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ORIGINAL INVESTIGATION

A follow-up study: acute behavioural effects of Δ^9-THC in female heterozygous *Neuregulin 1* transmembrane domain mutant mice

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Abstract
Rationale Heavy cannabis use is linked with an increased risk for schizophrenia. We showed previously that male heterozygous neuregulin 1 transmembrane domain (*Nrg1* HET) mice are more sensitive to some effects of the psychotropic cannabis constituent Δ^9-tetrahydrocannabinol (THC). We report data from a follow-up study in female *Nrg1* HET mice, investigating THC effects on behaviours with some relevance to schizophrenia.
Methods Mice were injected with THC (0, 5 or 10 mg/kg i.p.) 30 min before a test battery: open field, elevated plus maze, novel object recognition (set 1) or light–dark, social interaction (SI) and prepulse inhibition (PPI 1: variable interstimulus interval (ISI); set 2). Another set (set 3) was injected with the same doses of THC before a fixed interstimulus interval PPI test (PPI 2).
Results Female *Nrg1* HETs displayed the hallmark increased locomotor activity at 5 months and anxiolytic-like behaviour in the open field at 3 and 5 months. THC decreased locomotor activity in both genotypes. THC selectively reduced some SI behaviours in WT mice. Baseline PPI was enhanced in mutants under a variable ISI, while THC had no effect on PPI using either protocol.
Conclusions This study reports novel findings on the baseline PPI profile and resistance to THC-induced social withdrawal in female *Nrg1* HET mice. This is the first description of THC effects in females of this mouse model and suggests that the transmembrane domain *Nrg1* mutation

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does not appear to have a severe impact on the behavioural sensitivity to THC in female mice.

Keywords Cannabinoid · THC · Neuregulin 1 · Schizophrenia · Motor activity · Memory · Anxiety · Exploration · Prepulse inhibition · Sensorimotor gating

Introduction

The heterogeneous aetiology of schizophrenia is thought to comprise interactions between genetic susceptibility factor(s) (e.g. neuregulin 1 and *DISC1*) and adverse environmental factor(s) such as drug abuse or obstetric complications (van Os and Kapur 2009). While there is large overlap in the presentation of schizophrenia in males and females, there is evidence for delayed age of onset, fewer negative symptoms, more affective, paranoid and cyclical psychotic symptoms and a more rapid and greater response to antipsychotic therapy in women (Leung and Chue 2000; Rao and Kölsch 2003). Sex differences in behaviours such as smoking and medication compliance may account for some of these differences, but the sex steroid oestrogen has also been suggested to play a role in the structural and functional abnormalities of schizophrenia (Leung and Chue 2000). In particular, the decrease in oestrogen at the commencement of menopause is thought to be responsible for both the incidence of the first presentation of schizophrenia after 45 years of age in some women and for the reduction in treatment efficacy and deterioration of symptoms in older women with schizophrenia compared with their experiences at a younger age (Leung and Chue 2000). Furthermore, variants in genes such as reelin (Shifman et al. 2008) and *COMT* (Sazci et al. 2004) appear to have stronger associations with schizophrenia in women.

Neuregulin 1 is a candidate susceptibility gene for schizophrenia (Stefansson et al. 2002; for review and meta-analysis see Harrison and Law 2006; Munafo et al. 2006). Functional polymorphisms (Kircher et al. 2009; Mata et al. 2009) and changes in mRNA and protein isoform expression in postmortem brain tissue of schizophrenia patients (Chong et al. 2008; Law et al. 2006) provide converging evidence for its role in the pathophysiology of the disorder. Our laboratory has previously used the transmembrane domain neuregulin 1 heterozygous (*Nrg1* HET) mutant mouse to investigate behavioural domains relevant to schizophrenia. This mouse has partial face, predictive and construct validity for schizophrenia. Its adult behavioural phenotype is characterised by locomotor and exploratory hyperactivity (Stefansson et al. 2002; Karl et al. 2007) which is reversible by clozapine (Stefansson et al. 2002) and impaired response to social novelty and increased aggression in the resident–intruder test (O'Tuathaigh et al. 2007). However, prepulse inhibition of the startle response (PPI) in *Nrg1* HET mice has been reported to be disrupted (Stefansson et al. 2002) or unaltered (Boucher et al. 2007a; van den Buuse et al. 2009), depending on the protocol applied. Interestingly, some of the behaviours in *Nrg1* HET mice appear to be sex-specific, with only female mutants displaying increases in walkovers (O'Tuathaigh et al. 2008) and sifting of cage bedding (O'Tuathaigh et al. 2006).

Cannabis abuse is linked with an overall increase in the risk for schizophrenia (Moore et al. 2007), and it is important to understand the biological basis for how it might interact with other risk factors to bring about the disorder. It is possible that there is a subset of individuals with increased genetic susceptibility to the effects of cannabis, since cannabis use in adolescence has been associated with increases in psychosis-like symptoms in individuals with a Val/Met polymorphism in the *COMT* gene (Caspi et al. 2005). We have previously investigated the acute effects of the major psychoactive cannabis constituent Δ^9-tetrahydrocannabinol (THC) in *Nrg1* HET mice. Male *Nrg1* HET mice were more sensitive to the hypolocomotor and anxiogenic effects of THC and displayed prepulse inhibition enhancement after administration of THC (Boucher et al. 2007a). THC exposure selectively increased expression of c-fos, a marker of neuronal activation, in the ventral part of the lateral septum of *Nrg1* HET, but not wild-type-like mice (Boucher et al. 2007b).

Since behavioural effects of THC are often sex-specific [e.g. increased anti-nociception and catalepsy (Tseng and Craft 2001; Tseng et al. 2004) and greater spatial learning deficits (Cha et al. 2007) in female rats], and since *Nrg1* HET mice exhibit a partially sex-specific behavioural phenotype, we aimed to investigate the effects of THC in female *Nrg1* HET mice in a battery of behavioural tests with some relevance to schizophrenia. Here, we discuss these effects in the context of previous observations in male *Nrg1* HET mice which were tested under similar experimental conditions (Boucher et al. 2007a). This investigation is of particular importance in determining the risk of exacerbation or precipitation of schizophrenia following cannabis exposure in susceptible females.

