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AUTHOR(S)

Aine T. Behan, Magdalena Hryniewiecka, Colm MP O'Tuathaigh, Anthony Kinsella, Mary Cannon, Maria Karayiorgou, Joseph A. Gogos, John L. Waddington, David R. Cotter

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Chronic Adolescent Exposure to Delta-9-Tetrahydrocannabinol in COMT Mutant Mice: Impact on Indices of Dopaminergic, Endocannabinoid and GABAergic Pathways

Áine T Behan*[,1], Magdalena Hryniewiecka[1], Colm MP O'Tuathaigh[2,6], Anthony Kinsella[2], Mary Cannon[1], Maria Karayiorgou[3], Joseph A Gogos[4,5], John L Waddington[2] and David R Cotter[1]

[1]Department of Psychiatry, Royal College of Surgeons in Ireland, Dublin, Ireland; [2]Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland; [3]Department of Psychiatry and Genetics & Development, Columbia University, NY, USA; [4]Department of Physiology and Cellular Biophysics, Columbia University, NY, USA; [5]Department of Neuroscience, Columbia University, NY, USA

Q1

Cannabis use confers a two-fold increase in risk for psychosis, with adolescent use conferring an even greater risk. A high-low activity polymorphism in catechol-*O*-methyltransferase (COMT), a gene encoding the COMT enzyme involved in dopamine clearance in the brain, may interact with adolescent cannabis exposure to increase risk for schizophrenia. The impact of such an interaction on central neurotransmitter pathways implicated in schizophrenia is unknown. Male mice with knockout of the COMT gene were treated chronically with delta-9-tetrahydrocannabinol (THC) during adolescence (postnatal day 32–52). We measured the size and density of GABAergic cells and the protein expression of cannabinoid receptor 1 (CB1R) in the prefrontal cortex (PFC) and hippocampus (HPC) in knockout mice relative to heterozygous mutants and wild-type controls. Size and density of dopaminergic neurons was also assessed in the ventral tegmental area (VTA) across the genotypes. COMT genotype × THC treatment interactions were observed for: (1) dopaminergic cell size in the VTA, (2) CB1R protein expression in the HPC, and (3) parvalbumin (PV) cell size in the PFC. No effects of adolescent THC treatment were observed for PV and dopaminergic cell density across the COMT genotypes. COMT genotype modulates the effects of chronic THC administration during adolescence on indices of neurotransmitter function in the brain. These findings illuminate how COMT deletion and adolescent cannabis use can interact to modulate the function of neurotransmitters systems implicated in schizophrenia.
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INTRODUCTION

The traditional hypothesis, widely held for several decades, is that increased dopaminergic function underlies the pathology of schizophrenia (Carlsson, 1978). More recently, the dopamine hypothesis of schizophrenia has been revised, with excess mesolimbic dopamine being secondary to lower dopamine levels in the prefrontal cortex (PFC) (Davis *et al*, 1991). In this context, the catechol-*O*-methyltransferase (COMT) gene on chromosome 22q11, a region implicated in schizophrenia, is functionally significant. It encodes the COMT enzyme responsible for the degradation of dopamine, particularly in the PFC. Furthermore, a valine to methionine substitution (valine substitution for methionine at the 158/108 locus Val158Met) within COMT was shown to reduce enzymatic activity of COMT, and thus lead to a slower breakdown of dopamine (Chen *et al*, 2004; Lachman *et al*, 1996). Reports have shown that a functional polymorphism in the COMT gene can moderate the association between cannabis use and psychosis (Caspi *et al*, 2005; Harrison and Weinberger, 2005; Tunbridge *et al*, 2006; van Os *et al*, 2010), but not all studies support such associations (Zammit *et al*, 2011). One particular study (Caspi *et al*, 2005) demonstrated that psychosis was most likely in those who used cannabis during adolescence and were homozygous for the COMT Val allele with the Met allele generating a four-fold reduction in enzymatic activity.

Mutant mice with deletion of the COMT gene have further elucidated the role of COMT in terms of dopamine-related function (Babovic *et al*, 2007; Seamans and Yang, 2004) of

*Correspondence/Current address: Dr ÁT Behan, Department of Physiology, Royal College of Surgeons in Ireland, 123 St Stephens Green, Dublin 2, Dublin, Leinster D2 Ireland. Tel: +353 1 402 8512, Fax: +353 1 402 2447, E-mail: abehan@rcsi.ie
[6]Current address: School of Medicine, University College Cork, Cork, Ireland.
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PFC-mediated cognition (Babovic *et al*, 2007; Gogos *et al*, 1998) and of spatial working memory. Thus, they have proved valuable in demonstrating the complexities of the inverted U-shaped dose–response relationship between PFC-mediated function and cortical dopaminergic activity involving the COMT gene, whereby a narrow range of dopamine can benefit PFC function and either insufficient or excessive dopamine can impair cognitive function (Castner *et al*, 2000; Goldman-Rakic *et al*, 2000; Seamans and Yang, 2004; Tunbridge *et al*, 2006). Moreover, a companion study by the authors have shown that the effects of chronic adolescent exposure to delta-9-tetrahydrocannabinol (THC) on behaviors related to psychosis and memory are modified in COMT mutant mice (O'Tuathaigh *et al*, 2010). No complementary analysis on neuronal structure has been performed in COMT mutant mice. Although it is known that single polymorphisms involve smaller effects on enzyme activity in humans, the COMT mutant mice provide an adequate model to further explore the modulatory role of COMT, following adolescent cannabis use.

