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ARTICLE

Transmembrane domain *Nrg1* mutant mice show altered susceptibility to the neurobehavioural actions of repeated THC exposure in adolescence

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Abstract

Heavy cannabis abuse increases the risk of developing schizophrenia. Adolescents appear particularly vulnerable to the development of psychosis-like symptoms after cannabis use. To test whether the schizophrenia candidate gene neuregulin 1 (*NRG1*) modulates the effects of cannabinoids in adolescence, we tested male adolescent heterozygous transmembrane domain *Nrg1* mutant (*Nrg1* TM HET) mice and wild type-like littermates (WT) for their neurobehavioural response to repeated Δ^9-tetrahydrocannabinol (THC, 10 mg/kg i.p. for 21 d starting on post-natal day 31). During treatment and 48 h after treatment withdrawal, we assessed several behavioural parameters relevant to schizophrenia. After behavioural testing we measured autoradiographic CB_1, $5\text{-}HT_{2A}$ and NMDA receptor binding. The hyperlocomotor phenotype typical of *Nrg1* mutants emerged after drug withdrawal and was more pronounced in vehicle than THC-treated *Nrg1* TM HET mice. All mice were equally sensitive to THC-induced suppression of locomotion. However, mutant mice appeared protected against inhibiting effects of repeated THC on investigative social behaviours. Neither THC nor *Nrg1* genotype altered prepulse inhibition. Repeated adolescent THC promoted differential effects on CB_1 and $5\text{-}HT_{2A}$ receptor binding in the substantia nigra and insular cortex respectively, decreasing binding in WT while increasing it in *Nrg1* TM HET mice. THC also selectively affected $5\text{-}HT_{2A}$ receptor binding in several other regions in WT mice, whereas NMDA receptor binding was only affected in mutant mice. Overall, *Nrg1* mutation does not appear to increase the induction of psychotomimetic symptoms by repeated adolescent THC exposure but may attenuate some of its actions on social behaviour and schizophrenia-relevant neurotransmitter receptor profiles.

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Key words: Adolescence, cannabinoids, neuregulin 1, schizophrenia, social interaction.

Introduction

The 'two-hit' hypothesis of schizophrenia suggests that genetic and environmental risk factors interact to produce the disorder (Bayer *et al.* 1999). Onset of symptoms is usually during adolescence or early adulthood, periods of major change in brain morphology and neural connectivity and thus increased vulnerability to insult (Paus *et al.* 2008). Perturbation to normal brain maturation during the transition from adolescence to adulthood, for example, by drug abuse, might contribute to exacerbation or precipitation of schizophrenia onset. Cannabis abuse is linked with a moderate increase in the risk of developing schizophrenia (Moore *et al.* 2007). Interestingly, individuals predisposed to schizophrenia are more vulnerable to adverse effects of cannabis (i.e. hallucinations,

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confusion and learning and memory deficits; d'Souza *et al.* 2005; Peters *et al.* 2009). One mediator of this increased vulnerability appears to be the schizophrenia candidate gene catechol-*O*-methyltransferase (Caspi *et al.* 2005). Further research has to clarify whether other candidate genes have similar properties.

Neuregulin 1 (*NRG1*) is a proposed schizophrenia susceptibility gene (Stefansson *et al.* 2002; for review and meta-analysis respectively, see Harrison & Law, 2006; Munafo *et al.* 2006; but also see Sanders *et al.* 2008). One of several transgenic mouse models available for *Nrg1* (Duffy *et al.* 2008) contains a heterozygous mutation in the transmembrane domain (*Nrg1* TM HET). *Nrg1* TM HET mice show face and partial predictive validity for schizophrenia, exhibiting age-dependent locomotor and exploratory hyperactivity (Karl *et al.* 2007; onset at age 5 months; reversible by clozapine treatment; Stefansson *et al.* 2002) and impaired preference for social novelty (O'Tuathaigh *et al.* 2007). We have shown that male *Nrg1* TM HET mice possess increased sensitivity to neurobehavioural effects of acute Δ^9-tetrahydrocannabinol [THC; i.e. enhancement of prepulse inhibition (PPI), hypolocomotive and anxiogenic effects, selective increases in c-Fos expression; Boucher *et al.* 2007*a*, *b*]. This phenomenon is sex-specific, since female *Nrg1* TM HET mice do not show increased behavioural sensitivity to acute THC treatment (Long *et al.* 2010*a*). The role of *Nrg1* in the response to cannabinoid agonists is further exemplified by altered rates of tolerance in adult *Nrg1* TM HET mice to the locomotor and anxiogenic effects of the synthetic cannabinoid CP 55 940 (Boucher *et al.* 2011). Based on our findings, *Nrg1* might be another genetic mediator of increased vulnerability to cannabis-induced psychosis.

Importantly, earlier age of onset of cannabis use appears to confer increased susceptibility to detrimental effects in adulthood, such as cognitive deficits (Ehrenreich *et al.* 1999; Pope *et al.* 2003), and may also precipitate earlier onset of schizophrenia (Large *et al.* 2011). Rodent studies also suggest that cognitive deficits are induced by chronic THC exposure during adolescence, but not adulthood (Quinn *et al.* 2008; Rubino *et al.* 2009*a*, *b*). Such early cannabinoid exposure may have long-lasting behavioural and neurobiological consequences (Realini *et al.* 2009) and there is evidence that both genetic and age-associated factors might play a role in the link between cannabis use and psychosis. Therefore, we aimed to determine the effects of acute and chronic adolescent THC on behaviour and receptor binding density in the *Nrg1* TM HET mouse. As these mice exhibit an age-dependent phenotype, we hypothesized that male adolescent *Nrg1* TM HET mice, like adults (Boucher *et al.* 2007*a*), would be more sensitive to the behavioural effects of THC in a battery of tests relevant to schizophrenia, resulting in earlier onset of schizophrenia-relevant behaviours and a more severe phenotype. We expected that these behavioural changes would be accompanied by alterations in CB_1, 5-HT_{2A} and NMDA receptors (NMDARs). These receptors are relevant to the pharmacological effects of cannabis and the pathophysiology of schizophrenia (Dalton *et al.* 2011; Kang *et al.* 2009; Matsumoto *et al.* 2005; Zavitsanou *et al.* 2002, 2004) and levels and/or activation of which are altered in adult *Nrg1* TM HET mice (Bjarnadottir *et al.* 2007; Dean *et al.* 2008; van den Buuse *et al.* 2009).