Methods

Animals

The generation of heterozygous *Nrg1* transmembrane domain mutant mice has been described previously (Stefansson et al. 2002). Genotypes were determined after weaning (postnatal day 21) by tail biopsy and polymerase chain reaction [for details see (Karl et al. 2007)]. Test animals were

heterozygous *Nrg1*$^{+/-}$ (*Nrg1* HET) and wild-type-like control *Nrg1*$^{+/+}$ (WT) littermates (backcrossed for >10 generations onto a C57BL6/JArc background). Adult, female age-matched (±10 days) test animals were pair-housed in Polysulfone cages (Tecniplast, Rydalmere, Australia) under a 12:12 h light–dark schedule [light phase: white light (illumination: 80 lx)–dark phase: red light (illumination: <2 lx)], with environmental enrichment in the form of a red, transparent, polycarbonate igloo (certified polycarbonate mouse igloo: Bioserv, Frenchtown, NJ, USA) and a metal ring (3 cm diameter) in the middle of the cage lid. Food and water were available ad libitum. Adult, female group-housed A/JArc mice (Animal Resources Centre, Canning Vale, Australia) aged 7 months at the commencement of testing were used as standard opponents in the social interaction test. All research and animal care procedures were approved by the Garvan Institute/St Vincent's Hospital Animal Experimentation Ethics Committee and were in agreement with the Australian Code of Practice for the Care and use of Animals for Scientific Purposes.

Drug treatment

THC (dronabinol; THC Pharm GmbH, Frankfurt, Germany) was suspended in a 1:1:18 mixture of ethanol–Tween 80®–0.9% saline. Mice were given an intraperitoneal (i.p.) injection of vehicle (1:1:18 ethanol–Tween 80–saline) or THC [5 or 10 mg/kg body weight (BW)] at a volume of 10 ml/kg BW. Injections were administered according to a quasi-randomised, counterbalanced, within-subjects design, so that mice received one of each treatment over three experimental sessions separated by 7 days. This treatment design was identical to the one used for male heterozygous *Nrg1* transmembrane domain mutant mice (Boucher et al. 2007a).

Behavioural testing

Mice in sets 1 and 2 were tested in the open field (OF) for 30 min at 13 and 21 weeks of age (baseline testing) to confirm the age-dependent hyperactive phenotype previously observed in male *Nrg1* HET mice (Karl et al. 2007). Testing with THC commenced at 25 weeks of age, when mice were divided into two sets with equal representation of genotypes. Set 1 (n=12–13) was tested in the OF, elevated plus maze (EPM), and novel object recognition (NOR) tests while set 2 (n=10–14) was tested in the light–dark (LD), social interaction (SI), Y maze and prepulse inhibition (PPI 1-using a variable interstimulus interval) tests (Table 1). Behavioural testing of set 3 commenced at 24–26 weeks of age, when mice (n=12–13) were tested in PPI [PPI 2-using a fixed interstimulus interval and identical to the protocol used for male *Nrg1* HETs (Boucher et al. 2007a)]. All behavioural testing was commenced at the start of the light cycle (0700 hours). Environmental odours were removed from test apparatus following each trial using 70% ethanol solution.

Open field

General locomotor activity was measured by placing mice into an OF activity chamber (Med Associates Inc., St. Albans, VT, USA). The animal's total horizontal (distance travelled) and vertical activity (as an indirect measure of *rearing*) in central and peripheral zones were measured as described previously (Karl et al. 2007). The ratio of central

Table 1 Test biography of wild-type-like (WT) and heterozygous *Nrg1* transmembrane domain knockout (*Nrg1* HET) mice

13 weeks	OF (30 min)					
21 weeks	OF (30 min)					
24–26 weeks	Set 1 (n=12–13)		Set 2 (n=10–14)		Set 3 (n=12–13)	
	Time [min]	Test	Time [min]	Test	Time [min]	Test
	0	Injection	0	Injection	0	Injection
	30–60	OF	30–40	LD	80	PPI 1
	65–70	EPM	45–55	SI		
	75–80	NOR 1	65–70	Y maze 1		
	135–140	NOR 2	80	PPI 2		
			125–130	Y maze 2		

Mice in sets 1–2 were tested at 13 and 21 weeks in a 30-min open field test. Mice in sets 1–3 were injected with either vehicle or THC (5 or 10 mg/kg body weight) in a counterbalanced, quasi-randomised design over a period of 3 weeks. A washout period of 7 days occurred between each treatment and behavioural testing session. Thus, all test mice were observed repeatedly after injection in the respective behavioural test battery [set 1: open field (*OF*), elevated plus maze (*EPM*) and novel object recognition (*NOR*); set 2: light dark (*LD*), social interaction (*SI*), Y maze and prepulse inhibition (variable interstimulus interval: *PPI 1*); set 3: prepulse inhibition (fixed interstimulus interval: *PPI 2*)]

to total distance travelled ("OF" distance ratio) and the time spent in the central zone were taken as measures of anxiety (Denenberg 1969). Mice were placed in the OF chamber and allowed to explore freely for 30 min.

Light–dark test

Mice were placed into the opening of the dark compartment of the LD apparatus (Med Associates Inc.) as described previously (Karl et al. 2007) and allowed to explore freely for 10 min. The ratio of distance travelled in the light compartment to total distance travelled ("LD" distance ratio) and the time spent in the light compartment were taken as measures of anxiety.

Elevated plus maze

Mice were placed onto the central platform of the EPM [described previously; (Karl et al. 2007)] facing an enclosed arm and allowed to explore freely for 5 min. Arm entries (when the mouse entered an arm with all four paws), time spent in arms and in the central platform and the frequency of the behaviours *rearing*, *head dipping* and *stretch attend postures* were scored. Closed arm entries were recorded as measures of motor activity. Anxiety-related behaviour was measured by open arm entries, time spent on open arms (divided into inner and outer halves) and the percentage of open arm entries (open arm entry ratio) (Hogg 1996; Pellow et al. 1985).

Social interaction

SI between a pair of rodents is used to measure anxiety-like behaviours (File and Seth 2003; Kask et al. 2001). Furthermore, reduction in SI is thought to model aspects of social withdrawal observed in schizophrenia patients (Ellenbroek and Cools 2000; Rung et al. 2005). Test mice and A/JArc standard opponents were placed in opposite corners of a grey perspex arena (35×35×30 cm) and allowed to explore the arena and each other freely for 10 min. Frequency and duration of the active socio-positive behaviours *general sniffing*, *anogenital sniffing*, *allogrooming*, *following* and *climbing over* in the test mouse were recorded manually.

