMT and *SLC6A4* genes moderate the impact of cannabis use on executive functions.

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INTRODUCTION

Cannabis is the most consumed illegal drug among youths between 14 and 25 years old, with prevalence rates and treatment demands steadily increasing over the past years (EMCDDA Annual Report, 2011; NSDUH National Survey, 2010). Cannabis-induced reinforcing effects are triggered by direct activation of cannabinoid CB1 receptors (located in frontal–cingulate–striatal regions), and indirect stimulation

of striatal dopamine release (Camí and Farré, 2003). These brain networks and neurochemical systems are critical for the functioning of the executive control system, which is involved in the active maintenance, updating, and shifting of the higher order goals that guide adaptive efficient behavior. In accordance with different conceptual models, the executive system encompasses a range of dissociable functions, including sustained attention, working memory, response monitoring, and decision making (Robertson and Garavan, 2000; Robbins, 2007; Verdejo-Garcia and Perez-Garcia, 2007), and these functions largely operate on monoamine systems (Barnes *et al*, 2011). The long-term effects of heavy cannabis use have been clearly associated with learning and memory deficits in tandem with hippocampal attrition (Grant *et al*, 2003; Yücel *et al*, 2008). However, the potentially adverse effects of cannabis

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use on executive functions are still controversial, with some studies showing significantly reduced performance of heavy users *vs* controls in tests of sustained attention, response monitoring, or decision making (Fontes *et al*, 2011; Verdejo-García *et al*, 2007), but meta-analytic data yielding overall small or negligible effects (Grant *et al*, 2003). These controversial findings raise the possibility that moderator variables contribute to the presence or severity of executive deficits among cannabis users, with growing evidence showing that both earlier age at onset and heavier use are associated with poorer executive function (Bolla *et al*, 2002; Fontes *et al*, 2011) and greater frontostriatal deficits (Arnone *et al*, 2008; Zalesky *et al*, 2012). However, no studies to date have explored whether genetic variations in the monoamine systems that support frontostriatal networks and executive control relate to individual differences in cannabis-related executive deficits.

Animal models have demonstrated that the catechol-O-methyltransferase (*COMT*) gene modulates the harmful effects of cannabis use on the prefrontal and hippocampal brain structure (Behan *et al*, 2012) and working memory (ÓTuathaigh *et al*, 2010). An experimental challenge study has also shown that the *COMT* gene moderates the impact of THC on memory and sustained attention, such that individuals carrying the high-activity *val/val* genotype are more likely to show cannabis-induced cognitive deficits (Henquet *et al*, 2009). In healthy individuals, the neurobiological correlates of working memory encoding suggest an abnormally increased activation of the prefrontal–parietal–striatal network in *val vs met* carriers. As this network is also abnormally hyperactivated among cannabis users when performing working memory tests (Jager *et al*, 2006, 2010), it is plausible to hypothesize that carrying the *val* variant and using cannabis may have cumulative negative effects on working memory and sustained attention. Considerably less is known about cannabis \times *COMT* gene interactive effects on other executive functions, but there is consistent evidence from animal and human studies showing that the *met/met* genotype of this gene is associated with poorer response monitoring/shifting skills (Nolan *et al*, 2004; Tunbridge *et al*, 2004).

On top of cannabinoid and dopamine effects, there is also animal evidence showing that high doses of cannabinoid agonists induce reduction of platelet serotonin concentrations, accompanied by alterations of the functioning of the serotonergic system in cannabis chronic smokers (Velenovská and Fisar, 2007). The *5-HTTLPR* polymorphism is one of the main regulators of serotonin transporter function (Greenberg *et al*, 1999), and the *s/s* genotype (associated with lower serotonin levels) has been consistently associated with poor decision-making skills in animals and humans (van den Bos *et al*, 2009; He *et al*, 2010; Homberg *et al*, 2008; Sonuga-Barke *et al*, 2011). The *5-HTTLPR* polymorphism may be specifically associated with decision-making skills due to its prominent role in emotional processing, and its link with the ventromedial prefrontal cortex–amygdala network (Bevilacqua and Goldman, 2011), which is also impacted by heavy cannabis use (Vaidya *et al*, 2011). There is evidence that higher THC concentrations predict poorer decision-making quality in regular users (Hermann *et al*, 2009), such that in this case, along the same lines, it is plausible that carrying the *s/s*

variant and using cannabis may have cumulative negative effects on decision-making skills.