Method

Animals

Male heterozygous $Nrg1^{+/-}$ (*Nrg1* TM HET) and wild type-like control $Nrg1^{+/+}$ (WT) littermates (Karl *et al.* 2007) from 14 litters were used. To target adolescence, the study commenced at post-natal day (PND) 31 (±2; Spear, 2004). Standard social interaction (SI) opponents were age-matched male A/JArc mice (Animal Resources Centre, Australia). Mice were pair-housed with limited environmental enrichment [certified polycarbonate mouse igloo (Bioserv, USA) and a metal ring in the cage lid] under a 12 h light/dark schedule (lights on 08:30 hours). Food and water were available *ad libitum*. Research and animal care procedures were approved by the University of New South Wales Animal Care and Ethics Committee and were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Drug treatment

THC (THC Pharm GmbH, Germany) was suspended in a 1:1:18 mixture of ethanol:Tween 80® :saline and injected at a volume of 10 ml/kg. Mice received 21 consecutive daily i.p. injections of vehicle or THC (10 mg/kg; $n = 11$–16; Long *et al.* 2010*b*).

Behavioural testing

Mice were behaviourally tested as outlined in Table 1. Devices were cleaned between trials with 70% ethanol.

Body temperature

Mice were assessed for hypothermia (Compton *et al.* 1993). Body temperature was measured 5 min before

Table 1. Test biography of mice

Test day	Postnatal day	Test
1	31	Body temperature, catalepsy, OF, LD, PPI
13	43	OF
14	44	NORT habituation trials 1–2
15	45	LD, NORT habituation trial 3
16	46	NORT habituation trials 4–5
17	47	NORT test trials 1–2
19	49	Social interaction
21	51	PPI
23	53	OF, LD, PPI (withdrawal day)

OF, Open field; LD, light–dark test; PPI, prepulse inhibition; NORT, novel object recognition test.
Mice were injected with either vehicle or Δ^9-tetrahydrocannabinol (10 mg/kg body weight) once daily from test day 1 to day 21 ($n=11$–16).

and 30 min after injection using a lubricated rectal thermometer (SDR Clinical Technology, Australia).

Spontaneous locomotor activity

Locomotor activity was measured in an open field (OF) activity chamber (41 × 41 cm; Tru-Scan Photo Beam Activity System; Coulbourn Instruments, USA) for 10 min. Horizontal (distance travelled) and vertical activity (rearing) in central and peripheral zones was measured by the Tru-Scan system and ANY-maze$^{\text{TM}}$ video tracking software (Stoelting Co., USA). The ratio of central:total distance travelled (distance ratio) and time spent in the centre were taken as measures of anxiety (Denenberg, 1969).

Light–dark test (*LD*)

Mice were placed into the opening of a dark box insert (Coulbourn Instruments) in the OF activity chamber and allowed to explore freely for 10 min. The ratio of distance travelled in the light compartment to total distance travelled (distance ratio) and time spent in the light compartment were taken as measures of anxiety.

Novel object recognition test (*NORT*)

NORT apparatus was a grey Perspex arena (35 × 35 × 30 cm). Mice were habituated to the empty arena for 5 min twice daily for 2 d. The following day, mice were habituated to the test procedure (i.e. exposure to objects). The next day, mice were placed in the arena, which contained two identical objects placed in opposite corners, and allowed to explore freely (test trial 1). In test trial 2, 60 min later, the chamber contained one copy of these objects (familiar object) and one novel object, in the same positions as in test trial 1. Object exploration was scored for 5 min by the behaviours nosing (when the mouse directed its nose to an object at a distance of ≤ 1 cm) and rearing on the object.

Social interaction

SI between rodent pairs is used to measure anxiety-like behaviours (File & Seth, 2003). Reduction in SI models aspects of social withdrawal (WD), which also occurs in schizophrenia (Ellenbroek & Cools, 2000). Test mice and untreated A/JArc standard opponents were placed in opposite corners of the NORT arena and allowed to explore freely for 10 min. Frequency and duration of the active socio-positive behaviours general sniffing, anogenital sniffing, allogrooming, following and climbing over/under were scored (Boucher *et al.* 2007*a*). Distance travelled was measured by ANY-maze$^{\text{TM}}$.

Prepulse inhibition

PPI, an operational measure of sensorimotor gating, is the attenuation of the startle response by a non-startling stimulus (prepulse) presented before the startling stimulus (pulse). PPI is impaired in schizophrenia patients (Braff *et al.* 2001). Startle reactivity was measured using SR-LAB startle chambers (San Diego Instruments, USA). Sensitivity of the piezoelectric accelerometer was adjusted for the lower body weight of the adolescent mice. The PPI test consisted of 5 min acclimatization to 70 dB background noise, followed by 105 trials presented in a pseudorandom order: 5 × 70 dB trials (background); 5 × 80 dB trials; 5 × 100 dB trials; 15 × 120 dB trials (startle) and 15 sets of five trials comprising a prepulse of 74, 82 or 86 dB presented 32, 64, 128, 256 or 512 ms (variable inter-stimulus interval) prior to a startling pulse of 120 dB (PPI response). The inter-trial interval varied randomly from 10–20 s. Acoustic startle response (ASR) was calculated as the mean amplitude to all startle trials. Percentage PPI (%PPI) was calculated as [(mean startle response (120 dB) – PPI response)/mean startle response (120 dB)] × 100%. PPI was averaged across inter-stimulus intervals to produce a mean %PPI for each prepulse intensity.

Receptor autoradiography

A subset of mice ($n=4$–5 per factor) were killed after PPI testing on WD day. Brains were dissected, snap frozen and stored at $-80°$ C. Coronal sections (14 μm) were cut and thaw-mounted onto slides.

Autoradiographic binding

Ligand binding and quantification was performed as previously described. For CB_1 receptors (CB_1Rs) (Deng *et al.* 2007), sections were pre-incubated for 30 min in 50 mM Tris-HCl buffer (pH 7.4) containing 5% bovine serum albumin then incubated for 120 min in the same buffer containing 10 nM [^{3}H]CP-55, 940 (168 Ci/mmol; PerkinElmer, USA) in the presence (non-specific binding) or absence (total binding) of 10 μM CP 55 940. After incubation, sections were washed three times in ice-cold buffer (1×60, 1×180 and 1×5 min), dipped in distilled water and air dried.

For 5-HT_{2A} receptors (5-HT_{2A}R), sections were pre-incubated in 170 mM Tris-HCl buffer (pH 7.4) for 15 min then incubated for 120 min in the same buffer containing 4 nM [^{3}H]ketanserin (88 Ci/mmol; PerkinElmer) in the presence (non-specific binding) or absence (total binding) of 2 μM spiperone (Kang *et al.* 2009). After incubation, sections were washed in ice-cold buffer (2×10 min), dipped in distilled water and air dried.