Novel object recognition

The distinction between familiar and unfamiliar objects is an index of recognition memory and its measurement is aided by the innate preference of rodents for novel over familiar objects (Dere et al. 2007; Ennaceur and Delacour 1988). The apparatus for the NOR test was the OF activity chamber. The NOR procedure consisted of two trials separated by 60 min, in which mice were placed in the activity chamber and allowed to explore freely for 5 min. In trial 1, the chamber contained two identical objects placed in opposite corners, 5 cm away from the closest two walls. In trial 2, the chamber contained one copy of the object from the first trial (familiar object) and one novel object in the same positions as the objects in trial 1. Mice could not displace the objects from their positions. Each object was available in triplicate. Two out of these were presented in the first trial and a third one in the second trial, to prevent the use of odour cues. All objects were thoroughly cleaned before being used for a different test mouse. The different objects were a glass bottle (55×28×28 mm), a thin plastic tube with a pointed end (57×25×25 mm), a glass cup (63×50×50 mm), a plastic specimen jar (57×47×47 mm), a plastic tub (41×47×47 mm) and a wider plastic tube with a pointed end (56×35×35 mm). Combinations and position of novel and familiar objects were counterbalanced between treatments. Trials were scored manually. Object exploration was defined by *nosing* (when the mouse directed its nose to the object at a distance of ≤1 cm). *Rearing* on the object was scored but not included as object exploration. Measures of object recognition are reported as percentage exploration (time spent exploring the novel object in trial 2 expressed as a percentage of the novel + familiar object exploration time in trial 2) and the discrimination index (the difference between the time spent exploring the novel and familiar objects in trial 2).

Y maze

Mice have a tendency to explore novel environments. The ability of mice to remember familiar arms of a Y maze was tested by comparing the levels of locomotion/exploration and time spent in a novel, unexplored arm and two familiar arms (Gray et al. 2009). The apparatus was a grey perspex Y-shaped maze with 30×10×17 cm arms joined by a triangular centre section. In trial 1, access to one arm was blocked by a grey perspex insert. Mice were placed into the centre of the maze facing the blocked arm and allowed to explore freely for 5 min. Trial 2 occurred 60 min later, when access to all three arms was available and mice were placed into the centre of the maze, facing the newly unblocked arm, and again allowed to explore freely for 5 min. Different patterns on each arm wall provided intra-maze directionality cues. Objects (e.g. a camera tripod) at the end of each arm provided extra-maze directionality cues. The blocked arm varied between each mouse in a counterbalanced manner. In trial 2, the time spent in and number of entries (defined when all four paws were inside the arm and expressed as a percentage of total entries into all arms) into the previously blocked arm were recorded manually.

Prepulse inhibition

PPI, an operational measure of sensorimotor gating, is the attenuation of the startle response by a nonstartling stimulus (prepulse) presented 30–500 ms before the startling stimulus (pulse). PPI is impaired in schizophrenia patients (Braff et al. 2001; Ludewig et al. 2003). Startle reactivity was measured in SR-LAB startle chambers (San Diego Instruments, San Diego, CA, USA). The 30 min PPI test session for set 2 (PPI 1) consisted of 5 min acclimatisation 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 interstimulus interval, ISI) prior to a startling pulse of 120 dB (PPI response). Intertrial intervals varied randomly from 10–20 s. Responses to each trial were calculated as the average mean amplitude detected by the accelerometer. The startle response was calculated as the mean amplitude to all startle trials and percentage PPI (% PPI) was calculated as [(mean startle response (120 dB)−PPI response)/mean startle response (120 dB)]×100%. PPI was averaged across ISIs to produce a mean % PPI for each prepulse intensity.

The PPI test protocol for set 3 (PPI 2) was identical to that used in the previous study with male *Nrg1* HET mice (Boucher et al. 2007a). It was similar to PPI 1 described above, except that the ISI was fixed at 80 ms and there were only 76 trials presented in a pseudorandom order: 8×70 dB trials (background), 10×90 dB trials, 18×120 dB trials (startle), two prepulse alone trials for each prepulse intensity (74, 78, 82 or 86 dB) and eight trials comprising a prepulse of 74, 78, 82 or 86 dB presented 80 ms before a startling pulse of 120 dB (PPI response).

Statistical analysis

Differences between treatment groups were analysed with two-way analysis of variance (ANOVA; between-subjects factors: 'treatment' and 'genotype'). For OF, NOR and PPI analysis, repeated measures (RM) three-way ANOVA was used [within-subjects factors: '5 min block' (OF) or 'object' (left/right or novel/familiar, NOR) or 'prepulse intensity' (PPI)]. This was followed by one-way ANOVA (factor: 'genotype', 'age' or 'treatment' split by the corresponding factor) and the Student–Newman–Keuls post hoc test, in which vehicle data were compared to each dose of THC (5 or 10 mg/kg) within each genotype. Differences were regarded as statistically significant when $P<0.05$. Degrees of freedom, F values and P values from two- and one-way ANOVA are presented in the "Results" section. P values from post hoc tests are indicated in Figures and Tables (single asterisk for effects of genotype; number sign for effects of treatment or age within a genotype). Data are presented as mean ± standard error of the mean (SEM). Analysis was performed using SPSS 17.0 for Windows.

Results

Locomotor and exploratory activity

Baseline (13 and 21 weeks of age)

Nrg1 HET mice showed an age-dependent difference in spontaneous locomotor activity in the OF compared to WT mice [two-way ANOVA distance travelled: age $F(1, 47)=83.2$, $P<0.001$, genotype $F(1, 47)=4.5$, $P<0.05$, age × genotype $F(1, 47)=7.2$, $P=0.01$; Table 2]. *Nrg1* HET mice displayed higher locomotor activity than WT mice at 21, but not 13 weeks of age as shown by one-way ANOVA split by age [21 weeks: $F(1, 47)=14.0$, $P=$

Table 2 Baseline locomotor activity and anxiety parameters: sets 1–2

Parameter	WT		*Nrg1* HET	
	13 weeks	21 weeks	13 weeks	21 weeks
Distance travelled [cm]	5,352.3±388.4	3,261.6±223.3$^{###}$	5,516.1±166.3	4,375.4±198.2 **, $^{###}$
Peripheral distance travelled [cm]	3,641.7±322.5	2168.5±148.2$^{###}$	3,523.3±120.9	2,810.3±129.2 **, $^{###}$
Time in centre [s]	475.0±38.7	474.2±48.0	560.7±29.0	531.1±33.0
Distance ratio	32.8±1.4	33.5±1.3	36.2±1.0*	35.6±1.1*
Rearing [*n*]	279.7±18.6	197.5±15.8$^{###}$	294.7±14.4	239.5±14.0$^{###}$

Measures of horizontal and vertical locomotor activity and anxiety in the open field test measured at 13 and 21 weeks in mice in sets 1 and 2. Data represent mean ± S.E.M, $n=22–27$

WT wild-type-like control, *Nrg1 HET* heterozygous *Nrg1* transmembrane domain mutant

*$P<0.05$, **$P<0.01$ (vs. WT of same age), $^{###}P<0.001$ (vs. 13 week group of same genotype)

0.0001]. Locomotion decreased with test experience, as shown by one-way ANOVA split by genotype [WT $F(1, 21)=41.6$, $P<0.001$, *Nrg1* HET $F(1, 26)=39.7$, $P<0.001$]. Consistent with this, peripheral distance travelled in the OF was also increased in *Nrg1* HET mice [two-way ANOVA: age $F(1, 47)=54.4$, $P<0.001$, age × genotype $F(1, 47)=6.6$, $P<0.05$]. The hyperactive phenotype of *Nrg1* HETs and the reduced locomotion rates of all mice at 21 weeks was confirmed by one-way ANOVA split by genotype [WT $F(1, 21)=30.0$, $P<0.001$, *Nrg1* HET $F(1, 26)=21.5$, $P<0.001$] or age [21 weeks $F(1, 47)=10.7$, $P<0.01$; Table 2]. The *rearing* frequency (i.e. vertical activity) of mice in the OF was lower at 21 weeks than at 13 weeks, but no genotype differences were detected [two-way ANOVA: age $F(1, 47)=37.8$, $P<0.001$; Table 2].