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Cannabis use alone is not necessary or sufficient to cause the development of schizophrenia, which suggests that the association may be because of a genetic vulnerability to the effects of cannabis. In support of this, patients with schizophrenia or with an established vulnerability to psychosis are much more sensitive to the effects of cannabis than control subjects (D'Souza *et al*, 2005; Henquet *et al*, 2005; Stefanis *et al*, 2004; van Os *et al*, 2002; Verdoux *et al*, 2003). Nonetheless, consumption of cannabis is a risk factor for developing schizophrenia (Arseneault *et al*, 2004; Henquet *et al*, 2005; Moore *et al*, 2007), and the risk of developing psychotic symptoms is highest when cannabis is consumed during adolescence (Arseneault *et al*, 2002; Caspi *et al*, 2005; Konings *et al*, 2008; McGrath *et al*, 2010; Moore *et al*, 2007), possibly because the brain is still developing (Ehrenreich *et al*, 1999; Pistis *et al*, 2004; Pope *et al*, 2003; Schneider and Koch, 2003). Cannabinoid receptors mature slowly, reaching maximal levels during adolescence that possibly reduce later in life due to post-adolescent pruning, in a similar manner to that described for dopamine receptors (Belue *et al*, 1995; McLaughlin *et al*, 1994; Seeman, 1999). Studies have shown that heavy cannabis use, especially during adolescence, could contribute to additional changes in global brain structures (Arnone *et al*, 2008; Matochik *et al*, 2005; Welch *et al*, 2010; Wilson and Cadet, 2009; Yucel *et al*, 2008). However, the effect of cannabis use during adolescence on specific cell populations and their structure, particularly those implicated in schizophrenia, is less well understood.

THC, the main psychoactive ingredient of cannabis, is known to mediate its effect by binding to the cannabinoid receptor 1 (CB1R) (Pertwee, 2005); this is the main cannabinoid receptor in the brain, being expressed predominantly in the hippocampus (HPC), PFC, cerebellum, and basal ganglia (Freund *et al*, 2003). Endocannabinoids, the endogenous ligands of CB1R, are released by post-synaptic neurons to activate CB1R on neighboring presynaptic neurons, which can in turn inhibit presynaptic neurotransmitter release of γ-aminobutyric acid (GABA) and glutamate, as well as modulating the dopamine system (Chevaleyre *et al*, 2006). It is also thought that dopaminergic midbrain neurons are modulated by endocannabinoid cortical neurons (Chevaleyre *et al*, 2006). Thus, the major neuronal populations modulated by cannabinoids are also ones implicated in schizophrenia (Laviolette and Grace, 2006; Lisman *et al*, 2008). How adolescent cannabis use and COMT genotype interact to modulate such structural changes, particularly in neuronal populations implicated in schizophrenia, is not yet understood.

Examination of morphological parameters such as neuronal cell size, cell density, and protein expression can provide complementary information on a neurons capacity to function (Behan *et al*, 2009; Cotter *et al*, 2002a, b). Recent reports have demonstrated deficits in GABAergic neuronal size and density (Cotter *et al*, 2002a) to be an indicator of pathophysiological deficits in schizophrenia and to be in line with functional assessments of the same neuronal populations (Hashimoto *et al*, 2003; Volk *et al*, 2001). In this study, we aimed to elucidate the impact of chronic THC treatment during adolescence on morphological indices of dopaminergic, cannabinoid, and GABAergic function in the brain and how they might be modified in COMT mutant mice.

MATERIALS AND METHODS

Animals

Mice containing the mutated COMT allele were generated at 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 as described previously (Babovic *et al*, 2007). After weaning on postnatal day (PND) 21, pups from litters of the same generation were housed in groups of three to five per cage and maintained at $21 \pm 1^{\circ}$C on a 12-h light/dark cycle (07:00 hours on; 19:00 hours off), with *ad libitum* access to food and water. Males of wild type (WT), COMT heterozygous (HET) and COMT knockout (KO) genotypes, 6–7 mice per treatment and genotype, were treated with THC 8.0 mg/kg subcutaneously or vehicle over 20 consecutive days (McKinney *et al*, 2008) from PND 32–52, a period corresponding to adolescence in mice (Vazdarjanova *et al*, 2011) . 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 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

Delta-9-tetrahydrocannabinol (Sigma Aldrich, St Louis, MO, USA) was dissolved in saline: cremaphor: ethanol (18 : 1 : 1); vehicle controls received identical injections of the final saline: cremaphor: ethanol solution.

Immunofluorescent Staining

Animals were killed at PND 150–160 by cervical dislocation. Whole brains were removed and post-fixed in 4%

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paraformaldehyde (24 h), cryoprotected in daily steps of 24 h in 10, 20, and 30% sucrose solution, and frozen in isopentane cooled on dry ice.

Serial coronal sections (20 μm) were collected at the PFC, Bregma 1.54−2.34 mm, the HPC, Bregma −1.34 to −3.52 mm, and the ventral tegmental area (VTA, Bregma −3.08 to −3.88 mm). Briefly, sections were incubated with primary antibodies against (1) monoclonal tyrosine hydroxylase (TH), a marker of dopaminergic neurons (1 : 500, Millipore, Cork Ireland) (2) monoclonal cannabinoid 1 receptor (CB1R, 1 : 2000, a kind gift from Dr Maurice Elphick), and (3) polyclonal parvalbumin (PV), a marker of a subset of GABAergic neurons (1 : 1000, Abcam, Cambridge, UK), diluted in 5% NGS/1 × PBS-T. PV and TH antibodies were revealed by fluorescence using the respective conjugated Alexa Fluor 488 secondary antibody (1 : 2000 in 5% NGS/PBS-T) and cover slipped using DAKO fluorescent mounting medium. CB1R antibody was visualized using the tyramide signal amplification kit (Perkin Elmer, Dublin, Ireland). All sections from each experimental group were immunostained in one single run under identical conditions with each run. A negative control, containing adjacent sections incubated in secondary antibody alone, that is, with the omission of the respective primary antibody, was included in every run. All morphometric measures were made by an observer blind to genotype and treatment.