For NMDARs, sections were incubated for 2.5 h in 30 mM Hepes buffer (pH 7.5) containing 100 μM glycine, 100 μM glutamate, 1 mM EDTA and 20nM [^{3}H]MK-801 (17.1 Ci/mmol; PerkinElmer) in the presence (non-specific binding) or absence (total binding) of 20 μM MK-801 (Newell *et al.* 2007). After incubation, sections were washed in ice-cold 30 mM Hepes containing 1 mM EDTA (pH 7.5, 3×20 min), dipped in distilled water and air dried.

Quantification

Slides were exposed to Kodak BioMax MR film (Kodak, USA) for 3 months and developed. Films were analysed with a computer-assisted image analysis system, Multi-Analyst, connected to a GS-690 Imaging Densitometer (Bio-Rad, USA). Brain regions were identified with reference to a mouse brain atlas (Paxinos & Franklin, 2004). Quantification of binding in each region was performed by measuring the average density in each region in two to three adjacent sections for both hemispheres and comparing the values against an autoradiographic standard (GE Healthcare, UK) (Kang *et al.* 2009).

Statistical analysis

Data were analysed with analysis of variance (ANOVA; between-subjects factors: 'treatment' and 'genotype'). For OF and PPI, repeated measures three-way ANOVA was used [within-subjects factor: 'interval' (OF) and 'prepulse intensity' (PPI)]. Linear contrasts identified differences between levels of prepulse intensity. For all analyses, initial ANOVA was followed by two- or one-way ANOVAs split by the corresponding factor(s) if appropriate. Main effects were regarded as statistically significant when $p < 0.05$. Degrees of freedom, *F* values and *p* values (* *vs.* WT; # *vs.* vehicle of corresponding genotype) are presented. Analysis was performed using SPSS 17.0 IBM, USA).

Results

At study commencement, WT and *Nrg1* TM HET mice weighed 14.9 ± 3.0 and 13.7 ± 3.8 g respectively. Throughout the study there was no significant difference in body weight between any groups of mice.

Body temperature

THC reduced body temperature in WT and *Nrg1* TM HET mice [thermic response (Δ°C): 0.2 ± 0.3 (vehicle WT), -1.1 ± 0.7 (THC WT), 0.5 ± 0.7 (vehicle *Nrg1* TM HET), -0.8 ± 0.4 (THC *Nrg1* TM HET); two-way ANOVA for 'treatment': $F_{1,46} = 5.9$, $p < 0.05$].

Locomotor activity

THC decreased locomotion in the OF on day 1 in both WT and *Nrg1* TM HET mice [$F_{1,53} = 17.8$, $p < 0.001$; Supplementary Table 1; Fig. 1*a*]. This effect was no longer present on day 13. On WD day, *Nrg1* TM HET mice in both treatment groups showed higher locomotor activity than WT controls ($F_{1,51} = 6.7$, $p < 0.05$). However, only THC-treated *Nrg1* TM HETs displayed significantly lower locomotor activity compared to vehicle-treated mutants ($F_{1,27} = 6.6$, $p < 0.05$; Supplementary Table 1; Fig. 1*a*).

THC also decreased locomotor activity in the SI test on day 19 [distance travelled (cm): 2176.7 ± 91.6 (vehicle WT), 3078.42 ± 158.0 (vehicle *Nrg1* TM HET), 911.8 ± 163.6 (THC WT); 1328.8 ± 120.1 (THC *Nrg1* TM HET); Supplementary Table 1].

When distance travelled was measured in 5 min intervals within the OF test, there was an effect of time on locomotor activity such that distance travelled was decreased in the second 5 min of the test on all test days (day 1: $F_{1,52} = 109.1$, $p < 0.001$; day 13: $F_{1,51} = 59.4$, $p < 0.001$; WD day: $F_{1,51} = 17.5$, $p < 0.001$; Fig. 1*c*). THC-treated mutant mice of both genotypes showed greater habituation than vehicle-treated mice after acute and chronic administration (interval × treatment interaction, day 1: $F_{1,52} = 9.3$, $p < 0.01$; day 13 $F_{1,51} = 26.7$, $p < 0.001$).

