

# Chronic Adolescent Exposure to $\Delta$ -9-Tetrahydrocannabinol in COMT Mutant Mice: Impact on Psychosis-Related and Other Phenotypes

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Cannabis use confers a two-fold increase in the risk for psychosis, with adolescent use conferring even greater risk. A high–low activity catechol-O-methyltransferase (COMT) polymorphism may modulate the effects of adolescent  $\Delta$ -9-tetrahydrocannabinol (THC) exposure on the risk for adult psychosis. Mice with knockout of the COMT gene were treated chronically with THC (4.0 and 8.0 mg/kg over 20 days) during either adolescence (postnatal days (PDs) 32–52) or adulthood (PDs 70–90). The effects of THC exposure were then assessed in adulthood across behavioral phenotypes relevant for psychosis: exploratory activity, spatial working memory (spontaneous and delayed alternation), object recognition memory, social interaction (sociability and social novelty preference), and anxiety (elevated plus maze). Adolescent THC administration induced a larger increase in exploratory activity, greater impairment in spatial working memory, and a stronger anti-anxiety effect in COMT knockouts than in wild types, primarily among males. No such effects of selective adolescent THC administration were evident for other behaviors. Both object recognition memory and social novelty preference were disrupted by either adolescent or adult THC administration, independent of genotype. The COMT genotype exerts specific modulation of responsivity to chronic THC administration during adolescence in terms of exploratory activity, spatial working memory, and anxiety. These findings illuminate the interaction between genes and adverse environmental exposures over a particular stage of development in the expression of the psychosis phenotype.

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**Keywords:** animal model; adolescence; psychosis; COMT;  $\Delta$ -9-tetrahydrocannabinol; gene  $\times$  environment interaction

## INTRODUCTION

Cannabis consumption has been associated with a doubling of the risk for schizophrenia (Arseneault *et al*, 2004; Henquet *et al*, 2005; Moore *et al*, 2007) and with a negative impact on the expression and course of schizophrenia (Martinez-Arevalo *et al*, 1994; Linszen *et al*, 1994). Systemic, acute administration of  $\Delta$ -9-tetrahydrocannabinol (THC), the psychoactive component of cannabis, has been shown to transiently induce positive symptoms, negative symptoms, and cognitive impairment, including deficits in working memory and executive function, in healthy subjects (D'Souza *et al*, 2004, 2009; Stefanis *et al*, 2004; Morrison *et al*, 2009) and to exacerbate these features in patients with

schizophrenia (D'Souza *et al*, 2005). Although cannabinoids produce a variable pattern of schizophrenia-like symptoms in healthy subjects, it has been proposed that the developmental timing of cannabis exposure and genetic factors may interact to modulate the effects of cannabis on the risk for psychosis (D'Souza *et al*, 2009).

It seems that the risk for psychosis is greater in those subjects who use cannabis in adolescence (Arseneault *et al*, 2002; Ashton, 2003). Adolescence may be a stage of particular vulnerability to the effects of THC, as cannabinoid receptors have been shown to mature slowly, reaching maximal levels during adolescence that may reduce subsequently because of postadolescent pruning, in a manner similar to that described for dopamine (DA) receptors (Rodriguez de Fonseca *et al*, 1993; McLaughlin *et al*, 1994; Belue *et al*, 1995; Seeman, 1999). Chronic THC administration in adolescent rats has been shown to induce persistent changes in cognitive and emotional processes in adulthood (Schneider and Koch, 2003; Cha *et al*, 2006, 2007; Rubino *et al*, 2009).

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The *catechol-O-methyltransferase* (*COMT*) gene, located on chromosome 22q11 (a region implicated in schizophrenia), encodes the COMT enzyme, which is involved in the degradation of DA, particularly in the prefrontal cortex (PFC). The human *COMT* gene contains a common functional polymorphism (valine (Val) substitution for methionine (Met)) at the 158/108 locus, with the Met allele resulting in a four-fold reduction in enzymatic activity (Tunbridge *et al*, 2006). *COMT* Val108Met allelic variation has been associated with differential performance on tasks measuring PFC-mediated cognition, including working memory and executive function (Egan *et al*, 2001; Tunbridge *et al*, 2006). Mutant mice bearing targeted mutations of *COMT* provide some insights into the role of COMT in several aspects of behavior, including PFC-mediated cognition (Gogos *et al*, 1998; Desbonnet *et al*, 2009; Kirby *et al*, 2010). Male *COMT* knockout (KO) mutants show improvement in spatial working memory (Babovic *et al*, 2008; Papaleo *et al*, 2008), whereas, conversely, a transgenic *COMT* mutant involving overexpression of the human *COMT*-VAL polymorphism shows deficits in attentional set shifting, recognition memory, and working memory performance; low-dose amphetamine restored recognition memory, confirming DAergic involvement and providing further support for an inverted U-shaped relationship between cognitive function and cortical DAergic activity (Seamans and Yang, 2004; Tunbridge *et al*, 2006).

A longitudinal birth cohort study showed that cannabis use was most likely to be followed by psychosis among those who used cannabis during adolescence and were homozygous for the *COMT* Val108Met allele (Caspi *et al*, 2005). Carriers of the Val allele are also more likely to show THC-induced psychotomimetic and amnesic effects than Met carriers, although this was conditional on increased 'psychosis proneness' as measured by psychometric tests (Henquet *et al*, 2006). Although the underlying mechanism is unknown, these gene  $\times$  environment findings have provided new clues to how adolescent cannabis use may lead to psychosis. It is proposed that exploration of a *COMT*-linked component underlying the induction of psychosis-relevant phenotypes after chronic adolescent *vs* adult THC treatment has the potential to provide insight into the lasting psychotomimetic effects of chronic cannabis use. The aim of this study was to investigate psychosis-relevant behavioral phenotypes in adult *COMT* KO mice after chronic adolescent *vs* adult THC exposure. It was hypothesized that knockout of the *COMT* gene would preferentially modify the long-term effects of chronic adolescent *vs* adult THC exposure on the expression of various psychosis-relevant cognitive, social, and anxiety-related behaviors during adulthood.