The aims of this study were: (i) to assess the executive functions of young daily cannabis users compared with non-drug-using controls, also exploring potential effects of age at onset and use severity; and (ii) to investigate possible interaction effects of cannabis use and *COMT val158met* and *5-HTTLPR* polymorphisms in explaining executive function performance in cannabis users. We hypothesized that: (i) cannabis users would have worse executive function performance than controls; (ii) cannabis users carrying the *val/val* genotype of the *COMT val158met* polymorphism would have worse working memory and sustained attention, whereas cannabis users carrying the *met/met* genotype would have worse monitoring/switching; and (iii) cannabis users carrying the *s/s* genotype of the *5-HTTLPR* polymorphism would have poorer decision-making performance.

MATERIALS AND METHODS

Participants

In all, 144 European-Caucasian participants: 86 cannabis users and 58 non-drug users (controls) participated in the study. The demographic and drug-use characteristics from both groups are summarized in Table 1.

Inclusion criteria for cannabis users were: daily use of cannabis (>7 joints per week) for a minimum duration of 3 years, meeting Diagnostic and Statistical Manual IV (DSM-IV) criteria for cannabis abuse or dependence, age between 18 and 30 years old, and having at least basic-level education (>8 years of education). The two latter inclusion criteria (and exclusion of the first two) were applied to controls. Volunteers were recruited through several sources: ‘word of mouth’, advertisement in the local university, and advertisement in the website of a local Non-Governmental Organization (Energy Control) specialized in providing harm reduction guidelines among drug users. All assessments were conducted in research-dedicated premises of the Clinical Research Unit (CRU) of IMIM-Hospital del Mar Research Institute.

Potential participants were medically screened at the CRU to rule out the presence of physical or neurological illness and of drug-related acute effects or withdrawal symptoms, as determined by standard physical examinations and biochemical determinations (supervised by the medical director). They were also carefully assessed to diagnose comorbid psychiatric disorders using a well-validated interview, the Psychiatric Research Interview for Substance and Mental Disorders (PRISM; Torrens *et al*, 2004), which provided DSM-IV diagnoses. In addition, the PRISM provided diagnoses for the whole spectrum of DSM-IV substance-use disorders, such that we were able to rule out the potential participants meeting the criteria for abuse or dependence on drugs different than cannabis. Therefore, all potential participants having medical illnesses or comorbid psychiatric/substance-use disorders (current or during the prior year) were excluded from the study. For non-drug user controls, additional exclusion criteria—based on the toxicological history—were the use of any illegal drug during the past year or in more than 10 occasions during lifetime. Toxicological history in the past 6 months was

Table 1 Descriptive Data on Demographic Variables and Drug-Use Characteristics

	Cannabis (n = 86) Mean (SD)	Control (n = 58) Mean (SD)	SMD ^a
Age	21.5 (2.9)	23.2 (4.8)	0.44
Vocabulary WAIS-III	11.3 (2.0)	12.6 (1.9)	0.67
Gender			0.08
Male	61 (70.9)	39 (67.2)	
Female	25 (29.1)	19 (32.8)	
University degree, n (%)			0.55
Yes	55 (64.0)	50 (87.7)	
No	31 (36.0)	7 (12.3)	
Employment status, n (%)			0.15
Employed	24 (28.2)	22 (38.6)	
Unemployed	23 (27.1)	11 (19.3)	
Student	38 (44.7)	24 (42.1)	
Smoker, n (%)			1.15
Current smoker	57 (66.3)	9 (16.1)	
Non-smoker/ex-smoker	29 (33.3)	47 (83.9)	
Age at first tobacco use, years	18.9 (3.0)	19.0 (4.2)	0.03
Cigarettes per day	9.4 (6.2)	6.6 (5.6)	0.46
Age at first alcohol use, years	14.8 (1.4)	15.6 (1.4)	0.59
Years of alcohol consumption	6.7 (3.0)	7.6 (4.4)	0.21
Age at first cannabis use, years	15.3 (1.5)		
Years of cannabis use	6.2 (2.8)		
Lifetime cannabis use (number of joints)	5645.1 (3908.6)		
Cannabis consumption during the past year	1002.2 (678.6)		

^aAbsolute value of standardized mean difference.

confirmed by hair testing (de la Torre *et al*, 2010; Pichini *et al*, 2006). These hair samples were also used to measure cannabidiol (CBD) and THC ratio concentrations pertaining to the last month of cannabis use by GC/MS (Falcón *et al*, 2011).