THC effects

THC decreased OF locomotor activity in all mice, and *Nrg1* HETs displayed increased locomotor activity, irrespective of treatment, compared to WT controls [two-way ANOVA distance travelled: treatment $F(2, 69)=82.8$, $P<0.001$, genotype $F(1, 69)=7.4$, $P<0.01$; two-way ANOVA peripheral distance travelled: treatment $F(2, 69)=54.1$, $P<0.001$, genotype $F(1, 69)=4.7$, $P<0.05$; Fig. 1a, b]. One-way ANOVA split by genotype confirmed that THC exerted locomotor depressant effects in WT and *Nrg1* HET mice [distance travelled: WT $F(2, 33)=32.7$, $P<0.001$, *Nrg1* HET $F(2, 36)=52.0$, $P<0.001$; peripheral distance travelled: WT $F(2, 33)=24.4$, $P<0.001$, *Nrg1* HET $F(2, 36)=30.4$, $P<0.001$] at both 5 and 10 mg/kg THC (post hoc analyses, Fig. 1a, b). One-way ANOVA split by treatment showed that total distance travelled was significantly higher in vehicle-treated *Nrg1* HETs than vehicle-treated WTs [vehicle $F(1, 23)=6.2$, $P<0.05$].

The locomotor-suppressant effects of THC were confirmed in the LD test [two-way ANOVA distance travelled: treatment $F(2, 66)=98.1$, $P<0.001$, genotype $F(1, 66)=4.0$, $P=0.05$]. THC (5 and 10 mg/kg) decreased distance travelled in both WT and *Nrg1* HET mice [one-way ANOVA split by genotype: WT $F(2, 27)=40.9$, $P<0.001$, *Nrg1* HET $F(2, 39)=63.1$, $P<0.001$; Fig. 1c].

THC decreased vertical activity/*rearing* in WT and *Nrg1* HET mice in the OF [two-way ANOVA: treatment $F(2, 69)=82.8$, $P<0.001$], LD [treatment $F(2, 66)=117.8$, $P<0.001$] and EPM tests [treatment $F(2, 61)=45.6$, $P<0.001$]. There were no genotype effects on these measures [$P>0.05$]. One-way ANOVA split by genotype confirmed that THC (5 and 10 mg/kg) decreased vertical activity/*rearing* in both genotypes in each test [vertical activity (OF): WT $F(2, 33)=19.3$, $P<0.001$, *Nrg1* HET $F(2, 36)=54.9$, $P<0.001$; vertical activity (LD): WT $F(2, 27)=41.9$, $P<0.001$, *Nrg1* HET $F(2, 39)=82.5$, $P<0.001$; *rearing* (EPM): WT $F(2, 29)=21.8$, $P<0.001$, *Nrg1* HET $F(2, 32)=25.2$, $P<0.001$; Fig. 2a–c]. In the EPM, THC also decreased *head dips* [two-way ANOVA: treatment $F(2, 61)=8.8$, $P<0.001$, genotype $P>0.05$]. Both 5 and 10 mg/kg THC exerted this effect in *Nrg1* HETs only [WT $P=0.05$, *Nrg1* HET $F(2, 32)=6.1$, $P<0.01$; Fig. 2d].

Anxiety

Baseline (13 and 21 weeks of age)

There were genotype differences in the OF distance ratio at 13 and 21 weeks [Two-way ANOVA distance ratio: genotype $F(1, 47)=4.3$, $P<0.05$; Table 2], with *Nrg1* HETs showing an anxiolytic-like phenotype. However, *Nrg1* HET and WT mice spent a similar length of time in the centre of the OF [time in centre: genotype $P>0.05$, age $P>0.05$; Table 2]. No age × genotype interactions were detected.

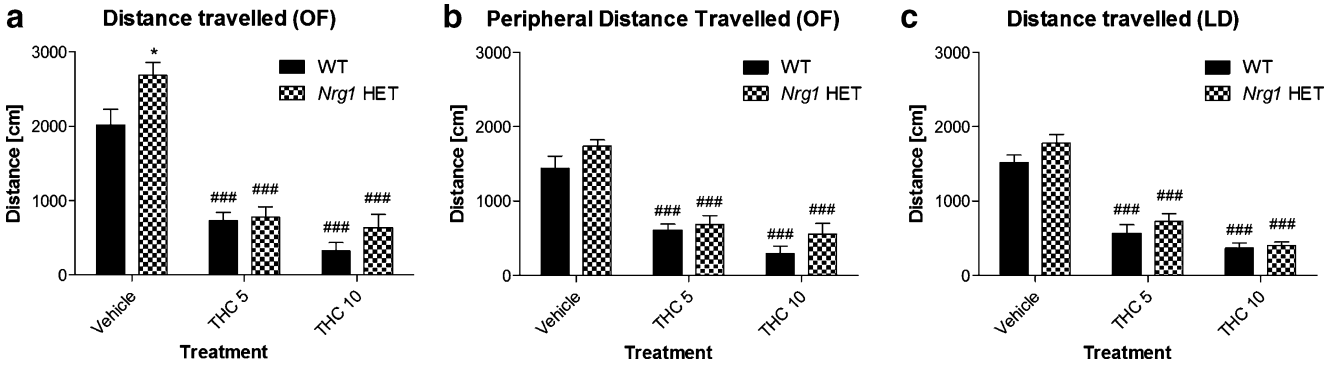

Fig. 1 Measures of horizontal locomotor activity after injection of THC (5 or 10 mg/kg). **a** Total and **b** peripheral distance travelled (OF, 30 min). **c** Total distance travelled (LD, 10 min). Data represent mean ± S.E.M., $n=10$–14. *Single asterisk* indicates $P<0.05$ (vs. vehicle-treated WT), and *triple number signs* indicate $P<0.001$ (vs. vehicle of same genotype). *THC* Δ^9-tetrahydrocannabinol, *OF* open field test, *LD* light–dark test, *WT* wild-type-like control, *Nrg1 HET* heterozygous *Nrg1* transmembrane domain mutant