## METHODS

### Animals

Mice containing the mutated *COMT* allele were generated at the Rockefeller University (New York, NY, USA) and backcrossed to C57BL6 mice for 10 generations (Gogos *et al*, 1998); breeding and genotyping at the Royal College of Surgeons in Ireland were performed as described previously (Babovic *et al*, 2007, 2008). After weaning on postnatal day

(PD) 21, pups from litters of the same generation were housed in groups of three to five per cage and maintained at  $21 \pm 1^\circ\text{C}$  on a 12-h light-dark cycle (0700 h on; 1900 h off), with *ad libitum* access to food and water. Males and females of wild-type (WT), *COMT* heterozygous (HET), and *COMT* KO genotypes, 8–15 mice per sex, treatment and genotype, were treated over 20 consecutive days, either PDs 32–52, corresponding to adolescence or PDs 70–90, corresponding to adulthood. These studies were approved by the Research Ethics Committee of the Royal College of Surgeons in Ireland. They were conducted under license from the Department of Health and Children, in accordance with the Irish legislation and the European Communities Council Directive 86/609/EEC for the care and use of experimental animals, and from the Environmental Protection Agency in relation to the contained use of genetically modified organisms. All efforts were made to minimize the number of animals used and their suffering.

### Drugs

THC (Sigma-Aldrich, St Louis, MO, USA) was dissolved in saline:cremaphor:ethanol (18:1:1) for subcutaneous (s.c.) injection at 4.0 and 8.0 mg/kg (s.c., 4 ml/kg); these doses were selected on the basis of previous studies of chronic THC effects in rodents (Quinn *et al*, 2008; Rubino *et al*, 2009). Vehicle controls received identical injection of the final saline:cremaphor:ethanol solution. The ethanol concentration in the THC solution results in ethanol doses of approximately 0.05–0.15 g/kg; this vehicle solution has customarily been used by many laboratories for the solubilization of cannabinoids, and does not induce any significant behavioral effects (Rubino *et al*, 2009). After both adolescent (PDs 32–52) and adult (PDs 70–90) treatments, there was an interval of 21 days before behavioral testing, to exclude withdrawal effects. Initially, 10–15 mice per group (genotype/treatment/sex) were examined for exploratory activity; thereafter, a randomly selected subset ( $n = 8$ –10 per group) was examined in the following order of testing: elevated plus maze (EPM); sociability and social novelty preference; spontaneous alternation; novel object recognition; delayed alternation. All animals were assessed by an observer blinded to genotype and treatment, before killing at PDs 150–160.

### Behavioral Measures

**Exploratory activity.** Each mouse was placed individually in the center of an open field apparatus ( $27.9 \times 27.9\text{ cm}^2$ ; ENV-510, Med Associates, St Albans, VT, USA). Total ambulatory counts, distance traveled (cm), and vertical activity were recorded over a period of 240 min.

**Spontaneous alternation.** Spatial working memory was assessed using the continuous variant of the Y-maze spontaneous alternation procedure (Lalonde, 2002; Hughes, 2004) as described previously (O'Tuathaigh *et al*, 2007; Babovic *et al*, 2008). Briefly, the Y-maze apparatus consisted of three identical arms (each  $40 \times 12.5 \times 40\text{ cm}^3$ ). Without previous habituation, each test mouse was placed at the center of the Y maze and allowed to move freely throughout the maze for a single 10-min period. Spontaneous alternation

was defined as successive entries into the three arms, in overlapping triplet sets. It is expressed as a percentage and refers to the ratio of arm choices differing from the previous two choices to the total number of arm entries:  $\text{percentage alternation} = ((\text{number of alternations} / \text{total number of arm entries}) - 2) \times 100$ .

**Delayed alternation.** Spatial working memory was assessed further in terms of the delayed variant of the Y-maze alternation procedure, using a manually operated, modified Y maze (Modular Maze, Med Associates), as described previously for T-maze alternation (Lai *et al*, 2006). All mice were gradually introduced to a food restriction regimen over a period of 7–10 days, until they had reached 85% of free-feeding weight. Over a subsequent period of 3 days, mice were habituated to the training environment. They were trained to retrieve reinforcing food pellets (12 mg; TestDiet, Richmond, IN, USA) at the end of each arm; this was followed by 10–12 days during which the mouse was rewarded with a food pellet when a successful alternation was achieved. After mice made at least 70% correct choices on 2 consecutive days, the testing phase began. For this, delays of 0, 15, or 30 s were randomly introduced between the sample run and choice run. Mice were given 4 trials of each delay per day of testing, for a total of 12 trials for each delay. Any animal that responded poorly to the food restriction regimen or failed to acquire the alternation task (one adolescent WT treated with vehicle, two adult COMT HET treated with 4 mg/kg THC) was excluded from the analysis. Owing to the labor intensity of the procedure, this assessment could be conducted only in males.

**Novel object recognition.** Recognition memory was assessed using the novel object recognition paradigm with two retention intervals (5 min and 1 h) and the same arena dimensions and novel objects as described previously (Babovic *et al*, 2008). The experiment was conducted under standard room lighting and proceeded according to the following stages:

(1) **Habituation phase:** Twenty four hours before testing, mice were placed in the arena and allowed to explore freely for two consecutive sessions of 30 min each, with an inter-session interval of 15 min.

(2) **Sample phase:** Mice were first allowed to explore the arena for two further sessions of 30 min, separated by an inter-session interval of 15 min. Two identical objects, A1 and A2, were then placed at the rear left and right corners of the arena, 5 cm from both walls to facilitate exploration around each object. Each test mouse was placed at the midpoint of the wall opposite to the sample objects, with nose facing the wall and away from objects, to remove any unintentional bias, and allowed to explore the arena for 5 min. Time spent interacting with each object was recorded manually. At the end of the sample phase, the test mouse was returned to its home cage.

(3) **Test phase 1:** After an inter-trial interval of 5 min, the test mouse was placed in the arena now containing familiar object A1 and novel object B1 for 5 min. Time spent interacting with each object was recorded manually for 5 min.

(4) **Test phase 2:** After an inter-trial interval of 1 h (ie, the period between completion of test phase 1 and start of test phase 2), the test mouse was placed in the arena now containing familiar object B1 and novel object C1. Time spent interacting with each object was recorded manually for 5 min. Both the sequence of object presentation and the position of familiar/novel objects were counterbalanced between animals. Time spent interacting with each object was determined as nose or whiskers touching the object or directed toward it within <1 cm, or touching the object with a paw. Accidental contact with object (ie, bumping or using the object as a platform to explore the arena) was not counted as interaction with the object.

**Sociability and social novelty preference.** This task has been used to provide an easily quantifiable measure of affiliative behavior and behavioral response to social novelty (Moy *et al*, 2004; Sankoorikal *et al*, 2006). Sociability and social novelty preference were assessed in a three-chamber apparatus using a two-stage paradigm, as detailed previously (O'Tuathaigh *et al*, 2007; Babovic *et al*, 2008):

(1) **Sociability:** Before the sociability phase, the test mouse was allowed a 15-min habituation period during which the two doors connecting the three chambers were opened, enabling free access to the entire apparatus. After this habituation phase, an unfamiliar same-sex C57BL6 mouse (Stranger 1) was placed in either the left or right chamber enclosed in a small, internal wire cage ( $10 \times 10 \times 12 \text{ cm}^3$ ), which allowed nose contact; placement of the Stranger mouse in the left or right chamber alternated between trials, with an empty but otherwise identical wire cage in the opposite chamber. Each Stranger mouse had been habituated to placement in the small, internal wire cage 24 h before testing. After placement of the Stranger mouse into the left or right chamber, both doors to these side chambers were then opened, and the test mouse was allowed to leave the center chamber and explore all three chambers of the apparatus for a period of 10 min. Entry into either chamber was defined as all four paws in that chamber.