Test Procedure

This study was approved by and conducted in accordance with the local ethics committee (CEIC—Parc de Salut Mar). Upon arrival to the research centre (IMIM-Hospital del Mar Medical Research Institute), participants were informed of the ensuing protocol and provided informed consent before participating in the study. All participants were subjected to an initial medical exploration that included a detailed medical history, biochemical analyses, physical examination, urine and hair drug testing, and a brief neurological examination. Eligible subjects underwent a psychiatric interview to exclude the presence of major psychiatric disorders, history of abuse, or drug dependence

(except for nicotine dependence) administered by a clinical psychologist. Afterwards, all participants were requested to observe a 72-h abstinence period (possible withdrawal effects were medically controlled), and urine and hair drug screens were carried out by immunoassay (CEDIA; Thermo Fisher Scientific) to confirm abstinence before neuropsychological testing. Drug classes screened for included: cannabis, MDMA, cocaine, opioids, amphetamine, and methamphetamine. All participants meeting the inclusion criteria underwent the neuropsychological assessment session of 120 min. All subjects were economically compensated for their participation (80 euros per participant).

Genotyping

Genomic DNA was extracted from the peripheral blood leukocytes of all the participants using Flexi Gene DNA kit (Qiagen Iberia SL, Sevilla, Spain) according to the manufacturer's instructions.

The *COMT val108/158met* (rs4680) single-nucleotide polymorphism (SNP) allelic variants were determined using the 59 exonuclease TaqMan assay with ABI 7900HT Sequence Detection System (Real Time PCR) supplied by Applied Biosystems (Foster City, CA). Primers and fluorescent probes were obtained from Applied Biosystems (TaqMan SNP Genotyping assays: assay ID C_2255335_10). Reaction conditions were those described in the ABI PRISM 7900HT user's guide. Endpoint fluorescent signals were detected on the ABI 7900, and the data were analyzed using the SDS software, version 2.3 (Applied Biosystems).

5HTTLPR genotyping was performed using PCR as described by Cuyas *et al*. (2011). The reaction mixture contained: 1 × PCR Amplification buffer and 1 × PCR Enhancer solution (Invitrogen, Carlsbad, CA), 1.5 mM MgSO₄, 300 mM dNTPs, 0.5 pmol of each primer, 0.5 U of Taq DNA polymerase (Invitrogen) and 50 ng of genomic DNA as template. The products of allelic-specific amplifications (allele L, 528 bp; allele S, 484 bp for *5-HTTLPR*) were detected on an automatic ABI 3730XL capillary sequencer and analyzed by the GeneMapper Software v.3.5 (Applied Biosystems).

Table 2 shows the genotype distribution for *COMT val158met* and *5-HTTLPR* polymorphisms as a function of group (cannabis users and controls).

Neuropsychological Assessment

We implemented a computerized neuropsychological assessment aimed to target specifically five different aspects of executive control using well-validated probes from the CANTAB (Robbins *et al*, 1994, 1998, see www.camcog.com) and other sources. The domains and tests used were: sustained attention (CANTAB Rapid Visual Information Processing Test, RVIP), working memory (N-back; Watter *et al*, 2001), response monitoring/set shifting (CANTAB Intradimensional/Extradimensional set shifting, ID/ED), planning/reflection impulsivity (CANTAB Stockings of Cambridge, SOC), and decision making (Iowa Gambling Task, IGT; Bechara *et al*, 2000). The main performance indices obtained from the different probes were the RVIP probability of hit (number of hits/(number of hits + number of misses)) indexing accuracy of vigilance/sustained attention; the

Table 2 Genotype Distributions of Participants in the Cannabis User and Control Groups

	Cannabis (n = 86) n (M/F)	Control (n = 58) n (M/F)	P-value
5HTTLPR			0.88
LL	22 (15/7)	16 (11/5)	
LS	47 (36/11)	31 (21/10)	
SS	17 (10/7)	11 (7/4)	
COMT val158met			0.09
Val/val	16 (9/7)	19 (13/6)	
Val/met	58 (46/12)	29 (20/9)	
Met/met	12 (6/6)	10 (6/4)	

Abbreviation: M/F, male/female distribution.