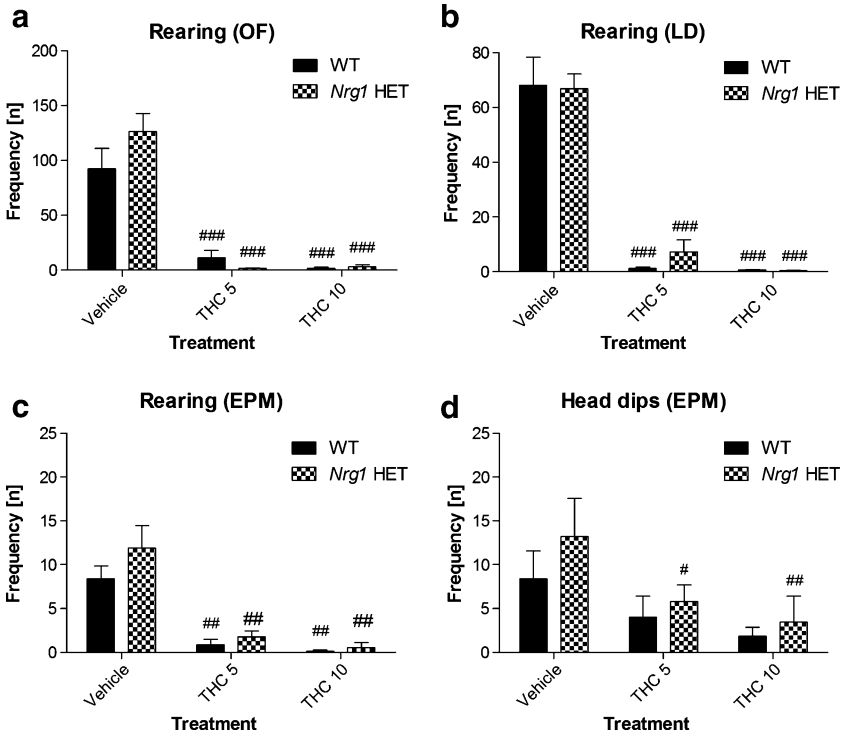

Fig. 2 Measures of exploratory activity after injection of THC (5 or 10 mg/kg). *Rearing* in **a** OF (30 min), **b** LD (10 min), **c** EPM (5 min). **d** Frequency of *head dips* (EPM). Data represent mean + S.E.M., n=10–14. *Single number sign* indicates P<0.05, *double number signs* indicate P<0.01, *triple number signs* indicate P<0.001 (vs. vehicle of same genotype). *THC* Δ^9-tetrahydrocannabinol, *OF* open field test, *LD* light–dark test, *EPM* elevated plus maze, *WT* wild-type-like control, *Nrg1 HET* heterozygous *Nrg1* transmembrane domain mutant

THC effects

THC produced anxiogenic effects in the OF for the parameters time in centre [two-way ANOVA: treatment $F(2, 69)$= 4.7, P<0.05] and distance ratio [treatment $F(2, 69)$=30.2, P<0.001]. No genotype effects were found, indicating that WT and *Nrg1* HET mice responded similarly to the anxiogenic effects of THC. There was no effect of treatment on time spent in the centre [ANOVA split by genotype: P>0.05; Fig. 3a], but 5 and 10 mg/kg THC

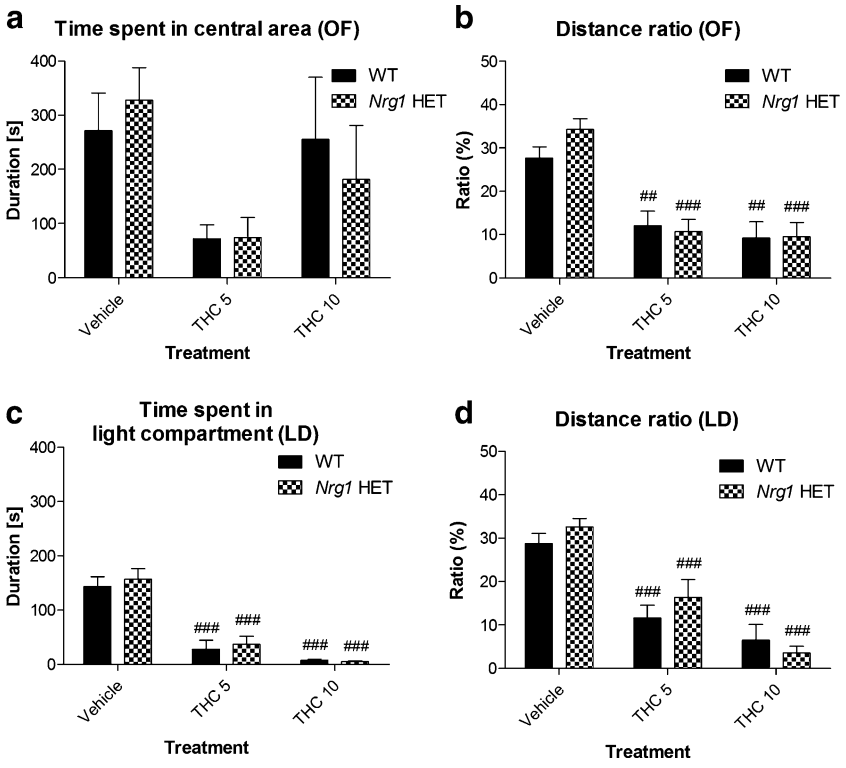

Fig. 3 Anxiety-related measures after injection of THC (5 or 10 mg/kg). **a** Time spent in the central area (OF, 30 min). **b** Distance ratio (OF). **c** Time spent in the light compartment (LD, 10 min). **d** Distance ratio (LD, 10 min). Data represent mean + S.E.M., n=10–14. *Double number signs* indicate P<0.01, *triple number signs* indicate P<0.001 (vs. vehicle of same genotype). *THC* Δ^9-tetrahydrocannabinol, *OF* open field test, *LD* light–dark test, *WT* wild-type-like control, *Nrg1 HET* heterozygous *Nrg1* transmembrane domain mutant

decreased the distance ratio in *Nrg1* HET [ANOVA split by genotype: $F(2, 36)=23.6$, $P<0.001$] and WT mice [$F(2, 33)=9.2$, $P=0.001$; Fig. 3b].