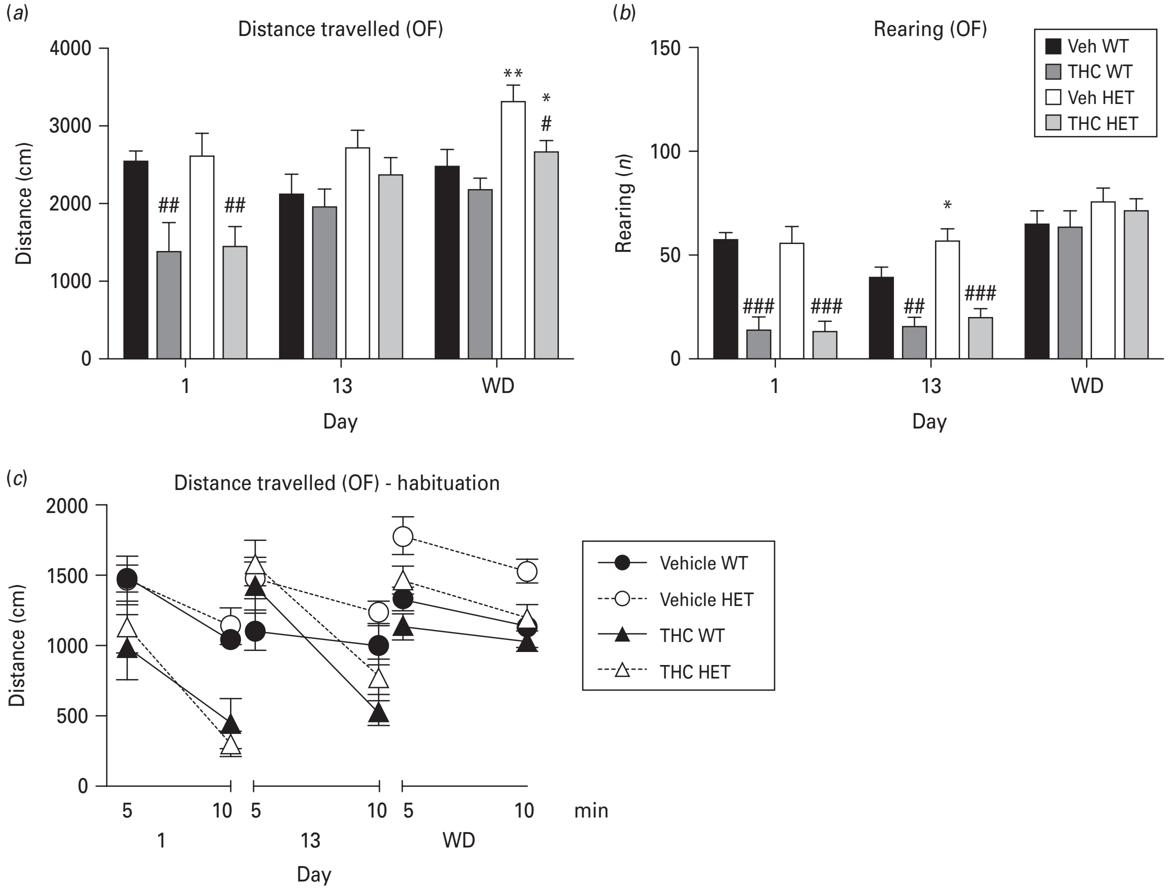

Fig. 1. Horizontal locomotor and vertical exploratory activity after injection of Δ^9-tetrahydrocannabinol (THC; 10 mg/kg). (*a*) Overall distance travelled; (*b*) rearing; (*c*) 5-min interval habituation of distance travelled in the open field (OF) on days 1, 13 and withdrawal (WD) day. Data represent means ± S.E.M.; $n = 12$–16. WT, Wild-type litter; HET, heterozygous; Veh, vehicle. $^{*} p < 0.05$, $^{**} p < 0.01$ *vs.* WT receiving corresponding treatment; $^{\#} p < 0.05$, $^{\#\#} p < 0.01$, $^{\#\#\#} p < 0.001$ *vs.* vehicle (analysis of variance).

Exploratory activity

THC reduced rearing in the OF in both genotypes on days 1 and 13 but not on WD day (e.g. day 1: $F_{1,53} = 49.4$, $p < 0.001$; Supplementary Table 1; Fig. 1*b*). Exploratory activity was higher in vehicle-treated *Nrg1* TM HET mice than in their WT counterparts in the OF on day 13 ($F_{1,26} = 5.1$, $p < 0.05$; Supplementary Table 1; Fig. 1*b*).

Anxiety

Acute administration of THC on day 1 induced task-specific anxiogenic-like behaviour in the OF in WT mice only, which showed more pronounced reductions in the time spent in the centre ($F_{1,24} = 11.2$, $p < 0.01$) and the distance ratio ($F_{1,24} = 9.4$, $p < 0.01$) compared to *Nrg1* TM HET mice (Supplementary Table 1; Fig. 2*a*, *c*). Chronic THC administration induced anxiogenic-like behaviour in the OF in both genotypes, as shown by the reduced OF distance ratio in WT and *Nrg1* TM HET mice on day 13 ($F_{1,51} = 20.2$, $p < 0.001$; Supplementary Table 1). In contrast, in the LD, there were no effects of 'treatment' or 'genotype' on anxiety-related parameters (Fig. 2*b*, *d*).

Learning and memory

Unexpectedly, exploration times were generally low in the NORT (<15 s/5-min trial) and object recognition bias was not highly prevalent in control mice, although the same NORT protocol previously yielded high recognition scores in our laboratory. Thus, NORT data are not shown.

Social interaction

There were no effects of THC treatment or genotype on total time spent in SI (data not shown). However, when examining individually scored behaviours, THC reduced the frequency and duration of general sniffing,

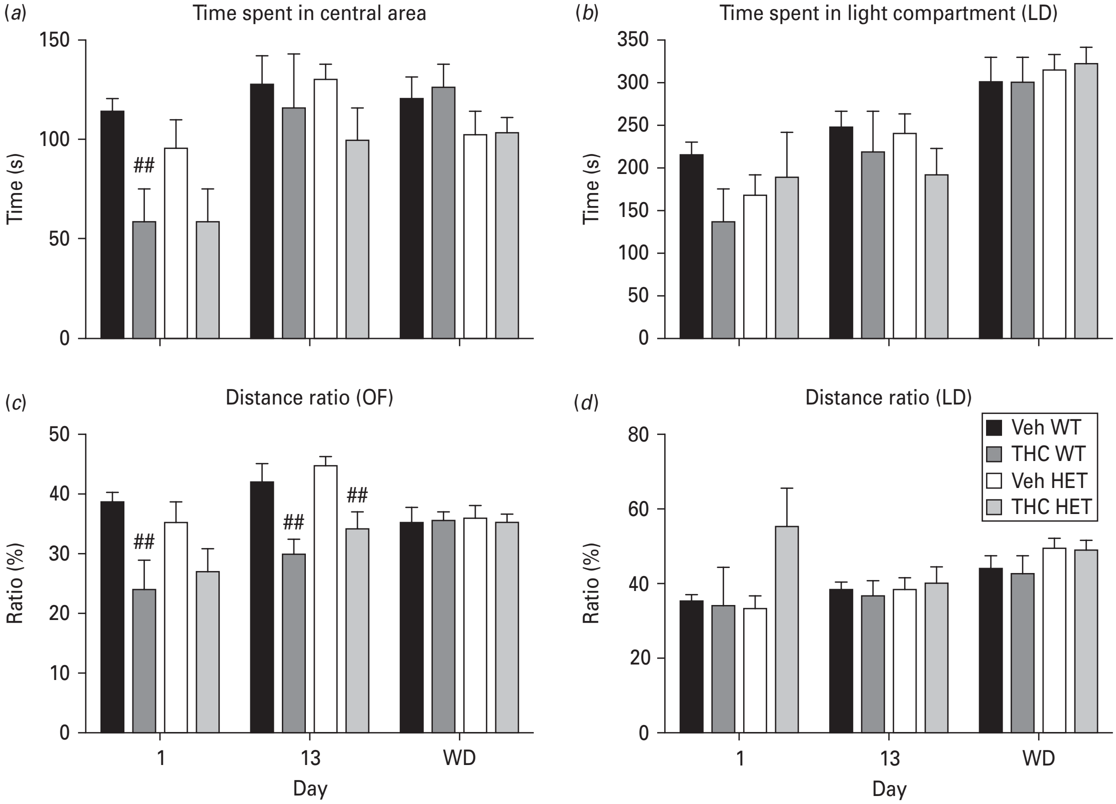

Fig. 2. Anxiety-related measures after injection of Δ^9-tetrahydrocannabinol (THC) (10 mg/kg). (*a*) Time spent in the central area of the open field (OF) on days 1, 13 and withdrawal (WD) day; (*b*) time spent in the light compartment of the light–dark (LD) on days 1, 15 and WD; (*c*), distance ratio (OF); (*d*) distance ratio (LD). Data represent means ± s.e.m.; $n = 11$–16. WT, wild-type litter; HET, heterozygous; Veh, vehicle. $^{\#\#}$ $p < 0.01$ *vs.* vehicle (analysis of variance).