Hardy–Weinberg equilibrium:

• 5HTTLPR: cannabis, $P = 0.515$; controls, $P = 0.793$.

• COMT val158met: cannabis, $P = 0.0022$; controls, $P = 1$.

N-back hit rate (proportion of hits minus proportion of errors) indexing working memory accuracy; the ID/ED total number of errors (adjusted by number of stages completed) indexing performance monitoring errors; the SOC initiation time and number of movements to correct, indexing reflection-impulsivity and planning skills, respectively; and the IGT net score ((sum of advantageous deck choices (C + D) minus sum of disadvantageous deck choices (A + B)) indexing quality of decision making. As both the N-back and the IGT tasks involve multiple series of trial blocks, the performance measures were calculated for N-back blocks 1, 2, and 3 (100 trials each), which index increasing working memory loads, and for IGT blocks 1–5 (20 trials each), which index progressive decision-making learning phases: pre-hunch (blocks 1 and 2), hunch (block 3), and explicit decision phases (blocks 4 and 5) (Bechara et al, 2005).

The RVIP decision-making pattern was further explored using the A' (discrimination) and B' (response bias) parameters derived from signal detection theory; the first indexing the ability to detect target sequences, and the second reflecting the tendency to respond, regardless of whether the target is present. The ID/ED error pattern was also further analyzed by calculating both intradimensional (indexing learning) vs extradimensional (indexing shifting) errors.

We also calculated a Z-scored total cognitive index by calculating and adding the standardized scores from the five main performance indices: RVIP probability of hit, N-back hit rate (in the harder 3-back condition), ID/ED total number of errors, SOC number of movements to correct, and IGT net score.

In addition to these tests, we used the vocabulary subtest from the Wechsler Adult Intelligence Scale as a proxy of verbal IQ.

Statistical Analyses

Sample characteristics are described by means of either mean and standard deviation (numerical variables) or absolute and relative frequencies (categorical variables). To quantify the differences between both study groups

(cannabis users and controls), the standard mean difference was computed. The χ^2 -test was applied to study the association between cannabis consumption and the *COMT val158met* and *5-HTTLPR* polymorphisms. In addition, it was used to check whether the Hardy–Weinberg equilibrium holds among both study populations. We used ANCOVA models (controlling for age, gender, estimated verbal IQ, and smoking status) to compare cannabis users and non-user controls, and to compare cannabis users with early vs later onset of cannabis use (before vs after 15 years old; Fontes et al, 2011). Pearson's correlation analyses were used to explore the association between estimates of cannabis use and THC/CBD concentrations and tests performance. Separate ANCOVA models were fitted to study the relation between cognitive performance and both cannabis consumption and the polymorphisms of interest while controlling for the covariates with significant impact on dependent measures. These models included study group and the respective genotype as predictive variables of interest as well as gender, age, estimated verbal IQ, and smoking status as potential covariates. Initially, all models did also include the two-way interaction between genotype and drug consumption. Whenever the interaction could be discarded, both factors were studied separately using the ANCOVA model excluding interaction. If, by contrast, the interaction was significant, the effect of drug consumption was studied separately for each genotype expression. In that case, the Tukey test for multiple comparisons was applied for the comparison of genotypes in the framework of the ANCOVA models including interaction. Statistical significance was set at 0.05. The statistical software package R (The R Foundation for Statistical Computing), version 2.13.1, was used for all analyses.

RESULTS

Group Comparisons

We found no significant differences between cannabis users and controls in any of the tasks' main performance indices or in the Z-scored cognitive score (see Table 3). We only found a significant difference between the groups on IGT block 3, which is considered to index the 'hunch' or learning phase of the task. The segregation of the cannabis group as a function of age at onset yielded the same pattern of results, and both subgroups of cannabis users (early and late onset) showed nonsignificant performance differences with controls.

Correlations

We did not find any significant correlation between self-report lifetime estimates of cannabis use and performance in executive tests. However, we found a significant positive correlation between CBD/THC ratio concentrations and SOC mean number of movements to correct, $r = 0.52$; 95% CI: 0.31, 0.68 ($P < 0.001$).