Quantitative Analysis of CB1R, PV, and TH Immunoreactivity

All analyses were carried out on a Leica DM2500 microscope (Leica Microsystems, Ireland), a cooled monochrome 12-bit camera QI-IMAGING CAMERA Fast 1394 (Media Cybernetics, UK) and a Prior × 100 *x*, *y*-motorized stage and stage controller (Media Cybernetics, UK) attached to a Heidenhain *z* axis depth gauge (Heidenhain, Germany). Cross-sectional areas of VTA, PFC, and HPC were captured at × 20 magnification and tiled using standardized exposure conditions. Boundaries of the VTA were defined by examining the size and shape of TH-IR neuronal groups and juxtaposition of other landmarks through reference to adjacent Nissl-stained VTA sections and a standard mouse atlas (Paxinos, 2008). The VTA was distinguished by the TH immunoreactive region bordered medially by the interpeduncular nucleus (IPN), laterally and ventrally by the substantia nigra pars compacta (SNpc) and medial lemniscus (ML), and dorsally by the parabrachial pigmented nucleus. Representative TH-immunoreactive sections of the VTA at −3.00 mm, −3.30 mm, and −3.60 mm with respect to Bregma are shown in Figure 1a. The location of PFC and HPC was defined using standard cytoarchitecture and subcortical landmarks as delineated in the Paxinos and Watson mouse brain atlas (Paxinos, 2008). Additionally, adjacent sections were stained with cresyl violet as described previously (Behan *et al*, 2010) and superimposed onto immunofluorescent images to accurately delineate PFC as well as the hippocampal formation, which included CA1, CA2/3, CA4, dentate gyrus, and subiculum.

Density and cell somal size of PV^+ neurons (Figure 1b) and TH^+ immunoreactive neurons (Figure 1d) were determined using morphometric methods, as outlined previously (Cotter *et al*, 2002a). Areal fluorescent intensity of CB1R staining was also measured (Figure 1c). Density (cells/mm^2), two-dimensional cell somal size (μm^2), and fluorescent intensity (mean fluorescent intensity/μm^2) were measured using Stereology-Pro 2.5 image analysis software (Kinetic Imaging, Liverpool, UK). From each animal, four cross-sectional areas of the PFC, six cross-sectional areas of the HPC, and four cross-sectional areas of the VTA were analyzed for the respective PV^+, $CB1R^+$, and TH^+ morphometric assessments (Figures 1b–d). The volume of the entire VTA (Figure 1a) was also estimated using stereological tools by the Cavalieri principle and the point-counting method (Behan *et al*, 2010; Gundersen *et al*, 1988). The coefficient of error for all morphometric measurements was $<5\%$.

Statistical Analysis

All morphological measurements were analyzed using analysis of variance (ANOVA) with post-hoc comparisons by Fisher's test and corrected for multiple comparisons using Bonferroni correction as outlined previously (Babovic *et al*, 2007; O'Tuathaigh *et al*, 2007). For these measurements, we hypothesized *a priori* that the effects of chronic THC treatment during adolescence on dopaminergic, cannabinoid, and GABAergic neuronal populations in PFC, HPC, and VTA, respectively, were modified by COMT genotype. All statistical analyses were carried out using the SPSS software package (Version 14, SPSS, Chicago, IL, USA).

RESULTS

See Table 1 for a summary of morphological and densitometric assessments carried out for this study.

TH Cell Counts in VTA

A genotype × THC treatment interaction for TH^+ neuronal cell somal size in VTA ($F_{2,25} = 8.12$; $p = 0.002$) reflected an action of THC to reduce TH^+ neuronal cell somal size in COMT KO ($94.43 \pm 7.86\ \mu m^2$) but not in WT ($111.20 \pm 12.90\ \mu m^2$), or COMT HET ($112.26 \pm 14.46\ \mu m^2$) (Figure 2a).

An effect of THC treatment on TH^+ cell density was observed in VTA ($F_{1,25} = 10.44$; $p = 0.003$), whereby THC reduced TH^+ cell density in a manner that did not differ between the genotypes (Figure 2b).

No effect of THC treatment on VTA volume was observed (Figure 2c).

CB1R Intensity in the PFC and HPC

An effect of genotype on CB1R intensity was observed in the PFC ($F_{5,25} = 8.07$; $p = 0.01$), whereby COMT HET mice (63.54 ± 5.90 intensity/μm^2) and COMT KO mice (64.85 ± 1.39 intensity/μm^2) expressed a higher CB1R intensity than WT (60.17 ± 5.14 intensity/μm^2) (Figure 3a). Conversely, an effect of genotype on CB1R intensity was observed in HPC ($F_{5,25} = 4.75$; $p = 0.015$) whereby COMT KO mice (33.76 ± 3.10 intensity/μm^2) expressed a lower CB1R intensity than WT (37.27 ± 3.30 intensity/μm^2) (Figure 3b). No effects of THC on CB1R intensity were observed in PFC or HPC.