the frequency of anogenital sniffing (e.g. anogenital sniffing: $F_{1,50} = 14.3$, $p < 0.001$; Supplementary Table 1; Fig. 3*a*, *b*) and the duration of allogrooming (data not shown). The effect of THC on the duration of general sniffing and frequency of anogenital sniffing was specific to WT mice and while the frequency of general sniffing was reduced by THC in both WT and *Nrg1* TM HET mice, it was significantly higher in THC-treated *Nrg1* TM HET mice ($F_{1,24} = 5.7$, $p < 0.05$) compared to THC-treated WT mice (Supplementary Table 1).

ASR and PPI

ASR

Chronic THC decreased the ASR in both WT and *Nrg1* TM HET mice ($F_{1,51} = 19.5$, $p < 0.001$; Supplementary Table 2; Table 2).

Prepulse inhibition

PPI increased with increasing prepulse intensity on each test day (Supplementary Table 2; Fig. 4*a*–*c*). Acute THC decreased PPI at the 74 dB prepulse intensity in *Nrg1* TM HET mice only (*Nrg1* TM HET 74 dB: $F_{1,27} = 4.8$, $p < 0.05$; Supplementary Table 2; Fig. 4*a*).

CB_1, 5-HT_{2A} and NMDAR receptor binding

Figure 5 depicts representative autoradiograms for [^{3}H]CP 55 940, [^{3}H]ketanserin and [^{3}H]MK-801 binding in WT and *Nrg1* TM HET mice. THC reduced [^{3}H]CP-55 940 CB_1R binding in the hippocampus (HPC) and ventromedial hypothalamus of WT and *Nrg1* TM HET mice. In the substantia nigra, CB_1R binding was reduced in vehicle-treated *Nrg1* TM HET mice compared to vehicle-treated WT littermates, while THC reduced CB_1R binding in WT mice but increased it in *Nrg1* TM HET mice [treatment × genotype interaction: $F_{1,13} = 28.2$, $p < 0.001$; Supplementary Table 3; Fig. 6*a*]. There was a trend towards a reduction in CB_1R binding by THC in the external globus pallidus in both genotypes ($p = 0.06$).

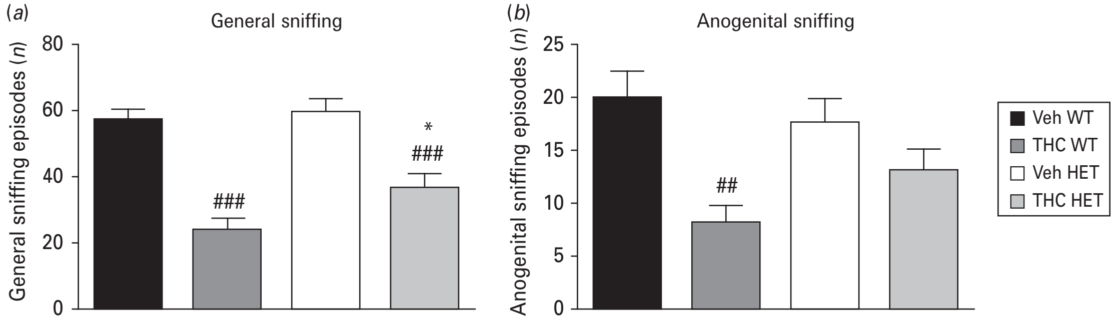

Fig. 3. Measures of social interaction after injection of Δ^9-tetrahydrocannabinol (THC) (10 mg/kg) on day 19. (*a*) Frequency of general sniffing; (b) anogenital sniffing. Data represent means ± s.e.m.; $n = 12$–14. WT, wild-type litter; HET, heterozygous; Veh, vehicle. * $p < 0.05$ *vs.* WT; ## $p < 0.01$, ### $p < 0.001$ *vs.* vehicle (analysis of variance).

Table 2. Acoustic startle response

	WT		*Nrg1* TM HET	
Day	Vehicle	THC 10	Vehicle	THC 10
1	51.7±5.8	45.6±5.0	45.3±3.5	34.0±3.8
21	73.2±5.7	46.8±4.3$^{\#\#}$	57.0±6.5	39.5±2.4$^{\#}$
WD	69.0±6.2	87.2±11.9	52.7±6.0	60.5±7.0

Nrg1 TM HET, heterozygous transmembrane domain *Nrg1* mutant; WT, wild-type-like littermate control; WD, withdrawal day.
Chronic Δ^9-tetrahydrocannabinol (THC; 10 mg/kg) reduces the startle response (arbitrary units) to a 120 dB acoustic stimulus.
Data represent means (± s.e.m.); $n = 12$–15.
$^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$ (*vs.* vehicle).

[^{3}H]Ketanserin 5-HT_{2A}R binding was also genotype and treatment dependent. Specifically, vehicle-treated *Nrg1* TM HET mice showed reduced 5-HT_{2A}R binding in the agranular insular and cingulate cortices but increased binding in the caudate putamen compared to WT controls. THC reduced 5-HT_{2A} binding in the anterior insula, cingulate cortex and ventral pallidum and increased binding in the caudate putamen of WT mice, but in *Nrg1* TM HET mice THC increased 5-HT_{2A}R binding only in the anterior insula ($F_{1,6} = 39.8$, $p < 0.001$; Supplementary Table 3; Fig. 6*b*).

The effects of THC on [^{3}H]MK-801 NMDAR binding were restricted to mutant mice, such that THC increased binding in the auditory cortex, cingulate cortex and hippocampus of *Nrg1* TM HET, but not WT mice (e.g. *Nrg1* TM HET in HPC: $F_{1,7} = 15.0$, $p < 0.01$; Supplementary Table 3; Fig. 6*c*).