Genotype \times Group interactions

Cannabis use \times *COMT val158met*. Results showed a significant drug \times gene interaction on RVIP probability of

Table 3 Neuropsychological Performance as a Function of Drug-Use Status: Cannabis Users vs Controls

	Cannabis (n = 86)	Control (n = 58)	F (1,138) ^a	P-value ^a
<i>Rapid visual processing</i>				
Probability of hit	67.6 (17.3)	72.6 (13.6)	0.005	0.94
Alpha	0.92 (0.05)	0.93 (0.04)	0.029	0.87
Beta	0.93 (0.09)	0.91 (0.09)	0.965	0.33
<i>N-back</i>				
Probability of hit, N = 1	90.7 (10.7)	93.3 (8.8)	0.189	0.66
Probability of hit, N = 2	81.5 (15.0)	83.9 (16.2)	0.019	0.89
Probability of hit, N = 3	60.9 (23.4)	65.0 (23.9)	0.001	0.97
<i>Intra/extradimensional set shifting</i>				
Total errors adjusted	25.8 (21.8)	22.4 (19.7)	0.037	0.85
Intradimensional errors	7.92 (5.43)	8.19 (6.37)	0.347	0.56
Extradimensional errors	10.86 (11.07)	9.08 (9.79)	0.146	0.70
<i>Stockings of Cambridge</i>				
Mean choices to correct	1.4 (0.3)	1.3 (0.2)	0.601	0.44
Mean latency to first choice	14 249 (4936)	16 247 (5808)	2.427	0.12
<i>Iowa Gambling Test</i>				
Block 1	-2.8 (4.4)	-2.7 (4.3)	0.182	0.67
Block 2	0.3 (5.4)	0.5 (4.6)	0.272	0.60
Block 3	1.1 (6.2)	3.6 (7.7)	6.339	0.01
Block 4	2.4 (7.2)	4.0 (8.8)	2.554	0.11
Block 5	3.2 (7.8)	5.0 (9.17)	1.728	0.19
Net score	4.56 (21.5)	10.33 (24.1)	3.549	0.06
Z composite score	-0.48 (2.97)	0.71 (2.94)	1.004	0.32

Results are presented as mean (SD).

^aFrom ANCOVA models adjusting for sex, age, smoking status, and IQ.

hits, $F(1,138) = 5.17$, $P < 0.05$, and ID/ED total number of errors, $F(1,138) = 6.06$, $P < 0.05$. Planned paired contrasts showed that, in the case of RVIP, the interaction was driven by lower probability of hits in cannabis users carrying the *val/val* genotype (see Table 4 and Figure 1); *val/val* cannabis users performed significantly worse than *val/val* controls ($P = 0.002$). Additional analyses of the RVIP signal detection parameters showed that *val/val* cannabis users exhibited significantly higher response bias (B') scores than *val/val* non-users ($P = 0.02$). Conversely, their discrimination scores (A') were lower, although this difference was not significant. No differences were found for *met* carriers. In the case of ID/ED, the interaction was driven by the significant difference between cannabis users carrying the

Table 4 Neuropsychological Performance as a Function of Gene \times Study Group Interaction

	Cannabis (n = 86)	Control (n = 58)	F (1,136) ^a	P-value ^a
<i>RVP: probability of hit</i>				
COMT <i>val/58met</i>			5.17	0.02
<i>Val/val</i>	61.6 (14.9)	76.6 (11.4)		
<i>Val/met and met/met</i>	69.0 (17.6)	70.7 (14.2)		
<i>RVP: alpha</i>				
COMT <i>val/58met</i>			2.93	0.09
<i>Val/val</i>	0.90 (0.04)	0.94 (0.03)		
<i>Val/met and met/met</i>	0.92 (0.05)	0.92 (0.04)		
<i>RVP: beta</i>				
COMT <i>val/58met</i>			4.16	0.04
<i>Val/val</i>	0.95 (0.05)	0.88 (0.12)		
<i>Val/met and met/met</i>	0.92 (0.10)	0.93 (0.08)		
<i>IED total errors adjusted</i>				
COMT <i>val/58met</i>			6.06	0.01
<i>Val/val and val/met</i>	28.0 (22.6)	21.0 (19.1)		
<i>Met/met</i>	12.3 (6.5)	29.1 (22.0)		
<i>IED extradimensional errors</i>				
COMT <i>val/58met</i>			6.51	0.01
<i>Val/val and val/met</i>	11.6 (11.3)	7.7 (9.01)		
<i>Met/met</i>	6.6 (6.5)	15.6 (11.3)		
<i>Iowa Gambling Test</i>				
5-HTTLPR			3.78	0.05
<i>L/l and l/s</i>	6.1 (21.4)	8.0 (23.5)		
<i>S/s</i>	-1.5 (21.3)	20.2 (25.3)		

Results are presented as mean (SD).