These effects were confirmed in the LD test using two-way ANOVA [time in light: treatment $F(2, 66)=52.5$, $P<0.001$; distance ratio: treatment $F(2, 66)=38.7$, $P<0.001$]. One-way ANOVA split by genotype revealed that THC (5 and 10 mg/kg) decreased time spent in the light compartment and the distance ratio in both genotypes [time in light: WT $F(2, 27)=25.8$, $P<0.001$, *Nrg1* HET $F(2, 39)=30.2$, $P<0.001$; distance ratio: WT $F(2, 27)=14.9$, $P<0.001$, *Nrg1* HET $F(2, 39)=26.9$, $P<0.001$; Fig. 3c, d]. There were no significant treatment or genotype effects on time spent in or entries into open arms or on the frequency of *stretch attend postures* in the EPM [data not shown].

Social interaction

Social interaction effects are reported in Table 3. Two-way ANOVA showed main effects of treatment, indicating that THC decreased both the frequency (fr) and duration (t) of *sniffing* [fr: $F(2, 62)=68.0$, $P<0.001$; t: $F(2, 62)=119.5$, $P<0.001$], *following* [fr: $F(2, 62)=5.8$, $P<0.01$; t: $F(2, 62)=5.7$, $P<0.01$], *anogenital sniffing* [fr: $F(2, 62)=22.3$, $P<0.001$; t: $F(2, 62)=49.0$, $P<0.001$] and total time spent in social interaction [fr: $F(2, 62)=65.1$, $P<0.001$; t: $F(2, 62)=77.4$, $P<0.001$]. *Nrg1* HETs showed an increased frequency of *climbing over* [genotype: $F(1, 62)=4.9$, $P<0.05$], but there was no main effect of genotype on any other social behaviour, and there were no significant treatment × genotype interactions. One-way ANOVA split by genotype confirmed that in both genotypes, THC (5 and 10 mg/kg) significantly decreased the frequency and duration of *general sniffing* [fr: WT $F(2, 25)=47.7$, $P<0.001$, *Nrg1* HET $F(2, 37)=35.4$, $P<0.001$; t: WT $F(2, 25)=26.2$, $P<0.001$, *Nrg1* HET $F(2, 37)=44.4$, $P<0.001$] and *anogenital sniffing* [fr: WT $F(2, 25)=26.8$, $P<0.001$, *Nrg1* HET $F(2, 37)=20.4$, $P<0.001$; t: WT $F(2, 25)=12.7$, $P<0.001$, *Nrg1* HET $F(2, 37)=9.0$, $P=0.001$] as well as the total frequency and duration of social interaction [fr: WT $F(2, 25)=45.2$, $P<0.001$, *Nrg1* HET $F(2, 37)=36.4$, $P<0.001$; t: WT $F(2, 25)=28.2$, $P<0.001$, *Nrg1* HET $F(2, 37)=38.2$, $P<0.001$; Table 3]. However, THC selectively decreased some behaviours in WTs only (one-way ANOVA split by genotype): frequency of *allogrooming* [fr: WT $F(2, 25)=4.1$, $P<0.05$, *Nrg1* HET $P>0.05$] and *climbing over* [fr: WT $F(2, 25)=5.4$, $P<0.05$, *Nrg1* HET $P>0.05$] and frequency and duration of *following* [fr: WT $F(2, 25)=7.4$, $P<0.01$, *Nrg1* HET $P>0.05$; t: WT $F(2, 25)=7.1$, $P<0.01$, *Nrg1* HET $P>0.05$; Table 3]. One-way ANOVA split by treatment showed that vehicle-treated *Nrg1* HETs spent significantly more time *climbing over* compared with vehicle-treated WTs [$F(1, 21)=4.5$, $P<0.05$].

Novel object recognition

Overall, mice displayed low levels of *nosing* and *rearing* on the objects (Table 4). In trial 1, THC decreased *nosing* [RM ANOVA: treatment $F(2, 36)=16.5$, $P<0.001$; Table 4], while there was no effect of genotype. After combining exploration times for both objects in trial 1, one-way

Table 3 Social interaction

Parameter	WT			*Nrg1* HET		
	Vehicle	THC 5	THC 10	Vehicle	THC 5	THC 10
General sniffing [*n*]	51.0±3.4	19.4±3.6$^{###}$	3.9±1.4$^{###}$	48.9±4.3	18.0±3.6$^{###}$	9.0±1.9$^{###}$
General sniffing duration [s]	32.2±3.3	14.7±2.9$^{###}$	2.8±1.1$^{###}$	30.3±2.9	13.7±3.0$^{###}$	5.3±0.9$^{###}$
Anogenital sniffing [*n*]	11.5±1.7	2.1±0.7$^{###}$	0.5±0.3$^{###}$	9.2±1.1	3.0±0.7$^{###}$	2.1±0.8$^{###}$
Anogenital sniffing duration [s]	8.7±1.8	2.3±0.9$^{###}$	0.2±0.1$^{###}$	6.9±1.0	3.3±1.0$^{##}$	1.4±0.7$^{###}$
Allogrooming [*n*]	2.1±0.7	0.5±0.4$^{#}$	0.2±0.2$^{#}$	1.7±0.4	1.6±0.7	1.7±1.1
Climbing over [*n*]	2.6±0.7	0.9±0.4$^{#}$	0.3±0.3$^{#}$	4.2±1.0	3.8±1.6	1.3±0.7
Climbing over duration [s]	1.1±0.3	0.9±0.6	0.0±0.0	2.8±0.7*	1.5±0.7	0.3±0.1
Following [*n*]	2.3±0.7	0.2±0.2$^{##}$	0.3±0.2$^{##}$	2.1±0.3	1.5±0.6	1.2±0.5
Following duration [s]	1.3±0.4	0.2±0.2$^{##}$	0.1±0.1$^{##}$	1.2±0.2	0.9±0.3	0.7±0.3
Total SI [*n*]	69.8±6.4	23.2±4.3$^{###}$	5.0±1.8$^{###}$	65.0±6.0	27.4±5.9$^{###}$	15.6±4.3$^{###}$
Total SI duration [s]	44.5±5.2	18.5±3.2$^{###}$	3.1±1.2$^{###}$	41.6±4.2	19.5±4.1$^{###}$	8.0±1.6$^{###}$

Frequency and duration of *general sniffing*, *anogenital sniffing*, *allogrooming*, *climbing over*, *following* and total social interaction (SI, 10 min) with a standard opponent A/JArc mouse after injection with THC (5 or 10 mg/kg). Data represent means (±S.E.M.), $n=10$–14

THC Δ^9-tetrahydrocannabinol, *SI* social interaction test

*$P<0.05$ (vs. vehicle-treated WT), $^{#}P<0.05$, $^{##}P<0.01$, $^{###}P<0.001$ (vs. vehicle of same genotype)