(2) **Social novelty preference:** Following the sociability phase, each test mouse was immediately returned to the center chamber and the doors to the side chambers were closed. There followed a second session to measure social preference for a novel Stranger. With the initial Stranger 1 (now Familiar) retained in its original chamber, a second, Stranger 2 mouse (Novel) was placed in the previously empty but otherwise identical small wire cage in the opposite chamber. After placement of the Novel mouse into the chamber opposite to that still containing the Familiar mouse, both doors to the side chambers were opened, and the test mouse was allowed to leave the center chamber and explore all three chambers of the apparatus for another period of 10 min. In the assessment of social novelty preference, all other parameters and measures were as described above for assessment of sociability.

**Elevated plus maze.** Emotional/anxiety-related behavior was assessed in the EPM (Pellow and File, 1986) as outlined previously (Babovic *et al*, 2010). The EPM consisted of two opposing arms surrounded by cream-colored, chip-board walls (closed arms; 12 cm high, 9 cm wide) and

two opposing arms devoid of walls (open arms) elevated 25 cm above ground level under dim lighting conditions (>50 lux). Cumulative time spent in open (aversive) and closed (nonaversive) arms was recorded during a 5-min session, together with the number of entries into each arm as defined by all four paws being placed in that arm; an increase or decrease in time spent in the open arm reflects, respectively, a decrease or increase in 'anxiety,' with total number of arm entries reflecting level of 'activity.'

### Statistical Analysis

This followed procedures similar to those described previously (O'Tuathaigh *et al*, 2007, 2010; Babovic *et al*, 2008), using analysis of variance (ANOVA) with *post hoc* comparisons by Dunnett's test, corrected for multiple comparisons: exploration in a novel environment was analyzed in terms of activity counts over 1–4 h of habituation; spontaneous alternation was analyzed in terms of percentage alternation and number of arm entries; delayed alternation was analyzed in terms of latency to reach alternation criterion and percentage correct alternation at each delay interval (mean across trials; 0, 15, 30 s); novel object recognition was analyzed in terms of time exploring the novel object during the sample phase, test phase 1, and test phase 2; sociability and social novelty preference were analyzed in terms of total time spent in and number of entries into each chamber; and the EPM was analyzed in terms of absolute or percentage time spent in and number of entries into each arm.

For the above phenotypic measures, we hypothesized *a priori* that drug treatment would be modulated by treatment age and sex (see the 'Introduction' section, Desbonnet *et al*, 2009; Kirby *et al*, 2010); thus, where appropriate, separate ANOVA analyses were also conducted within adolescent-treated, adult-treated, male and female groups. Although the focus of these analyses is on whether the effects of chronic THC were modified by the *COMT* genotype in relation to age at treatment, a number of ancillary findings on general responsivity to chronic THC in relation to age at treatment and sex, in a manner unrelated to genotype, are reported separately in Supplementary data. All statistical analyses were carried out using the SPSS software package (Version 14, SPSS, Chicago, IL, USA).

## RESULTS

### Exploratory Activity

All groups readily displayed habituation in terms of reduction in activity counts over 1–4 h of exploration (effect of hours:  $F(3, 269) = 410.00$ ,  $p < 0.001$ ). Among adolescent-treated mice, the effects of THC on activity differed between the genotypes over time in a manner that varied between the sexes (drug  $\times$  genotype  $\times$  hours  $\times$  sex interaction:  $F(12, 145) = 1.98$ ,  $p = 0.03$ ); over 1 h, THC increased exploratory activity in male *COMT* HET at 8.0 mg/kg and in male *COMT* KO at 4.0 and 8.0 mg/kg (Table 1). No such effects were evident in female adolescent-treated mice or in either male or female adult-treated mice. See also Supplementary data.

### Spontaneous Alternation

WT mice receiving vehicle displayed typical spontaneous alternation. In male adolescent-treated mice, THC disrupted alternation in a manner that differed between the genotypes (drug  $\times$  genotype interaction:  $F(4, 79) = 2.81$ ,  $p = 0.03$ ), whereas vehicle-treated *COMT* KO showed an increase in alternation, 8.0 mg/kg THC increased alternation in *COMT* HET but reduced alternation in *COMT* KO (Figure 1a). In female adolescent-treated mice, chronic THC disrupted alternation in a manner unrelated to genotype (effect of drug:  $F(2, 67) = 5.09$ ,  $p < 0.01$ ); 8.0 mg/kg THC reduced alternation in both WT and *COMT* KO (Figure 1c). No such effects on alternation were evident in either male or female adult-treated mice (Figures 1b and d), and no corresponding effects on number of arm entries were evident in either male or female adolescent- or adult-treated mice (data not shown). See also Supplementary data.

### Delayed Alternation

During training, there were no effects of genotype or treatment on time to reach at least 70% correct alternation. WT mice receiving vehicle displayed typical delayed alternation. Among experimental groups (males only; see the 'Methods' section), there were no effects of THC or genotype, or interactions with genotype, in terms of latency to reach the 70% correct criterion and alternation using either the 0-s or 30-s delay interval; vehicle-treated *COMT* KO exhibited an increase in alternation at the 15-s interval relative to WT ( $t(15) = 2.18$ ,  $p < 0.05$ ) (Figure 2a). However, at the 30-s delay interval, in adolescent-treated mice, THC disrupted alternation in a manner that differed between the genotypes (drug  $\times$  genotype  $\times$  treatment age interaction:  $F(4, 129) = 2.78$ ,  $p = 0.04$ ), whereas vehicle-treated *COMT* KO showed an increase in alternation at the 15-s interval relative to WT ( $t(15) = 2.18$ ,  $p < 0.05$ ) (Figure 2a). No such effects on delayed alternation were evident in adult-treated mice (Figure 2b).

### Novel Object Recognition

WT mice receiving vehicle displayed typical recognition of the novel object at the 5-min retention interval, which weakened at the 1-h retention interval. Among experimental groups, there were no effects of THC or genotype, or interactions with genotype, in terms of duration of object exploration during the sample phase or at the 5-min retention interval (test phase 1); at the 1-h retention interval (test phase 2), THC administered during either adolescence or adulthood disrupted recognition (effect of drug:  $F(2, 255) = 3.43$ ,  $p = 0.03$ ); but there were no effects of genotype or interactions with genotype (Table 2). See also Supplementary data.