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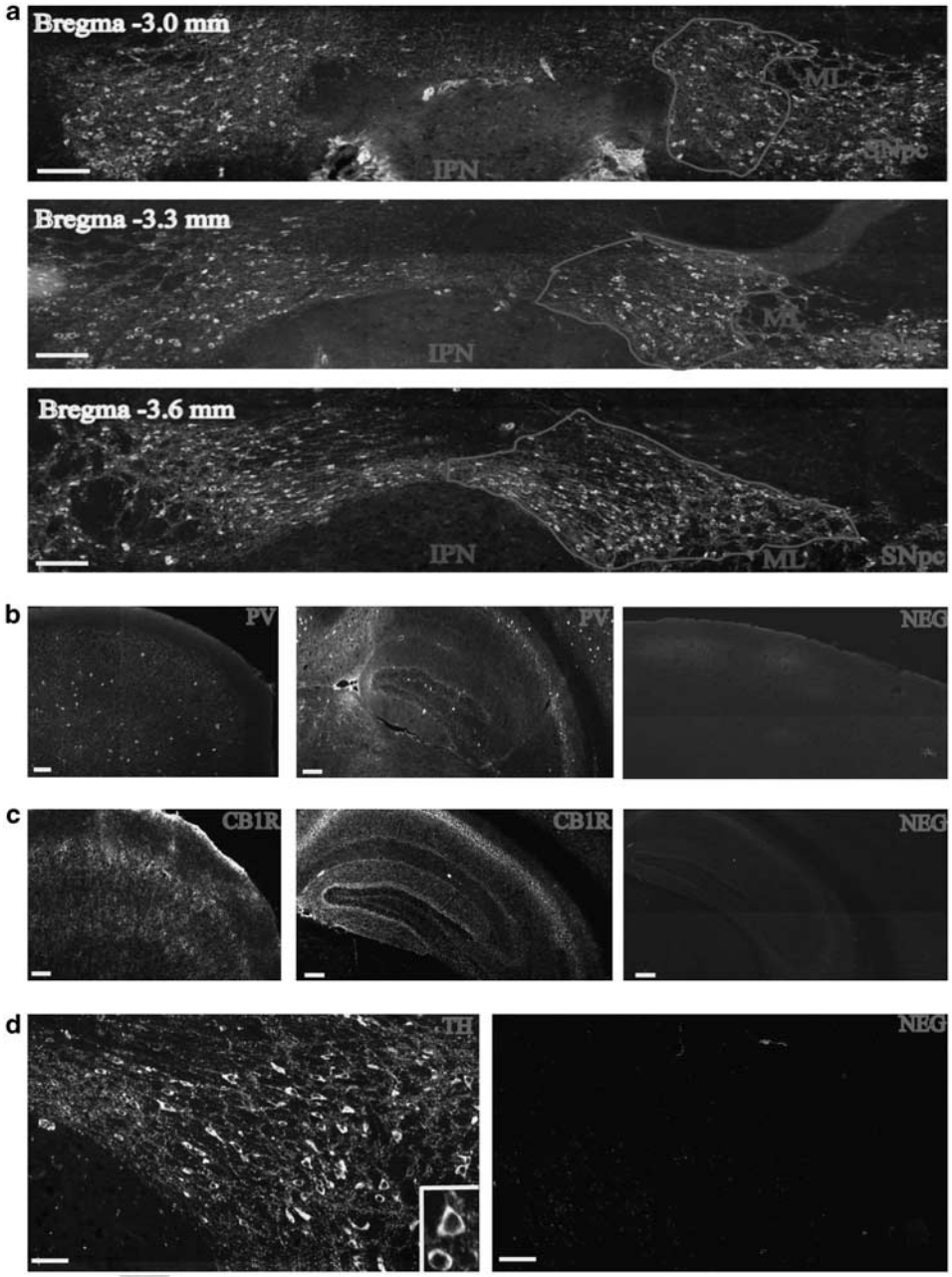

Figure 1 Delineation of ventral tegmental area (VTA) and representative immunofluorescent staining of PV, cannabinoid receptor 1 (CB1R), and TH in mouse brain. (a) Delineation of VTA using TH immunofluorescence at Bregma level −3.0 mm, −3.3 mm, and −3.6 mm. VTA boundary landmarks are the interpeduncular nucleus (IPN) at the ventromedial border, the medial lemnicsus (ML), and the substantia nigra pars compacta (SNpc) at its most lateral and ventrolateral border; scale bar, 200 μ. (b) PV immunoreactivity in prefrontal cortex (PFC) (left), hippocampus (HPC) (centre), and negative control (NEG) in HPC (right); scale bar, 200 μ. (c) CB1R staining in PFC (left), HPC (centre), and CB1R-negative control in PFC (right); scale bar, 200 μ. (d) TH immunoreactivity in VTA (left, and inset showing TH-positive cell soma of neuron) and TH-negative control in VTA (right); scale bar, 100 μ.

PV Cell Somal Size and Density in PFC and HPC

An effect of genotype on PV cell size was observed in the PFC ($F_{2,\ 25} = 6.36$; $p = 0.006$), whereby PV cells were smaller in COMT HET ($140.67 \pm 12.07\ \mu m^2$) than in COMT KO ($169.32 \pm 11.66 \mu m^2$), or WT ($146.42 \pm 7.89\ \mu m^2$) (Figure 3c). A genotype × THC treatment interaction for PV cell somal size in PFC ($F_{2,\ 25} = 7.04$; $p = 0.004$) reflected an action of THC to increase PV cell somal size in WT, to be without effect in HET and to decrease PV cell somal size in COMT KO (Figure 3c). An effect of genotype on PV cell size was observed in HPC ($F_{2,\ 25} = 8.021$; $p = 0.002$), whereby PV cells were smaller in COMT KO ($165.44 \pm 3.58\ \mu m^2$) and particularly in COMT HET ($154.83 \pm 11.91\ \mu m^2$) than in WT ($171.85 \pm 8.01\ \mu m^2$) (Figure 3d). There were no effects of genotype, THC treatment or gene × THC treatment interactions for PV cell density in PFC or HPC (Figures 3e and f).