Discussion

This study describes novel effects of adolescent THC on behaviour and receptor binding in a mouse model for the schizophrenia candidate gene *NRG1*. THC produced acute and chronic hypolocomotor and hypoexploratory responses in both *Nrg1* TM HET and WT mice, but only mutant mice showed residual hypolocomotion after THC WD. In contrast, mutant mice were less susceptible to other effects of THC, such as acute anxiogenic effects and reduction in investigative sniffing during SI elicited by repeated THC exposure. Repeated adolescent THC notably promoted differential effects on CB_1R and 5-HT_{2A}R density in the substantia nigra and insular cortex respectively, such that THC decreased binding density in WT but increased it in *Nrg1* TM HET mice. While repeated adolescent THC also affected 5-HT_{2A}R density in WT mice in the ventral pallidum, caudate putamen and cingulate cortex, no such effects were observed in *Nrg1* TM HET mice. Interestingly, the opposite profile was evident for NMDAR binding: repeated adolescent THC increased binding density in the hippocampus and auditory and cingulate cortices in mutants only.

Behavioural effects of Nrg1 *genotype and THC treatment*

Baseline hyperactivity in *Nrg1* TM HET mice emerged at an earlier age (PND 49–53) in this study than previously reported (Karl *et al.* 2007). As the *Nrg1* TM HET phenotype is sensitive to environmental modification (Karl *et al.* 2007), effects of the individually ventilated cage housing used in the present study and conventional housing used previously as well as

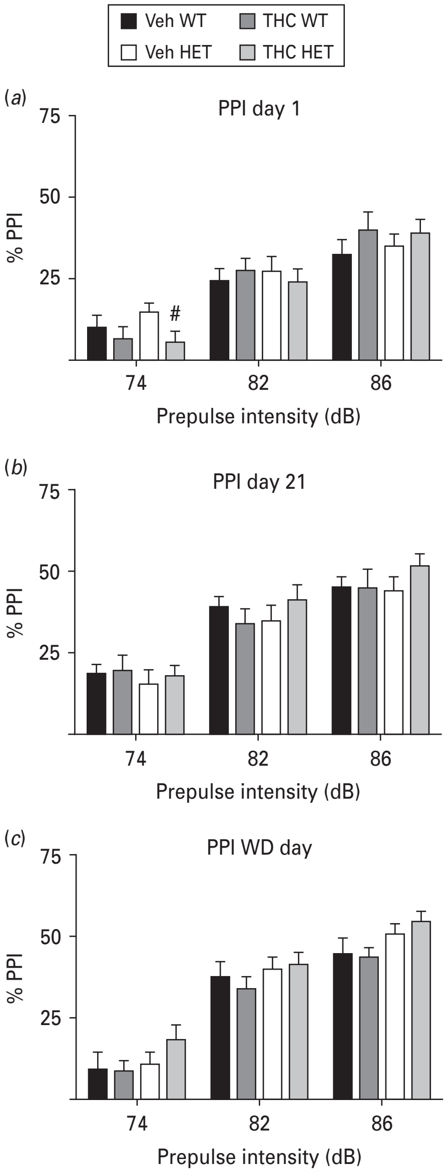

Fig. 4. Sensorimotor gating after injection of Δ^9-tetrahydrocannabinol (10 mg/kg). (*a–c*) % Prepulse inhibition (PPI) on days 1, 21 and withdrawal day. Data represent means ± s.e.m.; $n = 12–15$. WT, wild-type litter; HET, heterozygous; Veh, vehicle. $^{\#}p < 0.05$ *vs.* vehicle (analysis of variance).

the adolescent treatment procedure (i.e. stress of daily i.p. injections) might have contributed to the earlier phenotype onset (Kallnik *et al.* 2007). Repeated OF testing is unlikely to have contributed to the earlier onset as we have previously shown that locomotor activity is not altered by repeated exposure of *Nrg1* mutant mice to the OF paradigm (Boucher *et al.* 2011; Karl *et al.* 2007).

Acute THC elicited the typical cannabinoid effects of hypothermia and hypolocomotion in WT mice, similarly to our previous studies (Boucher *et al.* 2007*a*; Long *et al.* 2010*b*). These effects, observed at around PND 31, may represent one of the earliest manifestations of activation of CB_1Rs, which are suggested to be functionally immature until around PND 23, i.e. after weaning (Fride & Mechoulam, 1996). THC induced hypolocomotion in adolescents of both genotypes, which contrasts with the heightened susceptibility to THC-induced hypolocomotion of adult *Nrg1* TM HET mice (Boucher *et al.* 2007*a*). It is possible that using a lower, less sedative dose of THC in the present study, such as 1 mg/kg, may have unmasked some subtle differences in locomotor effects of THC between genotypes at this younger age.

Adolescent mice developed an overall tolerance to THC-induced hypolocomotion in the OF after chronic exposure. While tolerance to cannabinoid agonist-induced hypolocomotion is common (Boucher *et al.* 2011; Howlett *et al.* 2004), adolescent rats have been reported to be less susceptible than adults to the development of tolerance (Wiley *et al.* 2007). Interestingly, in THC-treated mice of both genotypes, hypolocomotor tolerance was evident only in the first half of the 10 min OF test on day 13, suggesting that the rate of tolerance to some THC effects may interact with its effects on habituation to the test environment. On the other hand, residual hypolocomotion was present in THC-treated *Nrg1* TM HET, but not WT mice in the OF and LD at WD. One possible interpretation for this phenomenon is that THC induced selective neurobiological alterations in *Nrg1* TM HET mice, which diminished the emergence of the typical hyperactive phenotype of these mice.

There were no baseline genotype differences in generalized anxiety-like behaviour in the adolescent mice, confirming the age-dependency of phenotypic features of this *Nrg1* mutant mouse model, since a task-specific, anxiety-related phenotype has been observed in adult *Nrg1* TM HET mice (Karl *et al.* 2007). Acute THC induced anxiety-like effects task-specifically in the OF, predominantly in WT mice, whereas chronic effects were detectable in all mice. The data suggest that adolescent WT and *Nrg1* TM HET mice have similar responses to chronic, but not acute, effects of THC on anxiety measures and that the effects of repeated adolescent THC exposure are not long lasting. This also appears to be an age-dependent phenomenon, as adult *Nrg1* mutants exhibited an increased sensitivity to acute THC (Boucher *et al.* 2007*a*).