### Sociability and Social Novelty Preference

During the sociability phase, all experimental groups demonstrated a preference for spending more time in, and making more entries into, the chamber containing the unfamiliar mouse relative to the opposite, empty chamber (effect of chamber: time,  $F(1, 244) = 58.10$ ,  $p < 0.001$ ; entries,

**Table 1** Exploratory Activity (Ambulatory Counts; Means  $\pm$  SEM,  $n = 8-10$  Per Group) in the Open Field During 1-4 h in Male and Female WT, COMT HET, and COMT KO Mice After Adolescent vs Adult THC (4.0, 8.0 mg/kg) or vehicle (VEH)

	Adolescent				Adult			
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 1	Hour 2	Hour 3	Hour 4
<i>Males</i>								
WT								
VEH	2855 $\pm$ 325	849 $\pm$ 171	298 $\pm$ 87	377 $\pm$ 249	3503 $\pm$ 456	694 $\pm$ 231	702 $\pm$ 154	641 $\pm$ 173
THC (4 mg/kg)	2386 $\pm$ 103	1079 $\pm$ 141	724 $\pm$ 134	704 $\pm$ 176	3080 $\pm$ 374	1130 $\pm$ 210	416 $\pm$ 70	404 $\pm$ 113
THC (8 mg/kg)	2703 $\pm$ 191	744 $\pm$ 75	450 $\pm$ 101	495 $\pm$ 160	3568 $\pm$ 339	1320 $\pm$ 490	659 $\pm$ 227	475 $\pm$ 188
HET								
VEH	2606 $\pm$ 229	635 $\pm$ 104	337 $\pm$ 76	416 $\pm$ 149	2615 $\pm$ 438	1009 $\pm$ 224	613 $\pm$ 251	463 $\pm$ 225
THC (4 mg/kg)	2649 $\pm$ 309	625 $\pm$ 74	669 $\pm$ 140	564 $\pm$ 156	2722 $\pm$ 460	1389 $\pm$ 312	487 $\pm$ 187	324 $\pm$ 163
THC (8 mg/kg)	3359 $\pm$ 380*	791 $\pm$ 122	654 $\pm$ 141	722 $\pm$ 202	3269 $\pm$ 185	814 $\pm$ 182	591 $\pm$ 108	337 $\pm$ 124
KO								
VEH	2291 $\pm$ 175	838 $\pm$ 84	661 $\pm$ 133	473 $\pm$ 150	3806 $\pm$ 653	804 $\pm$ 109	289 $\pm$ 54	302 $\pm$ 123
THC (4 mg/kg)	3257 $\pm$ 191*	735 $\pm$ 154	358 $\pm$ 116	456 $\pm$ 126	3425 $\pm$ 347	1770 $\pm$ 463	928 $\pm$ 477	693 $\pm$ 225
THC (8 mg/kg)	3387 $\pm$ 240*	948 $\pm$ 307	774 $\pm$ 316	616 $\pm$ 250	3481 $\pm$ 743	1410 $\pm$ 774	1228 $\pm$ 772	792 $\pm$ 329
<i>Females</i>								
WT								
VEH	2943 $\pm$ 177	735 $\pm$ 134	515 $\pm$ 155	709 $\pm$ 280	3166 $\pm$ 194	629 $\pm$ 88	366 $\pm$ 112	513 $\pm$ 188
THC (4 mg/kg)	2656 $\pm$ 485	813 $\pm$ 129	374 $\pm$ 78	530 $\pm$ 160	3054 $\pm$ 365	813 $\pm$ 129	502 $\pm$ 116	317 $\pm$ 201
THC (8 mg/kg)	2891 $\pm$ 203	1649 $\pm$ 489	1122 $\pm$ 359	1112 $\pm$ 191	2891 $\pm$ 203	889 $\pm$ 357	754 $\pm$ 219	616 $\pm$ 119
HET								
VEH	2808 $\pm$ 380	936 $\pm$ 184	617 $\pm$ 215	499 $\pm$ 140	2760 $\pm$ 276	1101 $\pm$ 277	837 $\pm$ 231	310 $\pm$ 77
THC (4 mg/kg)	3167 $\pm$ 399	996 $\pm$ 163	536 $\pm$ 169	579 $\pm$ 137	2897 $\pm$ 220	807 $\pm$ 218	438 $\pm$ 290	277 $\pm$ 117
THC (8 mg/kg)	3328 $\pm$ 523	1452 $\pm$ 435	903 $\pm$ 358	895 $\pm$ 377	3013 $\pm$ 412	1016 $\pm$ 399	692 $\pm$ 164	576 $\pm$ 289
KO								
VEH	3265 $\pm$ 373	1202 $\pm$ 119	520 $\pm$ 89	448 $\pm$ 111	3595 $\pm$ 465	1115 $\pm$ 344	886 $\pm$ 264	369 $\pm$ 121
THC (4 mg/kg)	3719 $\pm$ 442	812 $\pm$ 166	744 $\pm$ 229	474 $\pm$ 195	3643 $\pm$ 442	553 $\pm$ 165	694 $\pm$ 139	199 $\pm$ 45
THC (8 mg/kg)	3187 $\pm$ 499	808 $\pm$ 139	348 $\pm$ 93	379 $\pm$ 145	3843 $\pm$ 800	1009 $\pm$ 332	318 $\pm$ 120	290 $\pm$ 145

\* $p < 0.05$  vs VEH-treated controls.

$F(1, 244) = 191.00$ ,  $p < 0.001$ ); there were no effects of THC or genotype, or interactions with genotype, in relation to the sociability phase (data not shown).

During the social novelty preference phase, all experimental groups now displayed a preference for spending more time in, and making more entries into, the chamber containing the new, unfamiliar mouse relative to the opposite chamber containing the previous, now familiar mouse (effect of chamber: time,  $F(1, 244) = 71.12$ ,  $p < 0.001$ ; entries,  $F(1, 244) = 32.62$ ,  $p < 0.001$ ). THC treatment disrupted social novelty preference (chamber  $\times$  drug: time,  $F(2, 244) = 7.08$ ,  $p = 0.001$ ; entries,  $F(2, 244) = 9.68$ ,  $p < 0.001$ ), but there were no effects of genotype, or interactions with genotype (Figure 3). See also Supplementary data.