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Table 1 Morphological Measurements During Adulthood in PFC, HPC, and VTA of WT, COMT HET, and COMT KO Mice, Respectively, Following Treatment During Adolescence with 8.0 mg/kg THC or vehicle (0)

Genotype	THC (mg/kg)	CBIR PFC intensity (intensity/μm^2)	CBIR HPC intensity (intensity/μm^2)	PV PFC cell size (μm^2)	PV HPC cell size (μm^2)	PV PFC density (cells/mm^2)	PV HPC density (cells/mm^2)	TH VTA cell size (μm^2)	TH VTA density (cells/mm^2)	VTA volume (mm^3)
wt	0	60.17 ± 5.14	37.27 ± 3.31	146.42 ± 7.89	171.85 ± 8.01	81.08 ± 23.41	26.45 ± 3.20	107.76 ± 13.62	606.11 ± 70.25	0.23 ± 0.01
wt	8	51.38 ± 9.84	32.88 ± 2.38	162.24 ± 9.01	178.23 ± 6.14	84.07 ± 11.27	22.79 ± 2.40	111.20 ± 12.90	492.46 ± 76.66	0.21 ± 0.01
het	0	63.53 ± 5.99	37.48 ± 3.30	140.67 ± 12.07	154.83 ± 11.91	89.36 ± 15.93	27.46 ± 3.32	108.66 ± 14.42	561.49 ± 35.50	0.22 ± 0.01
het	8	63.20 ± 4.36	39.29 ± 3.67	143.64 ± 11.01	162.61 ± 15.53	76.85 ± 13.94	26.87 ± 3.67	112.26 ± 14.46	513.55 ± 19.29	0.22 ± 0.02
ko	0	64.86 ± 1.40	33.77 ± 3.10	169.32 ± 11.66	165.44 ± 3.58	95.16 ± 9.32	26.66 ± 2.14	118.14 ± 17.44	506.55 ± 25.14	0.23 ± 0.01
ko	8	66.11 ± 3.72	35.91 ± 1.16	148.68 ± 9.84	163.36 ± 4.73	82.56 ± 15.56	24.27 ± 2.88	94.43 ± 7.86	490.26 ± 53.64	0.23 ± 0.03

Data are means ± SEM.

It was noteworthy that CB1R intensity inversely correlated strongly with PV cell size in PFC ($r = -0.55$, $p < 0.001$) and HPC ($r = -0.46$, $p < 0.01$).

DISCUSSION

In this study, the effects on morphological indices of GABAergic, dopaminergic, and cannabinoid function were assessed in COMT mutant mice, following chronic THC treatment during adolescence. To our knowledge, this is the first study to assess the combined effects of COMT activity and adolescent THC administration on cannabinoid-modulated neuronal populations implicated in schizophrenia. As human polymorphisms elicit smaller effects on enzyme activity, our COMT mutant mice do not serve to replicate such findings here but to illuminate the modulatory role of COMT on other neuronal populations in the brain, following chronic THC administration. On the basis of previous findings, indicating a sexually dimorphic effect of COMT function on psychiatric phenotypes to be evident primarily in males (Gogos *et al*, 1998; O'Tuathaigh *et al*, 2010), we assessed male COMT mutant mice. On the basis of our behavioral studies on COMT mutant mice, indicating COMT genotype to influence the effects of adolescent THC administration on schizophrenia-related behavioral phenotypes (O'Tuathaigh *et al*, 2010), the same treatment regimen was employed in the present study. We examined morphological parameters such as neuronal cell size, cell density, and protein expression to obtain morphological correlates on the functional capacity of specific neuronal populations (Behan *et al*, 2009; Cotter *et al*, 2002a, b). Given that the COMT mutant model was based on a higher COMT activity being a risk factor for schizophrenia, our results show that COMT deletion is associated with: (1) release of an effect of adolescent THC treatment in VTA that reduced dopaminergic cell size, (2) increased CBR1 intensity in PFC, reduced CBR1 intensity in HPC, and a shift in effect of adolescent THC treatment in HPC and PFC from reduction to increase in CBR1 intensity, and (3) increased GABAergic cell size in PFC and HPC, and a shift in effect of adolescent THC treatment in PFC and HPC from increase to decrease in GABAergic cell size. Adolescent THC treatment also reduced dopaminergic cell density. COMT KO mice demonstrated the prominent structural neuronal deficits in this study.

COMT Modulation of Adolescent THC Effects on Neurotransmitter Systems

Dopaminergic neurons. A functional polymorphism in the COMT gene moderates the risk to develop psychosis, following adolescent cannabis use with individuals homozygous for the COMT Val allele possessing a higher risk for psychosis, following adolescent cannabis use (Caspi *et al*, 2005; Henquet *et al*, 2006). This is attributable to increased COMT activity associated with the COMT Val allele in the PFC leading to a reduction in PFC dopamine neurotransmission and increased mesolimbic dopamine signaling; the converse would be hypothesized for the COMT Met allele (Akil *et al*, 2003; Bilder *et al*, 2004). THC challenge studies in humans have demonstrated significant increases in striatal dopamine release after acute administration of