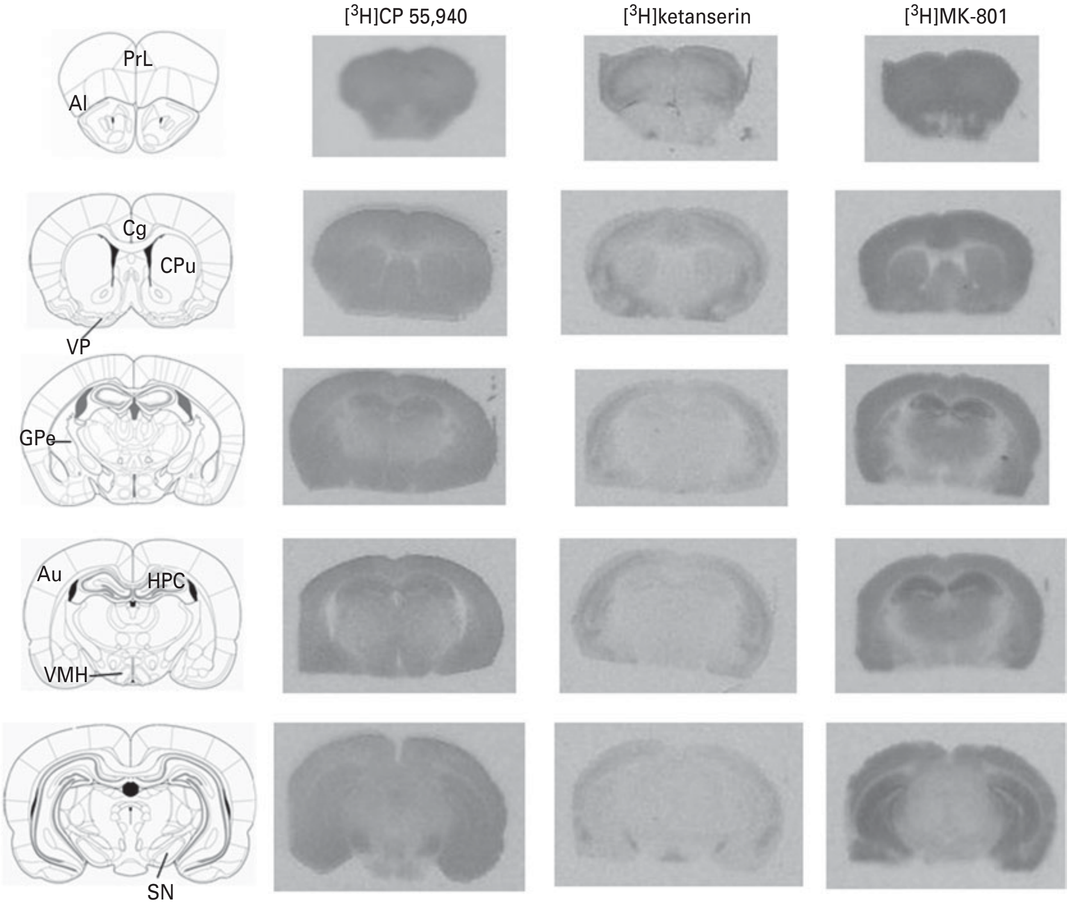

Fig. 5. Representative autoradiograms showing [^{3}H]CP 55 940, [^{3}H]ketanserin and [^{3}H]MK-801 binding in specific brain regions in wild-type litter and heterozygous transmembrane domain *Nrg1* mutant (*Nrg1* TM HET) mice. PrL, Prelimbic cortex; AI, agranular insular cortex; Cg, cingulate cortex; CPu, caudate putamen; VP, ventral pallidum; GPe, external globus pallidus; Au, auditory cortex; HPC, hippocampus; VMH, ventromedial hypothalamus; SN, substantia nigra.

Interestingly, adolescent *Nrg1* TM HET mice were resistant to THC-induced suppression of investigative social behaviours. There were no baseline differences between vehicle-treated mutant and WT mice in the SI test, which intends to model both social anxiety and social WD. While acute THC reduced total SI to a similar extent in adult *Nrg1* TM HET and WT mice (Boucher *et al.* 2007*a*), we now show that THC selectively reduced general sniffing and anogenital sniffing in adolescent WT mice. This selective reduction in investigative social behaviour cannot be explained by a locomotor suppressant effect of THC, since THC reduced locomotion to the same extent in both WT and *Nrg1* TM HET mice. It is possible that adolescent *Nrg1* TM HET mice undergo differential neurobehavioural adaptations to repeated THC that render them less susceptible to reductions in social behaviour induced by the drug – an effect worthy of consideration in light of the purported relief from negative symptoms experienced by cannabis users with schizophrenia (Smit *et al.* 2004).

There were no baseline genotype differences in startle response and PPI. Importantly, the PPI deficit of *Nrg1* TM HET mice initially reported (Stefansson *et al.* 2002) has not been replicated reliably (Boucher *et al.* 2007*a*; van den Buuse *et al.* 2009; but also see Boucher *et al.* 2011; Karl *et al.* 2011). Acute THC treatment selectively reduced PPI at the lowest prepulse intensity in *Nrg1* TM HET mice but did not alter the startle response, while chronic THC reduced the startle response in both genotypes but did not alter PPI during treatment or WD. These data suggest that THC has differential acute effects on sensorimotor gating in adult and adolescent *Nrg1* TM HET mice, since we have previously found that a single THC exposure enhanced PPI in adult *Nrg1* TM HET mice, while no

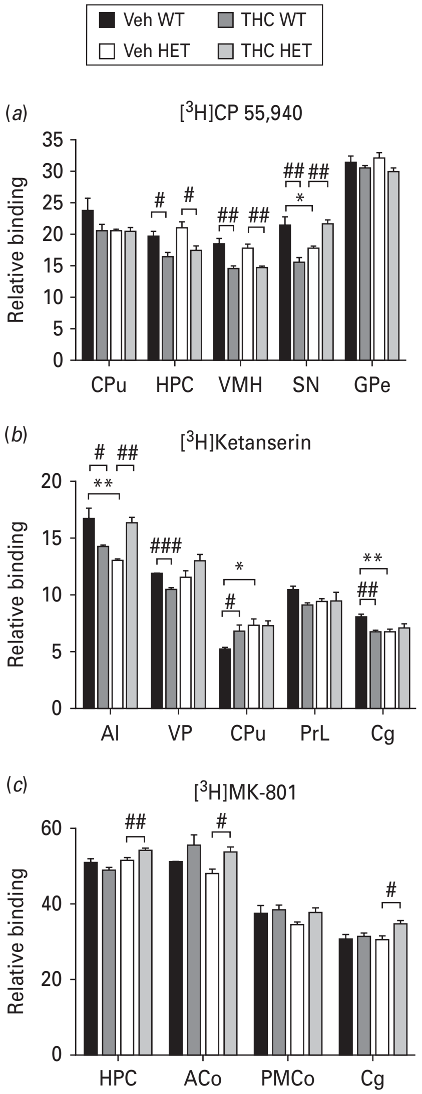

Fig. 6. Autoradiographic binding of (*a*) $[^3H]$CP-55 940, (*b*) $[^3H]$ketanserin and (*c*) $[^3H]$MK-801 on withdrawal day. Data represent mean binding density nCi/mg tissue (±S.E.M.); $n = 4$–5. WT, wild-type-like littermate control; HET, heterozygous; Veh, vehicle; CPu, caudate putamen; HPC, hippocampus; VMH, ventromedial hypothalamus; SN, substantia nigra; GPe, external globus pallidus; AI, agranular insular cortex; VP, ventral pallidum; PrL, prelimbic cortex; Cg, cingulate cortex; ACo, auditory cortex; PMCo, posteromedial cortical amygdaloid nucleus. * $p < 0.05$, ** $p < 0.01$ *vs.* WT; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ *vs.* vehicle (analysis of variance).