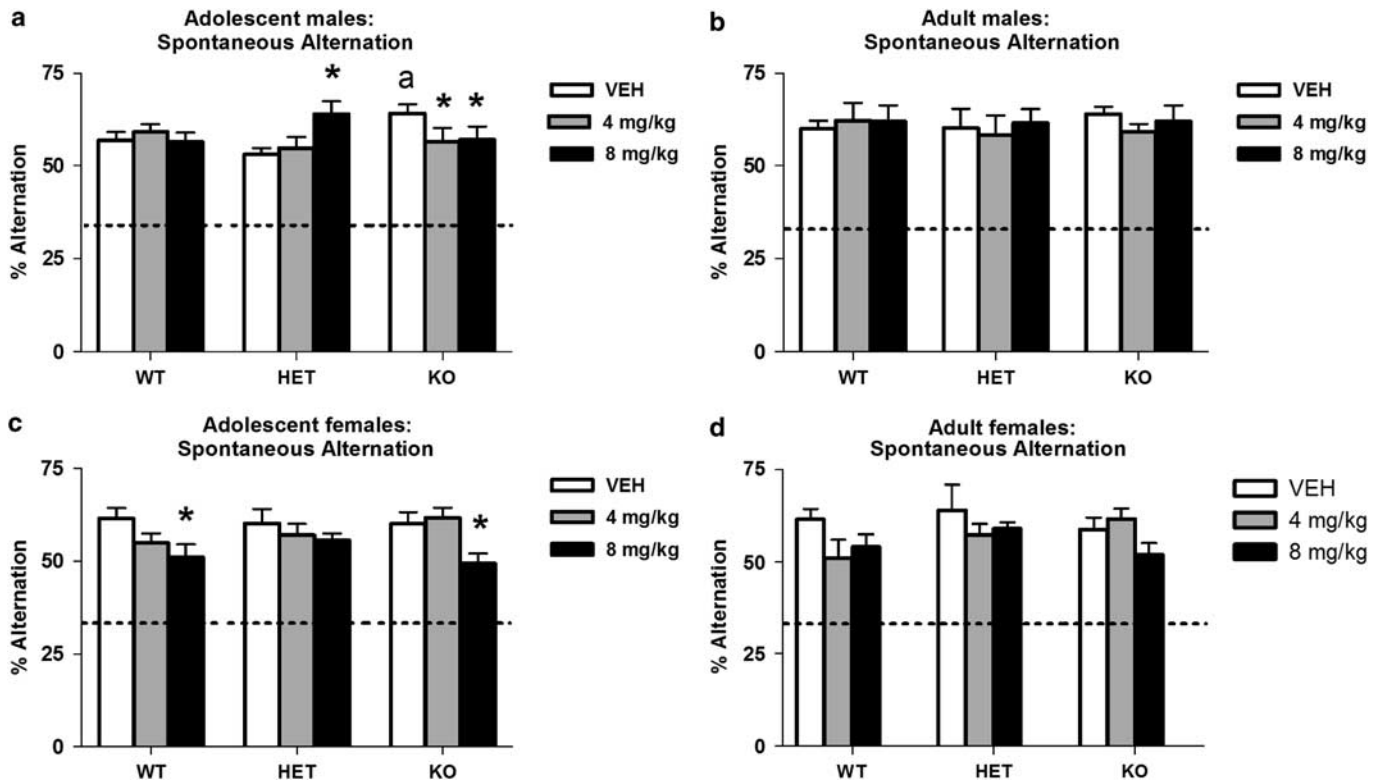
### Elevated Plus Maze

All experimental groups typically spent the majority of their time in the closed arms of the EPM. An effect of THC to increase time spent in the open arms (effect of drug:

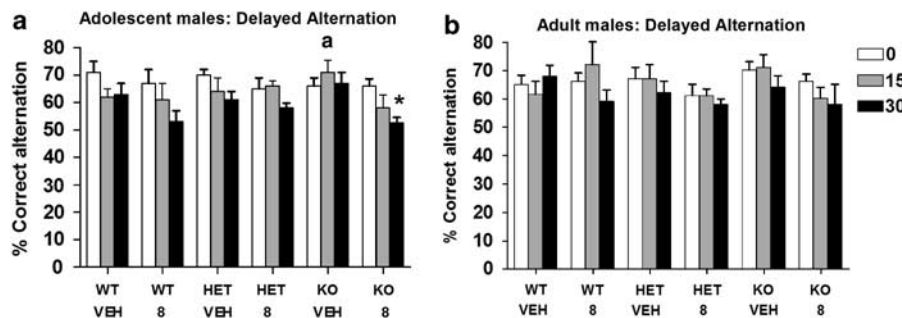
$F(2, 261) = 5.54$ ,  $p = 0.004$ ) differed between the genotypes in a manner that varied with treatment age and between the sexes (drug  $\times$  genotype  $\times$  treatment age  $\times$  sex interaction:  $F(4, 261) = 2.39$ ,  $p = 0.04$ ), whereas THC increased the time spent in the open arms in male, adolescent-treated COMT KO at 4.0 and 8.0 mg/kg, no such effects were evident in female adolescent-treated mice or in either male or female adult-treated mice (Table 3). THC increased the percentage of open arm entries (effect of drug:  $F(2, 261) = 3.81$ ,  $p = 0.02$ ) in a manner that did not differ between either the genotypes or the sexes; no effect of THC or genotype was evident on number of closed arm entries. See also Supplementary data.

### DISCUSSION

This study uses COMT HET and KO mice of both sexes to examine the effects of the COMT gene on behavioral responsivity to chronic THC administered over adolescence,



**Figure 1** (a–d) Spontaneous alternation (% alternation; means  $\pm$  SEM,  $n = 8$ –10 per group) in male and female WT, *COMT* HET, and *COMT* KO mice after adolescent male (panel a) vs adult male (panel b) or adolescent female (panel c) vs adult female (panel d) THC (4.0, 8.0 mg/kg) or vehicle (VEH); \* $p < 0.05$  vs VEH-treated controls; <sup>a</sup> $p < 0.05$  vs WT. The dotted line indicates chance performance levels in this task (33%).