^aP-value of interaction term in ANCOVA model adjusting for sex, age, smoking status, and IQ, with the exception of Iowa Gambling Task, in which age and smoking status were not associated with performance.

val allele and cannabis users carrying the *met/met* genotype ($P = 0.02$), the former showing a higher number of errors (Table 4). The detailed analysis of intra- vs extradimensional errors showed that differences were driven by extradimensional or shifting errors (Table 4).

The N-back task was analyzed using a 'group \times genotype \times working memory load' mixed model. Although the interaction effect was not significant, $\chi^2(1,138) = 2.08$, $P = 0.14$, visual inspection of the data shows that performance mildly varies between groups as a function of genotype and load: whereas controls carrying different genotypes have a uniform drop of performance as a function of load, *met/met* cannabis users have a smoother drop of performance as a function of load with respect to *val* carriers (see Supplementary Figure 1).

We found no drug \times gene interaction effects on the SOC, the IGT, or the total cognitive index.

Cannabis use \times 5-HTTLPR. Results showed a significant drug \times gene interaction on IGT, $F(1,138) = 3.78$, $P = 0.05$. Planned paired contrasts showed that the interaction was driven by significant differences between cannabis users and controls carrying the *s/s* genotype (see Table 4 and Figure 2); *s/s* cannabis users performed significantly worse than *s/s* controls ($P = 0.02$) (see Table 4). To further explore decision-making learning across the task, we analyzed the 'group \times block' interaction using a mixed model. The analysis showed evidence of an interaction effect, $\chi^2(1,138) = 3.59$, $P = 0.05$, and paired comparisons showed this effect was driven by the significant differences between *s/s* cannabis users and *s/s* controls ($P = 0.01$). Visual inspection of the group by block learning pattern show that, whereas *s/s* controls have a steep learning gain across blocks, *s/s* cannabis users take longer to learn the task (performance still < 0 by block 4) (see Figure 2). No differences were found for *l* carriers.

We found no drug \times gene interaction effects on the N-back, RVIP, ID/ED, SOC, or total cognitive index.

DISCUSSION

This study yielded two major findings: (i) daily cannabis consumption does not significantly impair executive functions among young users, not even in users with age of onset before 15 years old; but (ii) *COMT val158met* and *5-HTTLPR* genetic polymorphisms moderate the associa-

tion between cannabis use and executive functions. Specifically, cannabis users carrying the *val/val* genotype of the *COMT val158met* gene exhibited lower accuracy of sustained attention associated with a more strict response bias than non-users carrying the same genotype. The cannabis users carrying the *val* allele also committed more monitoring/shifting errors than cannabis users carrying the *met/met* genotype. Finally, cannabis users carrying the *5-HTTLPR s/s* genotype had worse decision-making skills than their non-user peers with the same genotype. Therefore, results indicate that certain genotypes of the *COMT* and the *SLC6A4* genes strengthen the impact of cannabis use of different executive functions.

With respect to the first finding, the absence of general adverse effects of cannabis use on executive functions is consistent with the outcomes from the most exhaustive meta-analyses conducted so far about the link between cannabis and cognition (Grant et al, 2003). They concluded that the main adverse effect of cannabis use on cognitive function was restricted to the domains of learning and memory, whereas effects were not significant for the attention/ executive control domain, which we addressed here. Previous studies have argued that the adverse effects on cannabis use on executive function may be dose-related (Bolla et al, 2002). Partially in agreement with this notion, we showed that the CBD/THC hair concentration correlated with a higher number of movements to achieve the correct response in the SOC, pointing to a mild dose-dependent effect of cannabis use on planning skills. Planning deficits have been previously reported as long-term effects of cannabis use in a remarkably well-controlled twin study, in which non-cannabis user twins served a control group for their cannabis user siblings (Lyons et al, 2004). Nonetheless, the overall results extend the notion that regular cannabis use is not substantially harmful for executive control among daily users. A complementary notion, which we further tested, is whether individual differences in genetic makeup may place only certain cannabis users at higher risk of developing executive impairment.