effect was observed in WT mice (Boucher *et al.* 2007*a*). Furthermore, repeated THC exposure had no effect on PPI in adolescent WT and *Nrg1* TM HET mice, similarly to our recent findings in adult mutants after repeated exposure to a synthetic cannabinoid agonist (Boucher *et al.* 2011).

Effects of **Nrg1** *genotype and THC treatment on autoradiographic receptor binding*

We report for the first time a decrease in CB_1R density in the substantia nigra of adolescent *Nrg1* TM HET mice. Since the binding ligand we used is a CB_1R agonist, it is possible that this finding reflects altered binding site affinity rather than overall receptor number. Nevertheless, we note with interest that using the same radioligand ($[^3H]$CP 55 940), CB_1R binding was modestly increased in the substantia nigra in adult *Nrg1* TM HET mice (Newell *et al.* unpublished observations), suggesting that the developmental trajectory of CB_1R expression between adolescent and adult mice differs between *Nrg1* TM HET and WT mice. Within the basal ganglia, CB_1R mRNA is synthesized in striatal medium spiny neurons and the receptor protein is transported to terminals projecting to the substantia nigra via the direct pathway and the external globus pallidus via the indirect pathway (Julian *et al.* 2003; van der Stelt & di Marzo, 2003). These pathways facilitate and inhibit movement, respectively. The reduced CB_1R binding in vehicle-treated adolescent mutant mice appears specific to the direct pathway, since CB_1R binding was not altered in the external globus pallidus. Combined with the observation that mRNA encoding ErbB4 receptors for NRG1 is localized on dopaminergic neurons in the substantia nigra (Abe *et al.* 2009), it is possible to speculate that hyperactivity in *Nrg1* mutant mice involves altered nigral dopaminergic neurotransmission, possibly related to reduced CB_1R availability in the direct pathway. We also report for the first time genotype differences in the effects of repeated adolescent THC on CB_1R binding in the substantia nigra, such that it reduced CB_1R binding in WT mice (consistent with prior research; Romero *et al.* 1997) but increased binding in *Nrg1* TM HETs. Combined with a trend towards reduced CB_1R binding in the external globus pallidus of both WT and mutant mice treated with THC, this suggests an involvement of both direct and indirect pathway CB_1Rs in the locomotor effects of chronic THC in WT mice. The increased CB_1R binding in the substantia nigra of THC-treated *Nrg1* TM HET mice may represent a maladaptive or compensatory response underlying the persistent reduction in locomotor activity after THC WD in mutant mice.

$5\text{-HT}_{2A}R$ binding was reduced in the anterior insular and cingulate cortices and increased in the caudate putamen of drug-free, adolescent *Nrg1* TM HET mice. This is consistent with reduced $5\text{-HT}_{2A}R$ density in prefrontal and other cortical regions in schizophrenia (Kang *et al.* 2009; Matsumoto *et al.* 2005; Pralong *et al.*

2000). Given that our data are from mice in mid-adolescence, the findings are particularly relevant to observations in the early stages of schizophrenia in humans, such as reduction in cortical 5-HT_{2A}Rs in individuals at high risk for schizophrenia (Hurlemann *et al.* 2008) and in first-episode patients (Rasmussen *et al.* 2010), and increased subcortical 5-HT_{2A}Rs (Erritzoe *et al.* 2008) in first-episode patients. While this suggests that changes in 5-HT_{2A}Rs may be an early manifestation of schizophrenia neuropathophysiology, it is possible that genetic influences such as *Nrg1* mutation may alter the development of the expression of this receptor, since we have previously observed a global increase in 5-HT_{2A}Rs in adult *Nrg1* TM HET mice (Dean *et al.* 2008). The effects of THC on 5-HT_{2A}Rs, similar to CB_1Rs, also appear to be genotype-specific, such that THC reduced binding in the agranular insula and ventral pallidum in WT mice but increased or did not change it in mutants. Overall, our results suggest that *Nrg1* modulates 5-HT_{2A}R binding density in brain regions relevant to schizophrenia and social anxiety (Furmark, 2009; Vertes, 2006; Wylie & Tregellas, 2010), which may subserve the differential patterns in THC effects on social anxiety in the present study.

We observed no baseline genotype differences in NMDAR binding. This is in line with a previous report of hypophosphorylation, but no change in total protein levels, of the NMDAR NR2B subunit in adult *Nrg1* TM HET mice (Bjarnadottir *et al.* 2007). Meanwhile, NMDAR binding density was selectively increased in the hippocampus and auditory and cingulate cortices of THC-treated mutant mice. This may represent an adaptation to THC-induced changes in endocannabinoid control of synaptic transmission (Bodor *et al.* 2005; Brown *et al.* 2003; Hoffman *et al.* 2010). Since NMDAR antagonists generally induce hyperactivity, increased NMDAR density may also underlie the persistent hypolocomotion after THC WD in mutant mice.

Here, we add to the *Nrg1* mutant mouse model literature by reporting adolescence-specific and genotype-dependent differences in the neurobehavioural response of these mice to THC. We hypothesized that adolescent *Nrg1* TM HET mice would be more susceptible to the hypolocomotor, hyposocial and anxiogenic effects of THC. However, we found no difference in the sensitivity of mutant mice to locomotor reduction by THC and, in fact, mutants were less susceptible to THC-induced reduction in social behaviour and to induction of anxiety-like behaviour by acute THC. Furthermore, the changes in CB_1, 5-HT_{2A} and NMDAR receptors in adolescent mutants that we observe are different to those previously found in adult mice, supporting the implications of our behavioural data that there are developmental differences in both the baseline phenotype of these mice and in their behavioural and neurochemical response to chronic cannabinoid agonist exposure. Overall, these findings are consistent with evidence for differential effects of THC between adolescents and adults and between 'vulnerable' (i.e. genetically modified) and 'healthy' brains.

Note

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/pnp).

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Statement of Interest

None.

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