**Figure 2** (a and b) Delayed alternation (% correct alternation; means  $\pm$  SEM,  $n = 8$ –10 per group) at the 0-, 15-, and 30-s intervals in male WT, *COMT* HET, and *COMT* KO mice after adolescent (panel a) vs adult (panel b) THC (8.0 mg/kg) or vehicle (VEH); \* $p < 0.05$  vs VEH-treated controls; <sup>a</sup> $p < 0.05$  vs WT.

in comparison with chronic THC administered over adulthood, using various phenotypic assessments related to psychosis. As summarized in Table 4, in the present gene  $\times$  environment interaction paradigms, adolescent THC administration induces a larger increase in exploratory activity, greater impairment in spatial working memory, in terms of both spontaneous and delayed alternation, and a stronger anti-anxiety effect in *COMT* KO than in WT, primarily among males; no such genotype- or sex-specific effects of adolescent THC administration were evident for novel object recognition and social behavior, in terms of both sociability and social novelty preference; no such effects of adult THC administration were evident for any behavior. Rather, both adolescent and adult

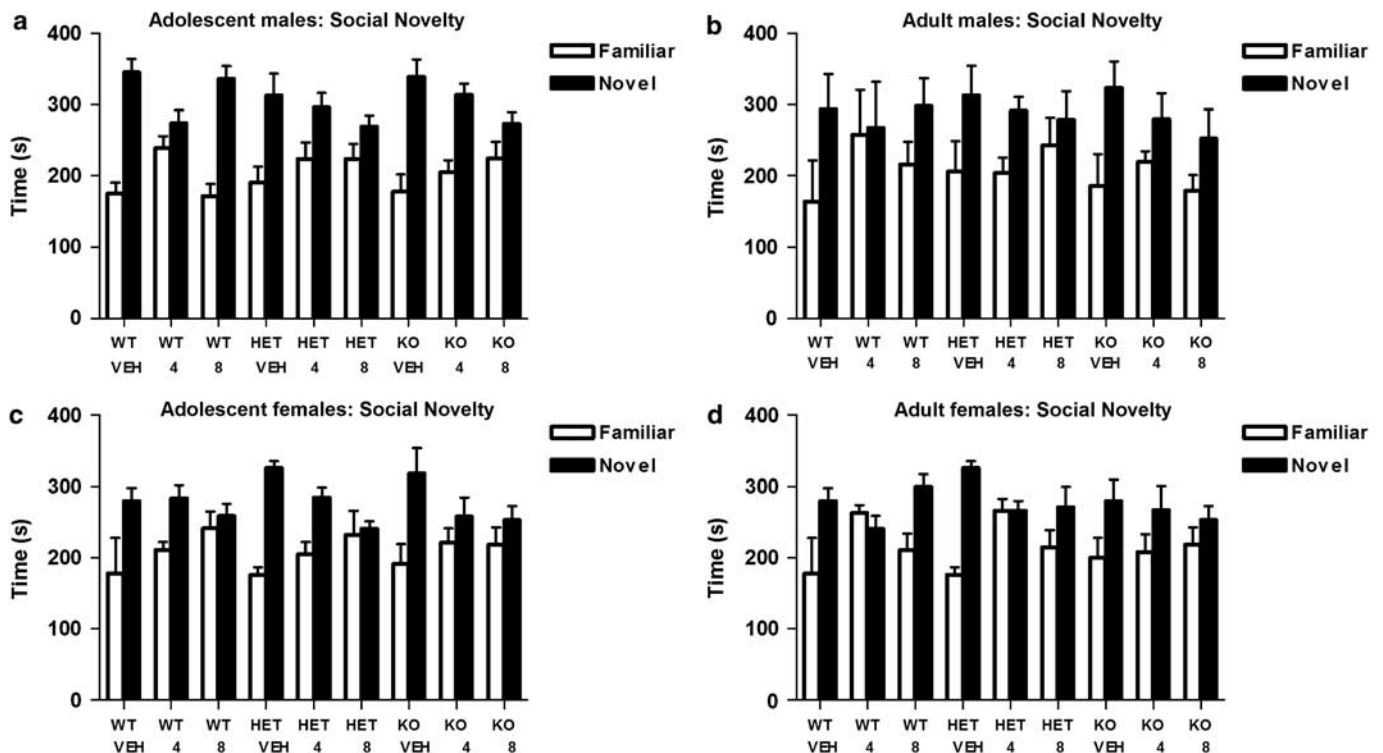
THC administration disrupted novel object recognition performance and social novelty preference, independent of genotype.

### COMT Modulation of Adolescent vs Adult THC Effects

**Exploratory Activity.** Spontaneous or psychostimulant-induced locomotor hyperactivity in a novel environment has long been associated with increases in mesolimbic DAergic transmission and is commonly adopted as a DA-linked ‘proxy’ measure of positive, psychotic symptoms (Rebec, 2006; Howes and Kapur, 2009; Perry *et al*, 2009; Van den Buuse, 2010). Although acute cannabis administration increases DAergic neuronal activity and DA release

**Table 2** Novel Object Recognition (% Novel Object Preference; Means  $\pm$  SEM,  $n = 16$ – $30$  Per Group) at the 5-min (Test Phase 1) and 1-h Retention Interval (Test Phase 2) in WT, *COMT* HET, and *COMT* KO Mice (Males And Females Composited) After Adolescent vs Adult THC (4.0, 8.0 mg/kg) or Vehicle (VEH)

	Adolescent			Adult		
	Sample phase	Test phase 1	Test phase 2	Sample phase	Test phase 1	Test phase 2
WT						
VEH	14.7 $\pm$ 4.0	61.5 $\pm$ 5.0	54.4 $\pm$ 2.1	12.3 $\pm$ 2.0	66.1 $\pm$ 5.6	57.0 $\pm$ 3.8
THC (4 mg/kg)	11.5 $\pm$ 1.9	64.3 $\pm$ 6.5	55.8 $\pm$ 3.8	25.0 $\pm$ 9.3	72.0 $\pm$ 9.0	49.7 $\pm$ 3.8
THC (8 mg/kg)	13.2 $\pm$ 1.9	70.6 $\pm$ 6.4	46.4 $\pm$ 4.5	12.8 $\pm$ 3.8	70.6 $\pm$ 5.4	54.0 $\pm$ 4.4*
HET						
VEH	16.4 $\pm$ 7.4	71.3 $\pm$ 7.9	52.2 $\pm$ 9.1	13.3 $\pm$ 3.1	59.0 $\pm$ 5.7	61.2 $\pm$ 7.0
THC (4 mg/kg)	19.4 $\pm$ 4.8	68.3 $\pm$ 1.8	53.4 $\pm$ 1.8	11.0 $\pm$ 4.1	68.5 $\pm$ 8.0	66.3 $\pm$ 10.1
THC (8 mg/kg)	24.1 $\pm$ 6.5	60.4 $\pm$ 4.6	44.6 $\pm$ 4.7*	15.8 $\pm$ 1.9	60.3 $\pm$ 4.5	58.0 $\pm$ 4.4*
KO						
VEH	10.8 $\pm$ 3.0	61.0 $\pm$ 6.0	58.4 $\pm$ 5.0	9.1 $\pm$ 6.0	59.3 $\pm$ 6.0	61.1 $\pm$ 6.0
THC (4 mg/kg)	15.8 $\pm$ 3.3	56.6 $\pm$ 3.8	52.4 $\pm$ 5.2	13.0 $\pm$ 7.8	63.0 $\pm$ 4.2	52.4 $\pm$ 3.2
THC (8 mg/kg)	35.7 $\pm$ 8.5	63.8 $\pm$ 5.4	52.3 $\pm$ 7.8	17.0 $\pm$ 6.2	63.8 $\pm$ 5.4	57.0 $\pm$ 6.0*