Table 4 Novel object recognition

Parameter		WT			*Nrg1* HET		
		Vehicle	THC 5	THC 10	Vehicle	THC 5	THC 10
Trial 1							
Nosing [s]	Left	1.2±0.5	0.1±0.1	0.4±0.3	1.9±0.5	0.3±0.2	0.1±0.1
	Right	1.7±0.8	0.2±0.2	0.3±0.1	2.7±1.1	0.3±1.1	0.1±0.1
	Left+Right	2.9±1.2	0.3±0.2$^{\#}$	0.7±0.4$^{\#}$	4.6±1.5	0.6±0.3$^{\#\#\#}$	0.2±0.1$^{\#\#\#}$
Trial 2							
Nosing [s]	Novel	1.5±1.0	1.1±0.7	0.5±0.4	3.5±1.1	0.4±0.3	0.3±0.3
	Familiar	1.4±0.5	1.0±0.6	0.3±0.2	2.9±0.8	1.0±0.5	0.2±0.1
	Novel+Familiar	2.9±1.1	2.1±1.3	0.8±0.6	6.4±1.6	1.4±0.6$^{\#\#}$	0.5±0.4$^{\#\#}$
Nosing (%)		31.4±13.0	42.2±11.2	67.1±4.3	53.6±10.1	33.1±19.8	21.8±21.8
Discrimination index		−37.3±26.0	−15.7±22.5	34.3±8.7	7.2±20.2	−33.7±39.5	−56.3±43.7

Time spent *nosing* objects (NOR, 2 × 5 min) after injection with THC (5 or 10 mg/kg). Data represent means (±S.E.M.), n=2–9

THC Δ^9-tetrahydrocannabinol, *NOR* novel object recognition test

$^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 (vs. vehicle of same genotype)

ANOVA split by genotype showed that 5 and 10 mg/kg THC decreased object exploration in both WT [F(2, 19)= 4.9, P=0.01] and *Nrg1* HET [F(2, 17)=12.2, P=0.001] mice. There was a main effect of treatment on *nosing* of the objects in trial 2 [RM ANOVA F(2, 33)=7.4, P<0.01], but no main effect of genotype or object and no interactions [Table 4], showing that there was no difference in the time mice spent with the novel and familiar objects in any treatment group. RM ANOVA split by genotype showed that THC (5 and 10 mg/kg) decreased total object *nosing* in *Nrg1* HETs [F(2, 15)=10.1, P<0.01] but not WTs in trial 2. There were no effects of treatment or genotype on the time spent *nosing* the novel object expressed as a percentage of total object exploration or on the discrimination index in trial 2 [data not shown].

Y maze

There were no effects of treatment or genotype on percentage of entries into the novel arm or time spent in the novel arm [data not shown].

Startle response and prepulse inhibition

PPI 1 (variable ISI)

Data from protocol PPI 1 are depicted in Fig. 4. THC decreased the startle response [two-way ANOVA: treatment F(2, 66)=13.8, P<0.001]. One-way ANOVA split by genotype confirmed that startle was decreased by THC [WT F(2, 27)=4.8, P<0.05, *Nrg1* HET F(2, 39)=12.2, P<0.001]: 10 mg/kg THC had a significant effect in WT and *Nrg1* HET mice while the effect of 5 mg/kg THC was only significant in *Nrg1* HET mice (post hoc analyses, Fig. 4, inset).

A main effect of prepulse intensity [RM ANOVA F(2, 132)=470.4, P<0.001] and a linear contrast between levels of prepulse intensity [P<0.001] indicated that PPI increased with increasing prepulse intensity (Fig. 4).

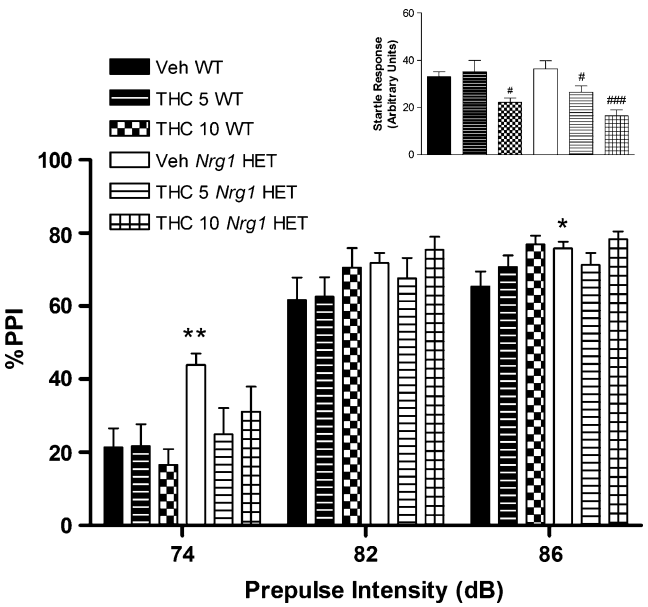

Fig. 4 Sensorimotor gating using a variable interstimulus interval after injection of THC (5 or 10 mg/kg). *Inset*: startle response [arbitrary units] to 120 dB acoustic stimuli (PPI, 30 min). *Main figure*: percentage PPI. Data represent mean+S.E.M., n=10–14. *Single asterisk* indicates P<0.05, *double asterisks* indicate P<0.01 (vs. vehicle-treated WT), *single number sign* indicates P<0.05 and *triple number signs* indicate P<0.001 (vs. vehicle of same genotype). *THC* Δ^9-tetrahydrocannabinol, *PPI* prepulse inhibition, *WT* wild-type-like control, *Nrg1 HET* heterozygous *Nrg1* transmembrane domain mutant

Three-way RM ANOVA revealed no main effect of THC on PPI [F(2, 66)=0.78, $P>0.05$] but there was a main effect of genotype [F(1, 66)=5.9, $P<0.05$; Fig. 4]. There was also a prepulse×genotype interaction [F(2, 132)=4.2, $P<0.05$] and one-way ANOVA split by treatment and prepulse intensity showed that PPI was higher in vehicle-treated *Nrg1* HETs than vehicle-treated WTs at the 74 and 86 dB prepulse intensities [vehicle WT 74 dB F(1, 22)=14.0, $P=0.001$, vehicle WT 86 dB F(1, 22)=6.5, $P<0.05$; Fig. 4].

PPI 2 (fixed ISI)

Data from protocol PPI 2 are shown in Fig. 5. THC decreased the startle response [two-way ANOVA: treatment $F(2, 67)=20.9$, $P<0.001$], but there was no effect of genotype. One-way ANOVA split by genotype confirmed that THC (10 mg/kg) decreased startle in *Nrg1* HETs and WTs while THC (5 mg/kg) decreased startle only in WTs [$F(2, 34)=14.7$, $P<0.001$, *Nrg1* HET $F(2, 33)=7.5$, $P<0.01$; Fig. 5, inset].