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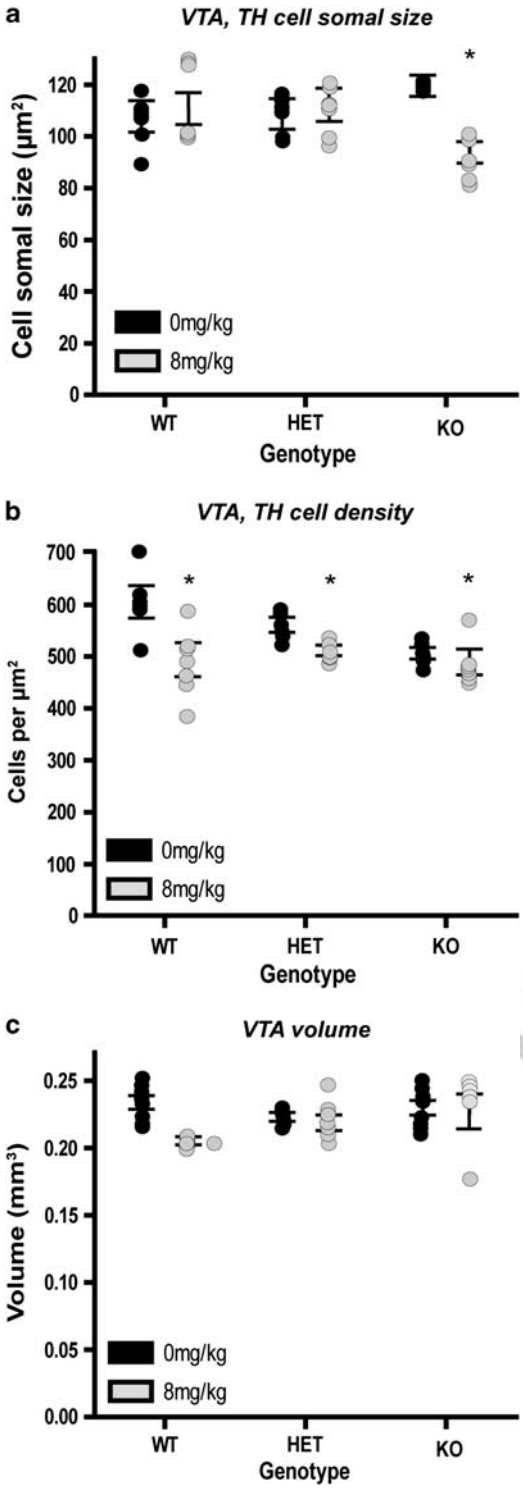

Figure 2 Somal size (a), cell density (b), and ventral tegmental area (VTA) volume (c) for TH-positive cells in VTA during adulthood, following adolescent treatment with 8.0 mg/kg delta-9-tetrahydrocannabinol (THC) or vehicle (0). Data are means ± SEM; $*p<0.015$.

inhaled THC (Bossong *et al*, 2009); however, such findings could not be replicated in a larger cohort (Stokes *et al*, 2010, 2011). In animal studies, THC increased dopaminergic neuronal firing and dopamine release in the striatum and PFC (Cheer *et al*, 2004; Chen *et al*, 1990; French, 1997; French *et al*, 1997; Pistis *et al*, 2002; Tanda *et al*, 1997). PFC is the dominant COMT site in the brain for modulating dopamine metabolism whereas the dopamine transporter (DAT), found at a much higher density in the striatum, is mainly responsible for regulating striatal dopamine levels (Scatton *et al*, 1985; Yavich *et al*, 2007) . Male COMT KO mice used in this study have previously demonstrated higher basal levels of dopamine release, dopamine levels, and its metabolites in PFC of COMT KO mice (Gogos *et al*, 1998; O'Tuathaigh *et al*, 2011). Given the evidence of COMTs regulatory role in the PFC (and not the striatum) (Scatton *et al*, 1985; Yavich *et al*, 2007), we would speculate that changes to COMT activity in PFC are having a knock-on effect on the activity and output of dopaminergic cortical neurons. It is possible that our observations of smaller tyrosine hydroxylase-labeled dopaminergic neurons in VTA in COMT KO mice following THC treatment occurs in response to altered input from cortical dopaminergic neurons (Akil *et al*, 2003). This further highlights a role for prefrontal COMT levels in the regulation of and communication between cortical and subcortical dopaminergic neuronal populations.

The aforementioned pattern of midbrain dopaminergic signaling is similar to that proposed to be present among subjects carrying the COMT Met allele (Akil *et al*, 2003; Bilder *et al*, 2004). We propose that lower cortical COMT activity may lead, through increased cortical dopaminergic activity, to reduced midbrain dopaminergic function, and hence, neuronal size. No changes in the structure of VTA dopaminergic neurons were observed in male COMT WT and HET mice. Our findings indicate that COMT genotype and adolescent THC exposure together can modulate dopaminergic neuronal structure in the mesolimbic system. Changes in dopaminergic neuron structure may reflect a cellular pathology that accompanies changes in dopaminergic activity as have been observed in other neuronal populations implicated in schizophrenia (Cotter *et al*, 2002a). Relating such structure–function findings are relevant to our understanding of the pathophysiology of schizophrenia, as excessive activity of dopaminergic neurons in VTA has also been proposed to have a role in the emergence of psychotic symptoms in schizophrenia (D'Souza *et al*, 2005; Henquet *et al*, 2005; Stefanis *et al*, 2004; van Os *et al*, 2002; Verdoux *et al*, 2003).

Cannabinoid 1 receptor and the endocannabinoid pathway. Dysfunction in PFC is strongly implicated in schizophrenia (Lafourcade *et al*, 2007) and within it, CB1R expression is reported to be reduced (Eggan *et al*, 2010). Our observation of reduction in CB1R expression in PFC and HPC of WT mice following adolescent THC treatment is in accordance with previous CB1R binding and protein expression studies in animals, following THC treatment (Romero *et al*, 1997, 1998). Furthermore, this finding is in line with what we would have predicted from our model based on a higher COMT activity and THC administration being a risk factor for schizophrenia (Caspi *et al*, 2005). Conversely, we observed a shift in this pattern, from reduction to increase in CB1R expression in PFC and HPC, in COMT KO mice following adolescent THC treatment. This affirms structural significance and interdependence of