\* $p < 0.05$  vs VEH-treated controls.**Figure 3** (a–d) Social novelty (Familiar vs Novel) preference times (s; means  $\pm$  SEM,  $n = 8$ – $10$  per group) in male and female WT, *COMT* HET, and *COMT* KO mice after adolescent male (panel a) vs adult male (panel b) or adolescent female (panel c) vs adult female (panel d) THC (4.0, 8.0 mg/kg) or vehicle (VEH).

in terminal fields, both in animals and humans (Murray *et al*, 2007; Bossong *et al*, 2009), the absence here of any overall effect of chronic cannabis administration over adolescence on exploratory activity in adulthood is in keeping with a recent report (Rubino *et al*, 2008).

Previous studies in *COMT*-deficient mice have shown few changes in DA metabolism or overflow in the dorsal striatum (Yavich *et al*, 2007). However, although the role of *COMT* in subcortical DA function seems to be lower than in the PFC, it may have an important role in regulating the

**Table 3** Time Spent in the Open Arm (% Time in Open Arm; Means  $\pm$  SEM,  $n = 8-10$  per Group) in the Elevated Plus Maze in WT, COMT HET, and COMT KO Mice After Adolescent vs Adult THC (4.0, 8.0 mg/kg) or Vehicle (VEH)

	% Time in open arm	
	Adolescent	Adult
<i>Males</i>		
WT		
VEH	6.1 $\pm$ 2.1	6.3 $\pm$ 1.3
THC (4 mg/kg)	10.1 $\pm$ 1.6	8.5 $\pm$ 2.1
THC (8 mg/kg)	6.3 $\pm$ 1.5	7.9 $\pm$ 1.8
HET		
VEH	5.9 $\pm$ 2.7	7.2 $\pm$ 1.3
THC (4 mg/kg)	10.5 $\pm$ 2.5	9.4 $\pm$ 3.1
THC (8 mg/kg)	9.7 $\pm$ 2.4	10.6 $\pm$ 2.0
KO		
VEH	8.2 $\pm$ 2.1	9.0 $\pm$ 2.8
THC (4 mg/kg)	17.5 $\pm$ 3.0*	11.4 $\pm$ 3.1
THC (8 mg/kg)	25.1 $\pm$ 5.0*	11.6 $\pm$ 2.0
<i>Females</i>		
WT		
VEH	9.6 $\pm$ 3.0	12.3 $\pm$ 2.8
THC (4 mg/kg)	20.5 $\pm$ 2.3	19.5 $\pm$ 2.3
THC (8 mg/kg)	18.1 $\pm$ 4.8	16.1 $\pm$ 4.8
HET		
VEH	16.1 $\pm$ 2.7	9.0 $\pm$ 0.9
THC (4 mg/kg)	18.6 $\pm$ 4.5	12.3 $\pm$ 2.2
THC (8 mg/kg)	12.8 $\pm$ 4.1	11.6 $\pm$ 1.9
KO		
VEH	16.9 $\pm$ 4.8	14.8 $\pm$ 3.1
THC (4 mg/kg)	25.6 $\pm$ 9.0	21.8 $\pm$ 4.8
THC (8 mg/kg)	18.2 $\pm$ 3.0	15.9 $\pm$ 2.0

\* $p < 0.05$  vs VEH-treated controls.**Table 4** Summary of Phenotypes of WT, COMT HET, and COMT KO Mice After Adolescent or Adult THC Treatment

Phenotype	Adolescent			Adult		
	WT	HET	KO	WT	HET	KO
Exploratory activity	=	=	$\uparrow^a$	=	=	=
Spontaneous alternation	=	=	$\downarrow^a$	=	=	=
Delayed alternation	=	=	$\downarrow^a$	=	=	=
Novel object recognition	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$
Sociability	=	=	=	=	=	=
Social novelty preference	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$
Elevated plus maze	=	=	$\downarrow^a$	=	=	=

Summary of phenotypes for WT, COMT HET, and COMT KO mice after adolescent vs adult THC (4.0, 8.0 mg/kg) or vehicle (VEH); = no significant difference relative to VEH;  $\downarrow$  lower than VEH;  $\uparrow$  greater than VEH.<sup>a</sup>Effect observed in males only.

balance and interplay of cortical vs subcortical DA transmission, primarily in males (Gogos *et al*, 1998; Huotari *et al*, 2004). Furthermore, DA content, fiber density, and receptor density reach peak levels during adolescence and thereafter undergo pruning in several brain regions, including the striatum and PFC (Hohn and Wuttke, 1979; Kalsbeek *et al*, 1988; Teicher *et al*, 1995; Tarazi *et al*, 1999). Therefore, although some effects of neophobia cannot be excluded, adolescent THC exposure in COMT mutants and the associated sex-specific hyper-exploratory phenotype in adulthood may, at least in part, reflect this DAergic developmental trajectory and the increased basal level of PFC DA observed in male COMT KO (Gogos *et al*, 1998).

### Spatial Working Memory

The fact that vehicle-treated male COMT KO evidence modest but consistent improvement in spatial working memory, as assessed using both spontaneous and delayed alternation, is in accordance with recent phenotypic studies in COMT mutants (Babovic *et al*, 2008; Papaleo *et al*, 2008). Studies in both rodents and humans show that cortical DA signaling, specifically DAergic activity through PFC D1 receptors, affects cognitive performance with an inverted U-shaped relationship, such that either deficient or excessive DAergic activity can exert deleterious effects (Goldman-Rakic *et al*, 2000; Mattay *et al*, 2003; Seamans and Yang, 2004; Tunbridge *et al*, 2006). In COMT KO, tissue levels, turnover, and overflow of DA are increased in the PFC but not in the striatum (Gogos *et al*, 1998; Huotari *et al*, 2002; Yavich *et al*, 2007). Thus, the present finding of improvement in spatial working memory in vehicle-treated male COMT KO is consistent with their having higher PFC DA levels.