A main effect of prepulse intensity [RM ANOVA: $F(3, 201)=110.0$, $P<0.001$] and a linear contrast between levels of prepulse intensity [$P<0.001$] indicated that PPI increased with increasing prepulse intensity. There were no main effects of or interactions between THC treatment and genotype on PPI in this paradigm [Fig. 5].

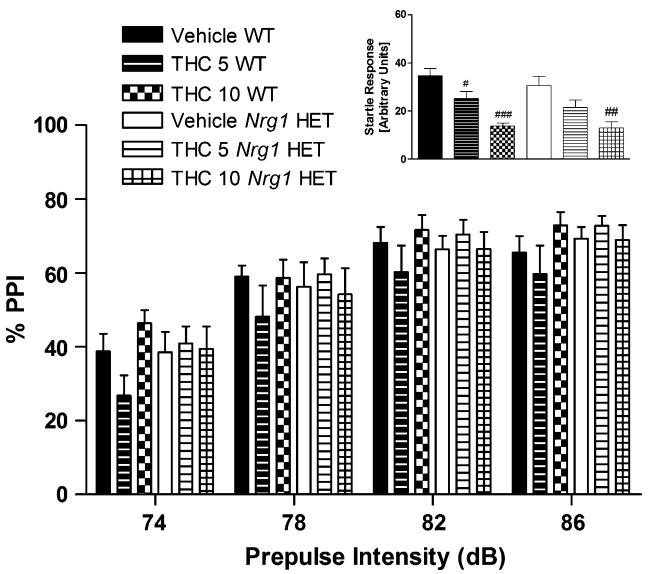

Fig. 5 Sensorimotor gating using a fixed interstimulus interval after injection of THC (5 or 10 mg/kg). *Inset*: Startle response [arbitrary units] to 120 dB acoustic stimuli (PPI, 30 min). *Main figure*: percentage PPI. Data represent mean+S.E.M., $n=11–13$. *Single number sign* indicates $P<0.05$, *double number signs* indicate $P<0.01$, *triple number signs* indicate $P<0.001$ (vs. vehicle of same genotype). *THC* Δ^9-tetrahydrocannabinol, *PPI* prepulse inhibition, *WT* wild-type-like control, *Nrg1 HET* heterozygous *Nrg1* transmembrane domain mutant

Discussion

The present study was a follow-up investigation to our previous study in male *Nrg1* HET mice (Boucher et al. 2007a). Here, we evaluated the effects of THC on a number of behavioural domains in female *Nrg1* HET mice, including some associated with schizophrenia symptoms and with the known behavioural properties of THC. At baseline, we observed hyperactivity at 5 months of age and an anxiolytic-like profile at 3 and 5 months. THC decreased locomotor and vertical exploratory activity and produced anxiogenic effects in the open field and light–dark tests to a similar extent in female mice of both genotypes. While THC decreased total social interaction time and frequency, some social behaviours were selectively decreased in WT mice. There were no effects of THC on models of spatial and object recognition memory. The screening of female test mice in two PPI paradigms revealed a PPI protocol-dependent sensorimotor gating performance: in protocol PPI 2 (fixed ISI), baseline PPI of *Nrg1* HET females was unaltered; however, baseline sensorimotor gating was enhanced in female *Nrg1* HET mice in the PPI protocol that incorporated a variable ISI (PPI 1). THC treatment had no impact on PPI in either protocol. Overall, these findings in female mice provide an important addition to the literature on effects of genetic and environmental (i.e. exposure to cannabis constituents) manipulations in mouse models, since the majority of behavioural studies use male test animals.

Female *Nrg1* HET mice displayed the hyperlocomotor phenotype associated with the transmembrane domain *Nrg1* mutation (Karl et al. 2007; Stefansson et al. 2002). This hyperactivity was age-dependent, in line with previous observations that locomotor activity in male *Nrg1* HET mice was comparable to WTs at 3–4 months of age but increased at 4–6 months (Karl et al. 2007). This suggests that in both sexes, the hyperactive phenotype of *Nrg1* HET mice emerges well after reaching adulthood. Both doses of THC suppressed locomotor and exploratory activity in female *Nrg1* HET and WT mice, a typical effect of cannabinoid CB_1 receptor agonists (Long et al. 2010). This suggests that female mice with a transmembrane domain *Nrg1* mutation do not show increased susceptibility to THC-induced sedation, which is interesting since male *Nrg1* HET mice are more susceptible to THC-induced locomotor suppression than WTs (Boucher et al. 2007a).

Female *Nrg1* HET mice showed anxiolytic-like behaviour in the open field at both 3 and 5 months of age prior to testing with THC, which is similar to the finding in male *Nrg1* HET mice raised in a similarly enriched environment to the females in the present study (Karl et al. 2007). The anxiety-related phenotype of *Nrg1* HET mice in the present study was task-specific, which is consistent with

previous studies (Karl et al. 2007; O'Tuathaigh et al. 2008). THC exerted anxiogenic-like effects in the open field and light–dark tests in females of both genotypes. This is in contrast to the anxiety-like response to acute THC that occurred in males, in which only *Nrg1* HET mice displayed lower distance ratios in the LD test and open arm entry ratios in the EPM. Together with the effects of THC on locomotor activity, these findings indicate that there may be task-specific differences in behavioural responses to THC between male and female *Nrg1* HET mice, possibly due to differences in receptor density, intracellular signalling, sex hormone function or other mechanisms in the neural pathways that modulate each behaviour.

Baseline social interaction was not significantly different between *Nrg1* HET and WT females. This is in contrast to a previous report that some social interaction behaviours were increased in mutants of both sexes while others were increased in female mutants only (O'Tuathaigh et al. 2008). The social interaction opponents used by O'Tuathaigh and colleagues were C57BL/6 mice, which are a less passive and more socially interactive strain than the A/J mice used in our study (Moy et al. 2004) and accordingly may have elicited different social interaction patterns. In the present study, THC generally decreased social interaction, but *climbing over*, *allogrooming* and *following* were decreased by THC only in WTs. While *Nrg1* HETs had a higher frequency of *climbing over* at baseline, it appears that the selective effect of THC on *allogrooming* and *following* in WT females might be independent of baseline behaviour. In similar experimental conditions in male *Nrg1* HET mice, THC also reduced total social interaction and *sniffing* and *anogenital sniffing* but did not decrease *allogrooming* and *following* (Boucher et al. 2007a). