Genetic vs. pharmacological inactivation of COMT influences cannabinoid-induced expression of schizophrenia-related phenotypes

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Abstract

Catechol-O-methyltransferase (COMT) is an important enzyme in the metabolism of dopamine and disturbance in dopamine function is proposed to be central to the pathogenesis of schizophrenia. Clinical epidemiological studies have indicated cannabis use to confer a 2-fold increase in risk for subsequent onset of psychosis, with adolescent-onset use conveying even higher risk. There is evidence that a high activity COMT polymorphism moderates the effects of adolescent exposure to cannabis on risk for adult psychosis. In this paper we compared the effect of chronic adolescent exposure to the cannabinoid WIN 55212 on sensorimotor gating, behaviours related to the negative symptoms of schizophrenia, anxiety- and stress-related behaviours, as well as ex-vivo brain dopamine and serotonin levels, in COMT KO vs wild-type (WT) mice. Additionally, we examined the effect of pretreatment with the COMT inhibitor tolcapone on acute effects of this cannabinoid on sensorimotor gating in C57BL/6 mice. COMT KO mice were shown to be more vulnerable than WT to the disruptive effects of adolescent cannabinoid treatment on prepulse inhibition (PPI). Acute pharmacological inhibition of COMT in C57BL/6 mice also modified acute cannabinoid effects on startle reactivity, as well as PPI, indicating that chronic and acute loss of COMT can produce dissociable effects on the behavioural effects of cannabinoids. COMT KO mice also demonstrated differential effects of adolescent cannabinoid administration on sociability and anxiety-related behaviour, both confirming and extending earlier reports of COMT x cannabinoid effects on the expression of schizophrenia-related endophenotypes.

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Introduction

According to gene × environment (G × E) hypotheses in psychiatry, genetic and environmental influences combine to influence both early CNS development and later function, producing pathophysiological deficits associated with the expression of disease-related endophenotypes (Cannon et al. 2003; Fatemi & Folsom, 2009; van Os et al. 2010; Waddington et al. 2011). A variety of environmental insults throughout the lifespan, ranging from viral infection during pregnancy, to psychosocial stressors and substance abuse over adolescence, have been associated with schizophrenia (van Os et al. 2010) and shown to produce behavioural and neurobiological changes reminiscent of schizophrenia in adult rodents (O’Tuathaigh et al. 2007, 2010a, b).

Cannabis consumption, particularly during adolescence, has been associated with a doubling of risk for schizophrenia (Arseneault et al. 2004; Henquet et al.
A longitudinal birth cohort study has shown that cannabis use was more likely to be followed by psychosis among those both exposed during adolescence and homozygous for the COMT Val108Met allele (Caspi et al. 2005). The catechol-O-methyltransferase (COMT) enzyme is expressed in pyramidal neurons of the prefrontal cortex (PFC) and hippocampus and plays a specific role in the catabolism of cortical dopamine (DA) (Papaleo et al. 2008). 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 4-fold reduction in enzymatic activity (Tunbridge et al. 2006). Its regional and functional specificity, together with the COMT gene lying within a chromosomal region of interest for psychosis (22q11), has made COMT the subject of extensive study in schizophrenia (Craddock et al. 2005). Although clinical data have not confirmed a direct link between COMT gene variation and schizophrenia (Allen et al. 2008; Okochi, 2009), functional polymorphisms of the COMT gene are associated with performance on frontal cortical tasks such as the Wisconsin card sort task in patients with schizophrenia and controls (Barnett et al. 2008). Alongside the original study by Caspi et al. (2005), several reports have confirmed an association between COMT genotype, cannabis consumption and psychosis (Costas et al. 2011; Henquet et al. 2006; Pelayo-Teran et al. 2010), although conflicting data indicate an interaction with either the low-activity (Costas et al. 2011; Zammit et al. 2007) or high-activity (Caspi et al. 2005) allele.

Studies employing COMT gene knockout (KO) mice have demonstrated a sex-specific (males only) decrease in PFC DA levels; this was accompanied by changes in psychostimulant sensitivity but no change in sensorimotor gating, a behavioural model linked with the psychotic symptoms of schizophrenia (Gogos et al. 1998; Haasio et al. 2003; Huotari et al. 2002; Powell et al. 2009). We have recently shown that in male COMT KO mice, genotype exerted specific modulation of responsivity to chronic Δ9-tetrahydrocannabinol (THC; the psychoactive constituent of cannabis) administration during adolescence in terms of exploratory activity, spatial working memory and anxiety; this illuminates the putative interaction between COMT × cannabis exposure over this particular stage of development in the expression of the psychosis phenotype (O’Tuathaigh et al. 2010c).

In the current study we seek to further clarify, at a preclinical level, the relationship between COMT activity and the psychotomimetic effects of adolescent cannabis exposure. In expt 1, male COMT KO mice that had received chronic treatment with the cannabinoid receptor agonist WIN 55212 during adolescence were subsequently tested for the following psychosis-related phenotypes: sensorimotor gating [habituation of acoustic startle response, prepulse inhibition (PPI)], behaviours relevant to negative symptoms of schizophrenia [sociability and preference for social novelty, forced swim test (FST)], and anxiety-related and thermal nociceptive behaviour (light/dark test, hot-plate/tail-flick paradigms). In expts 2 and 3, the effect of lifelong loss of COMT activity (expt 1) was compared to pharmacological inhibition of COMT activity, to clarify whether the COMT × cannabinoid interaction is also observed after acute inhibition of COMT or whether it is dependent upon adaptive changes observed following developmental absence of COMT. To this end, we examined, in C57BL/6j mice, whether acute pretreatment with the COMT inhibitor tolcapone would modify the acute behavioural effects of cannabinoid agonists WIN 55212 (expt 2) or CP 55940 (expt 3) in a test of habituation and PPI.

Materials and methods

Animals

In expt 1, mice containing the mutated COMT allele were generated at Rockefeller University, USA, as described previously (Gogos et al. 1998). Mice of a hybrid (129/J × C57BL6) strain with heterozygous deletion of the COMT gene were backcrossed to wild-type (WT) C57BL6 mice for 10 generations. Male WT and COMT KO mice were used at postnatal days (PND) 32–52 corresponding to adolescence. Experiments 2 and 3 employed adult CF7BL/6j mice (Harlan Laboratories, UK). Mice were housed in groups of 3–5 per cage and maintained at 21 ± 1 °C on a 12-h light/dark cycle (lights on 08:00 hours), with food and water available ad libitum. These studies were approved by the Research Ethics Committee of the Royal College of Surgeons in Ireland and were conducted under license from the Department of Health and Children in accordance with Irish legislation and the European Communities Council Directive 86/609/EEC.

Experimental design

In expt 1, male COMT WT and KO mice were treated with either vehicle (Veh) or WIN 55212 (2.5 mg/kg), administered once daily over 20 consecutive days during adolescence (PND 32–52), as described previously (O’Tuathaigh et al. 2010c). After adolescent treatment, there was an interval of 21 d before behavioural testing. All mice were then examined for startle
habitation and PPI \( n=7\text{–}10 \) per group (treatment/genotype condition), light/dark test \( n=7\text{–}12 \) per group), sociability and social novelty preference \( n=6\text{–}8 \) per group), hot-plate and tail-flick latency \( n=8\text{–}12 \) per group), and forced swim performance \( n=6\text{–}10 \) per group). All animals were assessed by an observer blind to genotype and treatment. After behavioural assessments were completed, all animals were sacrificed and brains harvested for neurochemical analysis at PND 135\text{–}150. In expt 2, adult male C57BL6/J mice (8\text{–}12 per treatment condition; aged 11\text{–}12 wk) were pretreated with either Veh or tolcapone (30 mg/kg) 60 min before administration of Veh or WIN 55212 (1.0 or 2.5 mg/kg). Twenty-five minutes later, mice underwent PPI testing. In expt 3, adult male C57BL6/J mice \( n=6\text{–}8 \) mice per treatment condition; aged 12 wk) were pretreated with either Veh or tolcapone (15 or 30 mg/kg) 60 min prior to treatment with vehicle or CP 55940 (0.4 mg/kg). A separate treatment group were pre-treated with Veh and subsequently administered CP 55940 (0.2 mg/kg). As in expt 2, mice were subjected to PPI testing 25 min after the second injection.

**Drugs**

WIN 55212 (Sigma-Aldrich, USA) and CP 55940 (Tocris Bioscience, UK) were dissolved in saline: cremaphor:ethanol (18:1:1) for subcutaneous (s.c.) injection at 1.0 or 2.5 mg/kg and 0.2 or 0.4 mg/kg, respectively; these doses were selected on the basis of previous studies of synthetic cannabinoid effects on cognition in rats and mice (Arguello & Jentsch, 2004; Baek et al. 2009; Boucher et al. 2011; Yim et al. 2008). Tolcapone, a gift from Professor Pekka Männistö (University of Helsinki), was dissolved in saline, with the addition of 1 ml cremaphor. Tolcapone doses were selected on the basis of both pilot data on the effects of the drug in tests of anxiety (Desbonnet et al. unpublished data) and previous studies on tolcapone effects on cognitive measures in rodents (Paterlini et al. 2005). All drugs were injected at a volume of 4 ml/kg.

**Behavioural measures**

**PPI**

Testing of PPI was performed in a startle chamber (SR-LAB, San Diego Instruments, USA), using a modified version of a procedure described previously (Yee et al. 2005). Each test session began with a 10-min acclimatization period where the mouse was placed into the cylinder and exposed to a constant background noise of 65 dB only. Subsequently, in a pseudorandom order, mice were presented with a block of 172 discrete test trials with inter-trial intervals ranging between 5 and 15 s; each block consisted of pulse-alone trials at three different intensities (100, 110 or 120 dB); these were combined with prepulse signals (4, 8, or 16 dB) to give nine different combinations of prepulse/pulse trials (4/100, 4/110, 4/120, 8/100, 8/110, 8/120, 16/100, 16/110, 16/120 dB). The first six and last six trials consisted of startle alone, comprising two trials of each of the three possible pulse intensities. Initial and habilitated startle response, at each pulse-alone intensity, was calculated on the basis of the average startle value for the first six and last six trials, respectively. Percentage PPI (%PPI) was calculated as

\[
\frac{(\text{mean startle response to pulse alone}) - (\text{mean startle response to prepulse + pulse response})}{\text{mean startle response to pulse alone}} \times 100\%
\]

%PPI was calculated for each combination of prepulse/pulse intensity.

**FST**

The FST has been used to assess anhedonic behaviour and has been offered as a model for the negative symptoms of schizophrenia in mice (e.g. Tejedor-Real et al. 2007). Mice were placed individually in one of four clear Pyrex cylinders (height 27 cm, diameter 8 cm; VWR International Ltd, Ireland) which were filled with water \((23 \pm 1^\circ C)\) to a depth of 16 cm for 6 min. Behaviours were monitored from above by video camera for subsequent analysis. Latency to immobility during the total test period of 6 min was recorded. Immobility was assigned when no additional activity was observed other than that required to keep the mouse’s head above the water.

**Sociability and preference for social novelty**

Social affiliative behaviour and preference for social novelty were assessed in a three-chamber apparatus and two-staged procedure as described previously (Nadler et al. 2004; O’Tuathaigh et al. 2007).

**Hot-plate/tail-flick test**

Thermal nociception measurements were conducted using the hot-plate test (model 7280, Ugo Basile, Italy) and the tail-flick assay (model 7360, Ugo Basile, Italy), as described previously (Walsh et al. 2010).

**Light/dark test**

Anxiety-related behaviour was assessed in the light/dark test (Bourin & Hascoet, 2003). Mice were placed into a test chamber \((43 \times 43 \times 33 \text{ cm})\) with a white
Plexiglas floor and clear walls (ENV-515-16; Med Associates, USA); infrared detection beams on the x-, y- and z-axes tracked the mouse’s position and activity over the course of the experiment. The chamber was equipped with a light-impermeable dark box insert, which covered half the area of the chamber. The light and dark compartments were connected via a small hole in the partition wall. Each compartment was differentially illuminated. A LED lamp (containing 9 × white Nichia LED bulbs; >15 lx) was placed 30 cm above the light chamber compartment. The time spent in, entries into, and ambulatory time and counts in the light vs. dark compartments were recorded for 10 min.

**High performance liquid chromatography (HPLC)**

Monoamines and metabolites were assayed 24 h following final behavioural testing, as described previously (Desbonnet et al. 2008; Harkin et al. 2001). Mice were sacrificed by decapitation, Brains were rapidly removed, dissected on ice, placed into chilled PBS buffer (0.1 M, pH 7.1) containing protease-inhibitor cocktail (Roche, UK) and spiked with N-methylserotonin, an internal standard (N-methyl-5-HT; 10 ng/20 µl; Sigma-Aldrich, Ireland); brain areas dissected included PFC and striatum. Samples were weighed, individually sonicated in 500 µl buffer and centrifuged (14,000 g, 4 °C, 15 min). Supernatant was collected and stored at −70 °C until analysis at which point it was diluted 1/5 in HPLC mobile phase. The mobile phase contained 0.1 M citric acid, 0.1 M sodium dihydrogen phosphate, 0.01 mM EDTA (Alkem/Reagecon, Ireland), 5.6 mM octane-1-sulphonic acid (Sigma) and 9% (v/v) methanol (Alkem/Reagecon), and was adjusted to pH 2.8 using 4 N sodium hydroxide (Alkem/Reagecon), and was adjusted to pH 2.8 using 4 N sodium hydroxide (Alkem/Reagecon). The monoamines and metabolites DA, homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline (NA) were measured using HPLC with electrochemical detection. Twenty microlitres of supernatant was injected onto the HPLC system which consisted of a SCL-10-Avp system controller, LC-10AS pump, SIL-10A autoinjector (with sample cooler maintained at 4 °C), CTO-10A oven, LEDC 6A electrochemical detector (Shimadzu) and an online Gastorr Degasser (ISS, UK). A reverse-phase column (Kinetex 2.6u C18 100 x 4.6 mm, Phenomenex) maintained at 30 °C was employed in the separation (flow rate 0.9 ml/min), the glassy carbon working electrode combined with an Ag/AgCl reference electrode (Shimadzu) was operated at +0.8 V and the chromatograms generated were analysed using Class-VP 5 software (Shimadzu). Monoamines were identified by their characteristic retention times as determined by standard injections which were run at regular intervals during the sample analysis. Concentrations were calculated using analyte:internal standard peak height ratios and expressed as nanograms of neurotransmitter per gram of fresh tissue weight.

**Data analysis**

Repeated-measures analysis of variance (ANOVA) was performed to analyse data for sociability and social novelty preference, startle habituation and %PPI for each pulse intensity (100, 110, 120 dB). Data for each nociception assay, forced swim latency, and each monoamine measure were analysed using between-subjects ANOVA with main factors of genotype and treatment. Based on a-priori hypotheses, genotypic differences in Veh-treated mice were examined separately using one-way ANOVA in expt 1. Post-hoc comparisons were performed using Bonferroni testing. Statistical significance was accepted at the 0.05 level of probability. Where the data were not normally distributed, analyses were conducted on square root transformations of the data. All statistical analyses were performed using the PASW software package (PASW version 18, SPSS Inc., USA).

**Results**

**Expt 1: effects of chronic adolescent exposure to WIN 55212 in COMT KO mice**

**PPI**

Startle response/startle habituation. Assessment of startle responsivity before and after trial blocks showed habituation at a pulse-alone intensity of 100 dB, with no effect of genotype or treatment (effect of trial block: $F_{1,45} = 8.79, p < 0.01$; data not shown). At the 110-dB pulse-alone intensity, a similar habituation profile was observed (effect of trial block: $F_{1,45} = 12.92, p < 0.01$); chronic adolescent treatment with WIN 55212 decreased overall startle amplitude in WT but increased startle amplitude in KO mice (genotype × startle interaction: $F_{1,45} = 3.85, p < 0.05$; Fig. 1a). At the 120-dB pulse-alone intensity, a similar habituation profile was evident (effect of trial block: $F_{1,45} = 3.92, p = 0.05$), together with a similar effect of WIN 55212 to reduce overall startle amplitude in WT but increase startle amplitude in KO mice (genotype × startle interaction: $F_{1,45} = 2.83, p = 0.07$; data not shown).

Prepulse inhibition. %PPI increased with prepulse intensity (4, 8, 16 dB) at pulse intensities of 100 dB
**COMT inactivation and schizophrenia phenotypes**

**Fig. 1.** Effects of chronic adolescent exposure to WIN 55212 (1.0 or 2.5 mg/kg) on the following behavioural measures in male COMT KO vs. WT mice: (a) startle responses to pre- and post-test 110-dB pulses; (b) % prepulse inhibition (PPI) at the 120-dB pulse intensity; (c) preference for time (s) spent with empty vs. mouse chamber (sociability); (d) latency to immobility in the forced swim test; (e) % time ambulating in the light compartment of the light/dark test; (f) latency to tail-flick (s) in the tail-flick test (s). Data are expressed as means (±S.E.M.). * p < 0.01 (treatment effect on overall startle amplitude); † p < 0.05 (effect of treatment); †† p < 0.01 (preference for time spent with mouse vs. empty chamber).

Sociability and preference for social novelty

During the sociability phase, mice spent more time in (effect of chamber: $F_{1,30}=86.78, p<0.001$) and made more entries into (effect of chamber: $F_{1,30}=14.35, p<0.001$) the chamber containing the unfamiliar mouse relative to the empty chamber; the effect of treatment with WIN 55212 to disrupt this profile in WT was absent in KO mice (genotype × treatment × chamber interaction: $F_{2,60}=3.06, p<0.05$; Fig. 1c). WIN 55212 increased the overall number of chamber entries in COMT KO but not in WT mice (genotype × treatment interaction: $F_{2,60}=3.54, p<0.05$).

During the social novelty preference phase, mice now spent more time in (effect of chamber: $F_{1,30}=6.35, p<0.05$) and made more entries into (effect of chamber: $F_{1,30}=7.49, p<0.001$) the chamber containing the new stranger mouse relative to the chamber.
containing the now familiar mouse; this profile was unaltered by genotype or treatment, other than an effect of WIN 55212 to decrease time spent with the new stranger mouse (effect of treatment: \( F_{2,38} = 3.14, p < 0.05 \); data not shown).

**FST**

The data from one WIN 55212 (2.5 mg/kg)-treated COMT KO mouse was lost due to a fatality. Latency to immobility in the FST was unaltered by treatment or genotype (Fig. 1d).

**Light/dark test**

WIN 55212 (2.5 mg/kg) increased % time in the light compartment (genotype × treatment interaction: \( F_{2,48} = 2.59, p < 0.05 \)) and % time ambulating in the light compartment (genotype × treatment interaction: \( F_{2,44} = 3.75, p < 0.05 \); Fig. 1e), i.e. indices of decreased ‘anxiety’, in COMT KO but not in WT mice. WIN 55212 (2.5 mg/kg) increased overall number of ambulatory counts across both compartments, i.e. an index of elevated ‘activity’, in COMT KO but not in WT mice (effect of treatment: \( F_{2,48} = 6.94, p < 0.01 \); genotype × treatment interaction: \( F_{2,48} = 5.82, p < 0.01 \); data not shown).

**Hot-plate/tail-flick tests**

Tail-flick latency was reduced in mice treated with 1.0 mg/kg WIN 551212 (effect of treatment: \( F_{2,48} = 3.34, p < 0.05 \); no effect of genotype or genotype × treatment interaction; Fig. 1f). Comparison of tail-flick latencies between Veh-treated COMT WT vs. KO mice revealed a marginal reduction in tail-flick latencies in KO relative to WT mice (\( t_{14} = 1.86, p = 0.08 \)). Hot-plate latencies were not modified by either genotype or treatment (data not shown).

**HPLC analysis**

In the PFC, COMT KO mice evidenced increased levels of DA (effect of genotype: \( F_{1,35} = 5.76, p < 0.01 \)) and DOPAC (effect of genotype: \( F_{1,35} = 212.48, p < 0.001 \)), with an increase in DOPAC:DA ratio (effect of genotype: \( F_{1,35} = 82.84, p < 0.001 \), in the absence of HVA or HVA:DA ratio (effect of genotype: \( F_{1,35} = 76.3, p < 0.001 \)). This profile was unaltered by treatment with WIN 55212 (see Table 1). In striatum, COMT KO mice

<table>
<thead>
<tr>
<th></th>
<th>COMT WT</th>
<th>WIN (2.5) (( n = 8 ))</th>
<th>COMT KO</th>
<th>WIN (2.5) (( n = 7 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prefrontal cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>2086 ± 194</td>
<td>2377 ± 441</td>
<td>2804 ± 396*</td>
<td>2529 ± 313</td>
</tr>
<tr>
<td>DOPAC</td>
<td>507 ± 49</td>
<td>490 ± 56</td>
<td>1715 ± 143*</td>
<td>1756 ± 63</td>
</tr>
<tr>
<td>HVA</td>
<td>648 ± 27</td>
<td>691 ± 54</td>
<td>0*</td>
<td>0</td>
</tr>
<tr>
<td>5-HT</td>
<td>1125 ± 37</td>
<td>994 ± 81</td>
<td>942 ± 71</td>
<td>956 ± 31</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>701 ± 131</td>
<td>690 ± 100</td>
<td>572 ± 57</td>
<td>562 ± 5.58</td>
</tr>
<tr>
<td>DOPAC/DA</td>
<td>0.25 ± 0.02</td>
<td>0.24 ± 0.02</td>
<td>0.72 ± 0.08*</td>
<td>0.74 ± 0.06</td>
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<tr>
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<td>0.36 ± 0.07</td>
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<tr>
<td>5-HIAA/5-HT</td>
<td>0.61 ± 0.10</td>
<td>0.76 ± 0.17</td>
<td>0.60 ± 0.03</td>
<td>0.59 ± 0.02</td>
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<td><strong>Striatum</strong></td>
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</tr>
<tr>
<td>Dopamine</td>
<td>22550 ± 1674</td>
<td>20094 ± 1120</td>
<td>32740 ± 9749</td>
<td>22179 ± 825</td>
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<tr>
<td>DOPAC</td>
<td>1782 ± 262</td>
<td>1599 ± 95</td>
<td>7854 ± 35*</td>
<td>5717 ± 174</td>
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<tr>
<td>HVA</td>
<td>2566 ± 117</td>
<td>2558 ± 117</td>
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<td>0</td>
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<tr>
<td>5-HT</td>
<td>1472 ± 171</td>
<td>1859 ± 118</td>
<td>1492 ± 382</td>
<td>1242 ± 65</td>
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<tr>
<td>5-HIAA</td>
<td>889 ± 31</td>
<td>949 ± 42</td>
<td>1152 ± 284</td>
<td>888 ± 39</td>
</tr>
<tr>
<td>DOPAC/DA</td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.00</td>
<td>0.24 ± 0.01*</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>HVA/DA</td>
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<td>0.13 ± 0.01</td>
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<td>0</td>
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<tr>
<td>5-HIAA/5-HT</td>
<td>0.64 ± 0.05</td>
<td>0.52 ± 0.03</td>
<td>0.78 ± 0.04</td>
<td>2.83 ± 2</td>
</tr>
</tbody>
</table>

DOPAC, Dihydroxyphenylacetic; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid.

Data are expressed as means (±S.E.M.).

*\( p < 0.01 \) (effect of genotype).
evidenced increased levels of DOPAC (effect of genotype: $F_{1,45} = 22.52$, $p < 0.001$), with increase in DOPAC:DA ratio (effect of genotype: $F_{1,45} = 18.65$, $p < 0.001$), in the absence of HVA or HVA:DA ratio; this profile was unaltered by treatment with WIN 55212. In both striatum and PFC, there were no effects of genotype or treatment with WIN 55212 on levels of 5-HT or 5-HIAA, or on 5-HIAA/5-HT ratio (Table 1).

**Expt 2: effects of WIN 55212 on PPI following pretreatment with tolcapone**

**Startle response/startle habituation.** Habituation of startle response was observed at each pulse-alone intensity of 100 dB (effect of trial block: $F_{1,45} = 12.26$, $p < 0.001$), 110 dB (effect of trial block: $F_{1,45} = 22.24$, $p < 0.001$) and 120 dB (effect of trial block: $F_{1,45} = 22.43$, $p < 0.001$). Overall startle amplitude at each of 110- and 120-dB pulse-alone intensities was decreased by 2.5 mg/kg WIN 55212 (110 dB, effect of WIN 55212: $F_{2,45} = 4.02$, $p < 0.05$; 120 dB, effect of WIN 55212: $F_{2,45} = 8.71$, $p < 0.01$; Fig. 2a,b). At 120 dB, WIN 55212 (2.5 mg/kg) disrupted habituation of startle response (trial block × WIN 55212 interaction: $F_{2,45} = 7.77$, $p < 0.05$; Fig. 2b). Pretreatment with tolcapone (30 mg/kg) disrupted habituation of startle at 100 dB (effect of tolcapone: $F_{1,45} = 6.10$, $p < 0.05$).

PPI. %PPI increased with prepulse intensity at pulse intensities of 100 dB (effect of prepulse intensity: $F_{2,102} = 2.88$, $p = 0.06$), 110 dB (effect of prepulse intensity: $F_{2,102} = 56.37$, $p < 0.001$) and 120 dB (effect of prepulse intensity: $F_{2,102} = 64.80$, $p < 0.001$). At 120 dB, 1.0 mg/kg WIN 55212 disrupted %PPI (effect of WIN 55212: $F_{1,41} = 6.45$, $p < 0.01$); this effect was unaltered by pretreatment with tolcapone (no effect of tolcapone or tolcapone × WIN 55212 interaction; Fig. 2c).

**Expt 3: effects of CP 55940 PPI following pretreatment with tolcapone**

**Startle response/startle habituation.** Habituation of startle response was observed at each pulse-alone intensity of 100 dB (effect of trial block: $F_{1,45} = 21.36$, $p < 0.001$), 110 dB (effect of trial block: $F_{1,45} = 35.29$, $p < 0.001$) and 120 dB (effect of trial block: $F_{1,45} = 24.54$, $p < 0.001$). At 110 and 120 dB, 0.4 mg/kg CP 55940 disrupted habituation of startle response (110 dB, trial block × CP 55940 interaction: $F_{2,40} = 4.72$, $p < 0.01$; 120 dB, trial block × CP 55940 interaction: $F_{2,40} = 6.56$, $p < 0.001$) and overall startle responsivity (110 dB, effect of CP 55940: $F_{2,40} = 8.34$, $p < 0.01$; 120 dB, effect of CP 55940: $F_{2,40} = 11.49$, $p < 0.001$);
additionally, when administered after pretreatment with 15 mg/kg tolcapone, 0.4 mg/kg CP 55940 reduced both habituation (110 dB, trial block × tolcapone × CP 55940 × interaction: \( F_{2,40} = 6.39, p < 0.001 \); Fig. 3a, b) and overall startle responsivity (110 dB, tolcapone × CP 55940 interaction: \( F_{2,40} = 12.33, p < 0.001 \); 120 dB, tolcapone × CP 55940 interaction: \( F_{2,40} = 15.95, p < 0.001 \); Fig. 3a, b).

PPI. %PPI increased with prepulse intensity at pulse intensities of 110 dB (effect of prepulse intensity: \( F_{2,80} = 48.66, p < 0.001 \)) and 120 dB (effect of prepulse intensity: \( F_{2,80} = 35.98, p < 0.001 \)). At 120 dB, pretreatment with tolcapone (30 mg/kg) reversed the increase in %PPI observed after 0.4 mg/kg CP 55940 (tolcapone × CP 55940 interaction: \( F_{2,40} = 3.71, p < 0.05 \); Fig. 3c).

Discussion

We have previously demonstrated that chronic adolescent, but not adult, administration of Δ⁹-THC produced deficits across the following areas in male mice with KO of the COMT gene: spatial working memory, basal activity levels, anxiety-related behaviour (O’Tuathaigh et al. 2010c). In the present study, the COMT KO mouse model was used to further examine the relationship between COMT genotype and cannabis exposure in modulating behaviours relevant to the onset of psychosis. In expt 1, COMT KO modified the effects of chronic adolescent administration of the synthetic cannabinoid WIN 55212 on the following behaviours in adulthood: (a) psychosis-related endophenotypes (PPI and habituation of acoustic startle response); (b) behavioural models relevant to negative symptoms of schizophrenia (sociability and social novelty preference); (c) anxiety- and pain-related measures (light/dark test, hot-plate/tail-flick test). In expt 2, pharmacological inactivation of COMT via acute administration of tolcapone marginally altered startle habituation but not PPI, nor did it alter the effects of acute WIN 55212 on either startle measure. However, in expt 3, while acute tolcapone treatment alone, did not affect any startle measure, tolcapone pre-treatment prevented the actions of acute administration of the cannabinoid receptor agonist CP 55940 to (a) decrease startle responsivity at both the 110 and 120 dB pulse intensities and (b) increase %PPI at 120 dB.

As shown previously (Gogos et al. 1998; Huotari et al. 2002), PPI was unaltered in Veh-treated COMT KO mice relative to WT (expt 1); additionally, as noted by others (Huotari et al. 2002), we also did not observe
any change in baseline startle reactivity in COMT KO mice. Therefore, while HPLC analysis confirmed the well-characterized genotypic increase in PFC DA in KO vs. WT mice (expt 1), this was not accompanied by any changes in acoustic startle. Similarly, transgenic mice containing the human COMT Val variant showed intact PPI (Papaleo et al. 2008). In contrast, studies in humans have repeatedly documented an association between COMT gene variants and modification of startle responsivity and PPI (Quednow et al. 2009, 2010; Roussos et al. 2009). In attempting to explain these discrepancies, some authors have referred to the absence of any directly comparable murine models of human COMT gene variation, as well as the predominant role played by monoamine oxidase vs. COMT in the breakdown of DA in mice (Powell et al. 2009).

Chronic administration of WIN 55212 during adolescence reduced overall startle amplitude (at 110 dB) in WT mice, while increasing startle reactivity in COMT KO mice. Reduction of startle amplitude has been documented previously in mice following both acute (Boucher et al. 2007, 2011) and chronic (Long et al. 2010) cannabinoid treatment. It has been noted that few studies of cannabinoid (acute or chronic) effects on startle have documented PPI deficits which are not confounded by concomitant changes in baseline startle reactivity (Boucher et al. 2011). In expt 1, we reported PPI deficits at the 120 dB pulse intensity in COMT KO mice administered WIN 55212 during adolescence, which were not accompanied by baseline changes in startle reactivity at this pulse level. PPI disruption without disruption of baseline startle has been reported previously in adult rats administered cannabinoids during adolescence (Schneider & Koch, 2003; Wegener & Koch, 2009). However, under the present PPI measurement parameters, COMT KO mice were particularly vulnerable to the PPI-disruptive effects of adolescent cannabinoid treatment.

In expts 2 and 3, acute injection with WIN 55212 or CP 55940 reduced startle amplitude. In expt 3, this decrease was accompanied by an increase in %PPI in CP 55940-treated mice. In expt 2, however, decrease in startle amplitude was observed at the highest dose of WIN 55212 (2.5 mg/kg), but PPI disruption was evident only following low-dose WIN 55212 (1.0 mg/kg), indicating some dissociation of cannabinoid effects on basic startle response and PPI. Tolcapone had no effect on startle amplitude in the present study. Data from human studies have shown that the direction of tolcapone-induced changes in startle amplitude is dependent upon functional polymorphisms in the COMT gene (Giakoumaki et al. 2008; Roussos et al. 2009), suggesting that the effects of acute pharmacological inhibition of COMT on startle may be dependent upon existing status of PFC DA availability. However, the highest dose of tolcapone prevented the disruptive effect of CP 55940 on startle response and PPI, suggesting that specific behavioural effects of this cannabinoid (which is more selective for the cannabinoid CB₁ receptor than is WIN 55212) may be dependent upon PFC DA levels. The absence of any acute tolcapone effects on PPI in C57BL/6 mice in either experiment is consistent with the limited data available (Paterlini et al. 2005). To our knowledge, the present study is the first to indicate that, under certain conditions (i.e. type of cannabinoid employed, dose of tolcapone), pharmacological inhibition of COMT activity may inhibit the acoustic startle-modifying effects of acute cannabinoid administration.

Aside from confirming previously documented effects of COMT KO on PFC DA and DA metabolite levels (Gogos et al. 1998; Huotari et al. 2002), expt 1 did not show any other genotypic or treatment effects on dopaminergic and serotonergic measures.

As reported previously (Babovic et al. 2008; O’Tuathaigh et al. 2010c), Veh-treated COMT mutant mice did not show any deficits in sociability or social novelty preference relative to WT animals. However, while chronic THC exposure during adolescence had no effect on sociability in our earlier report (O’Tuathaigh et al. 2010c), expt 1 demonstrated selective disruption of sociability in WT mice given WIN 55212 during adolescence. These data are in agreement with recent reports of disruption of social approach behaviour in rats treated chronically with WIN 55212 during adolescence (Leweke & Schneider, 2011; Schneider et al. 2008). As WIN 55212-induced disruption of sociability was not observed in COMT KO mice, the social deficit evoked by cannabinoids given during adolescence may be dependent upon an as yet uncharacterized, DA-related mechanism. As we observed with adolescent THC administration (O’Tuathaigh et al. 2010c), WIN 55212 disrupted social novelty preference in both genotypes; a comparable and long-lasting deficit in social recognition has been reported in rats administered WIN 55212 during adolescence (Leweke & Schneider, 2011).

In our previous study, COMT KO mice displayed a reduced anxiety phenotype in the elevated plus maze test following adolescent THC exposure (O’Tuathaigh et al. 2010c). This finding is supported by the result of expt 1, where the same profile was observed in the light/dark test in COMT KO mice after adolescent WIN 55212 administration. These conclusions are derived from time-based rather than activity-based
indices, excluding a generalized ‘increased activity’ explanation. Similarly, Veh-treated COMT KO mice demonstrated increased thermal pain sensitivity in the tail-flick test (but not in the hot-plate test), confirming data previously published by our laboratory and others (Papaleo et al. 2008; Walsh et al. 2010). Chronic adolescent WIN 55212 also decreased tail-flick latency in both WT and COMT KO mice, which is contrary to the observed anti-nociceptive properties of acute cannabinoid treatment documented elsewhere; the mechanism underlying this chronic treatment effect is not clear and warrants further investigation (Walsh et al. 2010).

In summary, these studies elaborate on our recent report indicating that psychosis-related behavioural effects are increased following adolescent THC exposure in COMT KO mice. Here, COMT KO mice are also more vulnerable to the PPI-disruptive effects of adolescent cannabinoid treatment. PPI has been proposed as a viable endophenotype to investigate in animal models of the genetics of schizophrenia (Powell et al. 2009). Thus, the present findings present a mechanism, centred on the balance of cortical DA activity, by which genetic vulnerability may interact with cannabis exposure to influence individual risk for schizophrenia. As noted previously (O’Tuathaigh et al. 2010c), the clinical data is equivocal with respect to an interaction between either the low-activity or high-activity COMT allele and increased risk for psychosis consequent to cannabis exposure. Alongside the present study using COMT KO mice, these complexities may reflect the inverted U-shaped relationship between PFC-mediated function and cortical dopaminergic activity (Tunbridge et al. 2006). Acute pharmacological inhibition of COMT in adult mice also modified the effects of acute cannabinoid administration on startle reactivity and PPI, suggesting that both developmental and acute loss of COMT can produce dissociable effects on the behavioural effects of cannabinoids. COMT KO mice also demonstrated differential effects of WIN 55212 on sociability and anxiety-related behaviour, elaborating earlier reports of chronic adolescent cannabinoid effects on the expression of schizophrenia-related endophenotypes.

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Statement of Interest

None.

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