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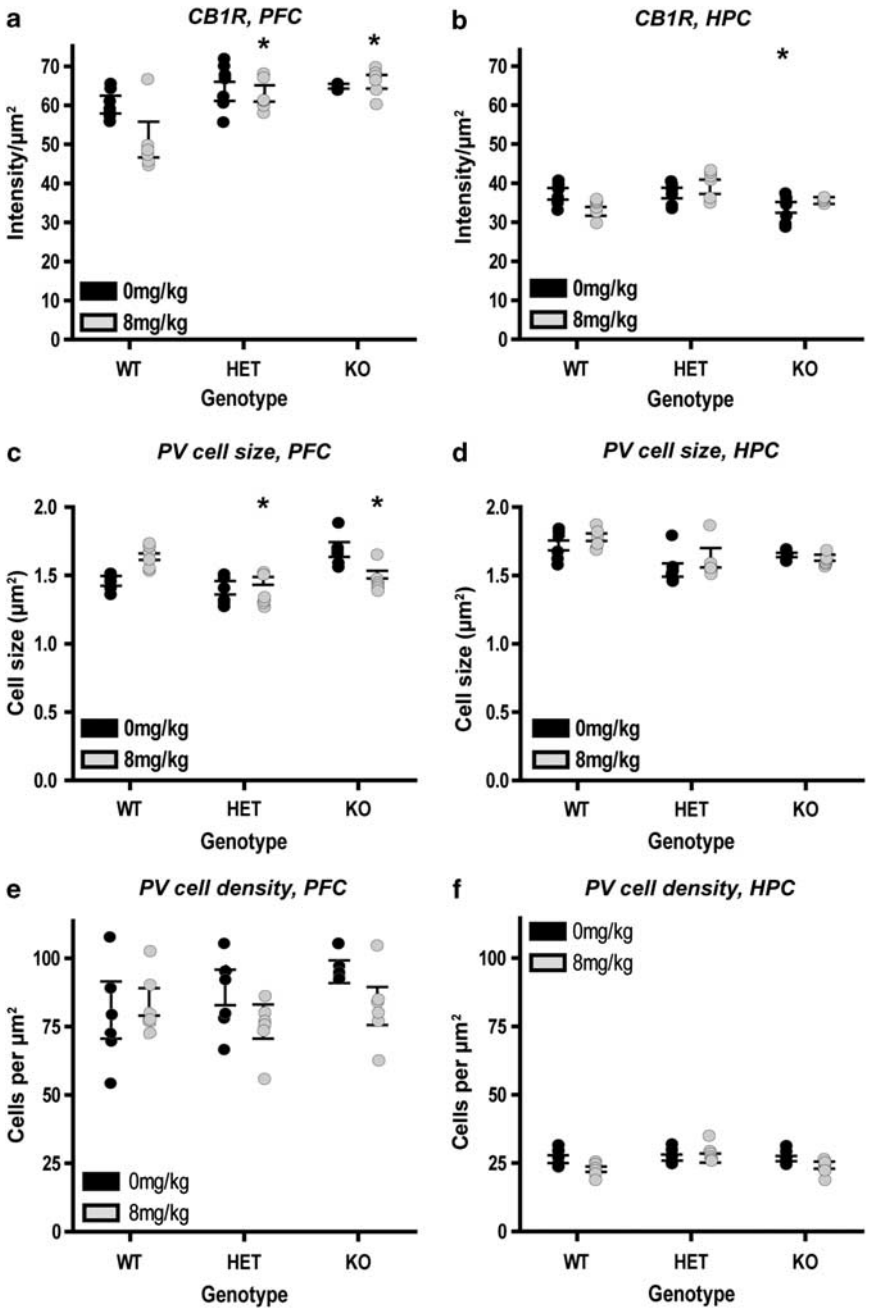

Figure 3 Cannabinoid receptor 1 (CB1R) intensity in prefrontal cortex (PFC) (a) and hippocampus (HPC) (b), PV cell size in PFC (c) and HPC (d), and PV cell density in PFC (e) and HPC (f) during adulthood, following adolescent treatment with 8.0 mg/kg delta-9-tetrahydrocannabinol (THC) or vehicle (0). Data are means ± SEM; *$p<0.015$.

COMT activity and the endocannabinoid system (Chevaleyre *et al*, 2006; Fride, 2005), and may be relevant to the known association of cannabis use with increased levels of psychotic symptoms (D'Souza *et al*, 2005; Henquet *et al*, 2005; Stefanis *et al*, 2004; van Os *et al*, 2002; Verdoux *et al*, 2003). It also emphasizes further the developmental period of adolescence and its vulnerability to subsequent effects of cannabis.

THC is known to affect endocannabinoid neurotransmission with components of this endocannabinoid pathway shown to be involved in regulating dopaminergic neurotransmission, particularly in the striatum (Cheer *et al*, 2004; Chen *et al*, 1990; Tanda *et al*, 1997). Furthermore, THC, which acts through activation of CB1 receptors, is known to increase firing of dopaminergic neurons in the midbrain (Diana *et al*, 1998; French *et al*, 1997) and these same midbrain dopamine neurons regulate excitatory and inhibitory inputs by retrograde endocannabinoid signaling (Chevaleyre *et al*, 2006; Lupica and Riegel, 2005; Matyas *et al*, 2008). The effects of cannabinoids such as THC on dopaminergic mesolimbic neurotransmission are usually indirect (Fernandez-Ruiz *et al*, 2010); it is thought that CB1R at GABAergic and

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glutamatergic synapses facilitate such indirect actions (Herkenham *et al*, 1990; Mailleux and Vanderhaeghen, 1992; Tsou *et al*, 1998). Therefore, our findings of smaller VTA dopaminergic neurons following THC treatment may occur in response to higher cortical CB1R levels in COMT KO mice (Akil *et al*, 2003). Our finding that adolescent THC treatment and COMT genotype interact to modulate VTA dopaminergic neuron size in adult mice may reflect an effect of endocannabinoid regulation on mesolimbic dopaminergic neuron populations following adolescent THC exposure, a critical period of brain development (Ehrenreich *et al*, 1999; Pope *et al*, 2003). On this basis, we propose that dysregulation of dopaminergic neurons in VTA in schizophrenia (Akil *et al*, 2003; Bilder *et al*, 2004) may be exacerbated by adolescent THC-induced, CB1R-mediated, long-term impairment in mesolimbic dopaminergic neuron structure and, hence, signaling.