According to our second finding, there are substantial moderation effects of the *COMT val158met* and the *5-HTTLPR* genetic polymorphisms on the potentially harmful effects of cannabis use on particular components of executive control. Importantly, these moderation effects are biologically plausible and in agreement with previous knowledge about the influence of these polymorphisms on

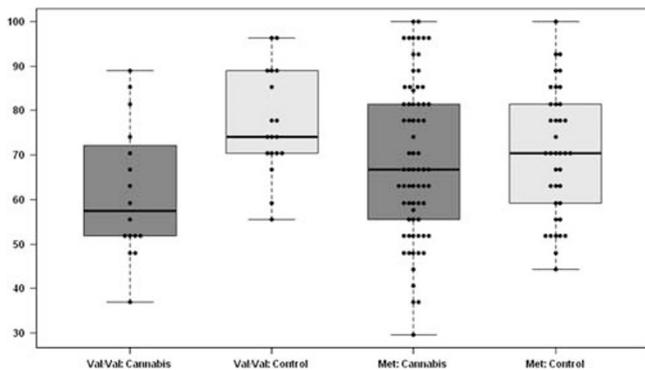


Figure 1 Rapid visual information processing's proportion of hits as a function of catechol-O-methyltransferase (*COMT*) gene and group.

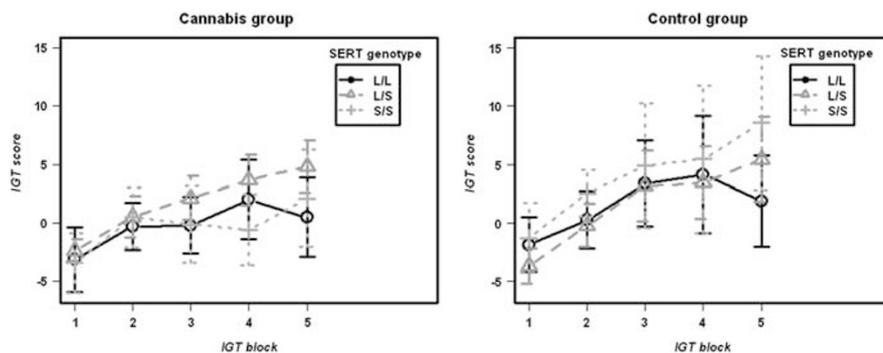


Figure 2 Block by block Iowa Gambling Task's net scores as a function of serotonin transporter (*SERT*) gene and group.

executive mechanisms. With regard to the *COMT val158met* polymorphism, we found that cannabis users carrying the *val/val* genotype exhibited lower accuracy in detecting target sequences during sustained attention, and more strict response bias (less proneness to respond). This genotype has been associated with altered functioning of dopamine richly innervated prefrontal–parietal networks (Tan *et al*, 2007), and more lapses during sustained attention in healthy volunteers (Hamidovic *et al*, 2010). On the side of cannabis use, animal models have demonstrated that cannabinoid agonists significantly increase omission errors and response latencies (but not commission errors) in the 5-choice serial reaction time task (5CSRTT) (Pattij *et al*, 2008), which is an animal analog of the continuous performance test used here (Chamberlain *et al*, 2011). Further evidence has shown that administration of dopamine D1 partial agonists and D2/D3 antagonists produce similar effects on the 5CSRTT, impacting sustained attention by increasing omission (or vigilance) errors (Barnes *et al*, 2012; Besson *et al*, 2010). These findings are in agreement with our results, which show that the *COMT val/val* genotype (associated with lower dopamine availability) and cannabis use (potentially impacting dopamine function) interactively impact vigilance accuracy in the context of a stricter response bias, which mainly promotes omission errors. Whereas the *val/val* genotype has been associated with abnormally lower tonic dopamine functioning, the other monozygotic variant (*met/met*) has been linked to reduced phasic dopamine turnover (Bilder *et al*, 2004), and set-shifting decrements in both animal and human studies (Nolan *et al*, 2004; Tunbridge *et al*, 2004). Our results replicated these patterns in the case of healthy individuals (*met/met* carriers committed more shifting errors than *val/val* carriers). However, the pattern of performance was reversed in cannabis users, in which the *val* carriers showed significantly higher errors than *met/met* cannabis carriers. This reversed pattern is consistent with structural imaging data of the *COMT* gene \times cannabis use interaction in regions relevant for stimulus–outcome learning and shifting, as we have recently shown that the *val* allele is associated with smaller ventral caudate volumes in cannabis users, whereas the opposite is true for controls (Batalla *et al*, 2012).

A second cannabis \times gene interaction effect was found for the link between cannabis use and decision making, moderated by the *5-HTTLPR* polymorphism: cannabis users carrying the *s/s* genotype took longer to learn and performed significantly more poorly the IGT than *s/s* control carriers. This effect is consistent with the association between the *s/s* genotype and poorer IGT performance, especially during initial phases of the task (van den Bos *et al*, 2009; He *et al*, 2010; Homberg *et al*, 2008), in which feedback-based learning about the emotional outcomes associated with each deck is critical to guide decisions towards safe options (Bechara *et al*, 2005). It is also consistent with previous evidence of drug-use interactions with the *s/s* genotype impacting emotion-based impulsive decision-making (Roiser *et al*, 2005). The link between cannabis use and serotonin transporter function is supported by evidence that high doses of cannabinoid agonists induce reduction of platelet serotonin concentrations, accompanied by alterations of the functioning of the serotonergic system in cannabis chronic smokers (Velenovská and Fisar, 2007). There is also evidence that the proposed link between

cannabis use and depression is only present in cannabis users carrying the *s/s* genotype (Otten and Engels, 2011). These findings are in agreement with the notions that: (i) cannabis use impacts serotonin function; and (ii) this interaction may exacerbate behavioral vulnerabilities associated with the *s/s* genotype. In this respect, recent studies have identified different—but related—behavioral phenotypes associated with the *s/s* genotype, including negative emotionality and hypervigilance (Homberg and Lesch, 2011). Overall, the evidence suggest that cannabis use may exacerbate *s/s* genotype-related behavioral phenotypes, such as negative emotionality or hypervigilance, which hamper the ability of cannabis users to learn adequately from negative feedback in the IGT.

In summary, we showed that cannabis users carrying the *COMT val/val* genotype exhibit lower accuracy of sustained attention than controls carrying the same genotype, that cannabis users *val* carriers commit more shifting errors than cannabis users *met/met* carriers, and that cannabis users carrying the *SERT s/s* genotype have poorer decision-making than controls carrying the same genotype. This study holds a number of important strengths and also limitations worth noting. Strengths include the thorough control of potential confounders associated with the effects of cannabis use and genetic makeup. These confounders were addressed first during the process of recruitment and assessment of inclusion/exclusion criteria, in which we excluded individuals with comorbid substance-use or psychiatric disorders (eg, psychosis), and then in statistical analyses, in which we controlled for potential effects of age, gender, IQ, and smoking. In addition, we ruled out potential effects of acute cannabis use and other illegal drugs use by means of combined urine and hair toxicological analyses and medical control of withdrawal effects. An additional strength was the representativeness of the sample, which extracted a large number of non-treatment-seeking daily cannabis users and matched non-drug user controls. As for the limitations, we should first acknowledge that our findings apply to small subsets of cannabis using individuals (*val* and *s/s* carriers), whereas we did not find main effects of any of the genotypes, and could not perform combined haplotype analyses. Nonetheless, our results inform about the molecular mechanisms by which cannabis use may exert a negative impact on executive functions, and they may entail important clinical implications. For example, the *COMT* inhibitor tolcapone has been proposed as a promising treatment for marijuana dependence (Weinstein and Gorelick, 2011), and its effect should be equivalent to a phenocopying of subjects' carriers of the *val/val* genotype to the *met/met* genotype, resulting in more sustained dopamine brain concentrations. Therefore, the poor performance of cannabis using *val* carriers may improve after this therapeutic approach. Because of the impact of the *COMT val* allele on the neurodegeneration of dopamine networks (Gennatas *et al*, 2012), future studies should also explore whether this gene variant is associated with accelerated cognitive aging in cannabis users. A second limitation we are aware of is the problem of the large number of multiple comparisons. However, we only corrected for multiple tests in case of pairwise group comparisons for a given cognitive task. The alternative option of globally applying the Bonferroni correction was

discarded because the resulting critical significance level for each test would have been extremely small, thus overly controlling for Type-1 error, but largely increasing the probability for Type-2 error. An additional shortcoming was the lack of significant correlations between cannabis use and cognition. This may be due, along with other factors, to the relative homogeneity of cannabis use patterns among participants, thus reducing variability of distributions and opportunities to detect associations.

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DISCLOSURE

The authors declare no conflict of interest.

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