Adolescent THC exposure has been reported previously to result in disruption of the spatial working memory in adulthood (Rubino *et al*, 2009). In this study, the improvement in spatial working memory in male COMT KO was found to be particularly sensitive to the disruptive effects of adolescent THC on cognitive function, as indexed by impairment in spontaneous and delayed alternation. The present heightening of this disruptive effect in male COMT KO is congruent with the effect of acute amphetamine on working memory in COMT KO (Papaleo *et al*, 2008), and with the effect of heightened mesoprefrontal DAergic activity by cannabinoid receptor 1 (CB1) activation to impair working memory (Jentsch *et al*, 1997; Pistis *et al*, 2001). Given that both deficient and excessive DAergic activity in the PFC can lead to impairment in PFC-related cognitive function, and the critical importance of adolescence in the development of DA systems, heightening of the disruptive effects of adolescent THC exposure on PFC-mediated working memory processes in male COMT KO is likely mediated by PFC DA dysfunction. On this basis, it is proposed that decreased availability of DA in the PFC, by sex-specific genetic deficiency in COMT function, may mediate adolescent THC-induced long-term impairment in cognitive processes, such as working memory, which are 'core' deficits in schizophrenia (Aleman *et al*, 1999).



## Recognition Memory

In a previous study, we showed a modest phenotypic reduction in object recognition memory in *COMT* HET relative to other genotypes (Babovic *et al*, 2008); although the present investigation failed to replicate this effect, different retention times were used between these studies. Both adolescent and adult THC exposure disrupted novel object recognition performance at the longest retention interval, but in a manner unrelated to genotype. These findings contrast with those above in relation to spatial working memory, in which the disruptive effects of THC were found to vary with the *COMT* genotype in a manner dependant on age at treatment and sex.

Although spatial working memory depends primarily on the PFC (Goldman-Rakic *et al*, 2000; Seamans and Yang, 2004; Tunbridge *et al*, 2006), recognition memory for novel vs familiar objects does not depend on the PFC but involves primarily medial temporal lobe structures, particularly the hippocampus (Hammond *et al*, 2004; Ainge *et al*, 2006; DeVito and Eichenbaum, 2009) and perirhinal cortex (Brown and Aggleton, 2001; Barker *et al*, 2007; Squire *et al*, 2007). Consistent with the present profile of differential cognitive deficits after adolescent THC exposure in mice, chronic THC exposure in rats has been associated with deficits in recognition memory, accompanied by changes in hippocampal proteomics (Quinn *et al*, 2008), and fewer synaptic contacts and/or less efficient synaptic connections throughout the hippocampus (Rubino *et al*, 2009). Interestingly, although in schizophrenia, impairment of spatial working memory is a 'core' cognitive deficit, recognition memory seems less impaired and varies greatly across studies (Aleman *et al*, 1999).

## Social Behavior

The present finding that *COMT* mutants show no alterations in either sociability or social novelty preference replicates our previous report (Babovic *et al*, 2008). Although chronic THC exposure over either adolescence or adulthood was without effect on sociability, both exposures disrupted social novelty preference; this effect of THC is similar to the phenotype of untreated mice mutant for the schizophrenia risk gene *neuregulin 1* (O'Tuathaigh *et al*, 2007). Residual social interaction deficits have also been reported after adolescent administration of a synthetic cannabinoid analog in rats (O'Shea *et al*, 2004) and of THC in mice (Quinn *et al*, 2008). We could not identify any material influence of the *COMT* genotype on the effects of THC for either sociability or social novelty preference.

## Anxiety

Overall, chronic THC exposure resulted in an 'anxiolytic' effect in the EPM. Acutely, cannabinoid agonists can induce anxiolytic-like effects at lower doses and anxiogenic-like effects at higher doses, in a manner that can differ between adolescent and adult animals (Viveros *et al*, 2005a, ). Rodent models of chronic exposure across diverse paradigms have produced conflicting results, with studies reporting anxiety-like behavior to be decreased, particularly using the EPM

(Biscaia *et al*, 2003; Schramm-Sapyta *et al*, 2007; Wegener and Koch, 2009), unaltered (Rubino *et al*, 2009) or increased (O'Shea *et al*, 2004, 2006). In humans, adolescent cannabis exposure has been associated with depressive and/or anxiety disorders, in addition to psychosis (Patton *et al*, 2002). Studies in *COMT* KO mice have indicated anxiety-like behavior to be either increased, particularly using the light-dark paradigm (Gogos *et al*, 1998; Papaleo *et al*, 2008), or unaltered (Haasio *et al*, 2003). In humans, the low-activity *COMT* Met allele has been linked to increased levels of anxiety (Drabant *et al*, 2006; Heinz and Smolka, 2006; Harrison and Tunbridge, 2008).

Our finding in the EPM, of an anxiolytic-like effect of THC that was most evident in male *COMT* KO exposed during adolescence, indicates a complex interaction between genotype, age at treatment, and sex. Sexual dimorphism in the *COMT* mutant phenotype has been reported for aggression, emotional reactivity, and aspects of cognition (Gogos *et al*, 1998; Babovic *et al*, 2008), and it has been suggested that an interaction between cannabinoid receptors and estrogen receptors in rodents may explain why adolescent males find cannabinoids less aversive than adult males (Quinn *et al*, 2008). Estrogen may have an important role in the downregulation of *COMT* transcription (Xie *et al*, 1999), such that sexual dimorphism in phenotypic effects may reflect the involvement of sex-specific compensatory mechanisms (a necessary factor to consider in relation to all constitutive KO mice) and/or transcriptional regulation by estrogens (Gogos *et al*, 1998; Harrison and Tunbridge, 2008). These data elaborate this evolving body of evidence to include responsivity to THC.

## Summary

These studies illuminate recent clinical finding that (1) the relationship between cannabis use during adolescence and subsequent psychosis is influenced by the human *COMT* genotype (Caspi *et al*, 2005); and (2) across young, mostly male patients, relatives, and unrelated controls, the association of acute THC administration with increased expression of psychosis liability and memory impairment, in terms of both immediate and delayed recall, is influenced by the *COMT* genotype (Henquet *et al*, 2006). In humans, the high-activity *COMT* Val108Met allele is associated with increased risk for psychosis consequent to THC exposure; thus, it might have been expected that in mutant mice, *COMT* KO would decrease psychosis-related behavioral effects of THC. The fact that *COMT* KO increased rather than decreased these effects may reflect complexities in the inverted U-shaped relationship between PFC-mediated function and cortical DAergic activity in human/nonhuman primates *vis-à-vis* rodents (Seamans and Yang, 2004; Tunbridge *et al*, 2006); thus, the directionality of the present findings may be less germane. It should be also noted that putative compensatory processes in *COMT* mutants may contribute to these phenotypes. Nonetheless, these findings indicate processes through which adolescent THC exposure could result in deleterious effects on several aspects of normal, adult functioning that are disrupted in schizophrenia, in a manner that is modulated by the *COMT* genotype. More importantly, they indicate how genetic,

developmental, and environmental factors interact to increase individual vulnerability to psychosis.

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## DISCLOSURE

The authors declare no conflict of interest.

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