GABAergic neurons. Dysfunction in GABAergic systems is evident in schizophrenia, with GABA synthesis and reuptake reduced in PFC and HPC (Heckers *et al*, 2002; Konradi *et al*, 2011; Volk *et al*, 2001). Furthermore, cell density of GABAergic neurons, in particular the number of PV-positive neurons, is decreased in PFC and HPC of patients with schizophrenia (Benes *et al*, 1991, 1998; Hashimoto *et al*, 2003; Konradi *et al*, 2011). Given these studies and based on a higher COMT activity being a risk factor for schizophrenia, one would have predicted deficits in cortical GABAergic neuronal populations in COMT WT mice, where the PFC would be hypodopaminergic and GABAergic inhibition would not be strong. Instead, GABAergic deficits were observed by a reduction in cell size in COMT KO mice and not COMT WT mice. The conventional inverted-U shape curve of dopaminergic modulation of the PFC activity may provide some understanding given that deficient or excessive dopamine activity exert disruptive effects on normal neuronal structure (Seamans and Yang, 2004). As dopamine is capable of having an inhibitory effect on cortical GABA neurons (Retaux *et al*, 1991), it is possible that an excessive release of prefrontal dopamine in COMT KO mice could lead to impairments in normal GABAergic neuronal structure. Conversely, GABAergic neurons in VTA are anatomically positioned to influence the activity of the dopamine system in the mesolimbic pathway (Laviolette and van der Kooy, 2001); so, changes in GABAergic neurons could be responsible for altering the activity and structure of neighboring dopaminergic neurons.

It is not yet clear how reduction in the size of PV^+ GABAergic neurons in COMT KO mice in response to adolescent THC treatment relates to the upregulation of cortical CB1R protein expression in COMT HET and KO mice, following adolescent THC exposure. However, given our findings of a significant inverse correlation between CB1R and PV cell size and that endocannabinoids are known to modify inhibition in GABAergic neurons (Lovinger, 2008), it is also possible that enhanced expression of cortical CB1R (by THC treatment) may alter the size of cortical GABAergic populations and, consequently, modify GABAergic signaling in COMT mutant mice. Our GABAergic findings in the COMT mutant mouse are interesting, given that most of the effects of the endocannabinoid system on dopaminergic neurotransmission are GABA- and/or glutamate-mediated (Herkenham *et al*, 1990; Mailleux and Vanderhaeghen, 1992; Tsou *et al*, 1998). Additionally, endocannabinoids such as THC are responsible for retrograde signaling of CB1R on GABAergic neurons and, through this mechanism, can prevent excess inhibition at GABAergic synapses (Lovinger, 2008). Upregulation of cortical CB1R protein expression in COMT HET and KO mice, following adolescent THC exposure, may thus mediate the reduction observed in midbrain dopaminergic neuronal cell size through CB1Rs cortical action on GABAergic neurons. How this occurs may have a functional origin arising from a dampened inhibition of GABAergic neurons brought, which, in turn, can be mediated by the increased expression observed in CB1R-enhanced endocannabinoid signaling (Lovinger, 2008).

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There is certainly a higher order of complexity in how these systems interact that should be interpreted with caution. Nonetheless, the GABAergic changes observed in this gene × environmental mouse model and their relationship to schizophrenia indicate further the vulnerability to, and consequent dysregulation of, neurotransmitter systems by developmental insults such as THC treatment during adolescence.

Summary

These studies elaborate and suggest putative mechanisms underlying recent clinical findings that cannabis use during adolescence, and subsequent psychosis is influenced by the human COMT genotype (Caspi *et al*, 2005). The higher activity COMT Val allele has demonstrated in some, but not all, human studies to be associated with increased risk of schizophrenia following THC exposure during adolescence (Caspi *et al*, 2005; Zammit *et al*, 2011). It might have been predicted, based on a higher COMT activity being a risk factor for schizophrenia, that COMT KO mice exposed to adolescent THC would evidence fewer schizophrenia-related neuronal changes than WT mice, that is, relative hyperdopaminergia in mesolimbic systems (Akil *et al*, 2003), reduced density of cortical and hippocampal GABAergic neurons (Cotter *et al*, 2002a), and reduced CB1R expression (Eggan *et al*, 2010). However, the opposite was observed; COMT KO mice demonstrated the more prominent structural neuronal deficits. These findings may reflect complexities arising from the inverted U-shaped relationship between cortical-mediated function and cortical dopaminergic activity in human/non-human primates *vis-à-vis* rodents, such that deficient or excessive dopamine activity could each exert disruptive effects on normal neuronal structure (O'Tuathaigh *et al*, 2010, 2011; Seamans and Yang, 2004; Tunbridge *et al*, 2006). Additionally, future comparative studies investigating these same paradigms in mice expressing variants of human COMT are required to rule out any probability of our COMT KO findings arising from developmental compensation in dopaminergic, CB1R, and GABAergic systems.

Our findings indicate disruptive effects of adolescent THC exposure on several neuronal populations, in a manner that can be modulated by COMT genotype. They demonstrate how genetic, developmental, and environmental factors can interact to modulate the cellular structure of neuronal

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populations implicated in schizophrenia such as the dopaminergic, endocannabinoid, and GABAergic systems.

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DISCLOSURE

The authors declare no conflict of interest.

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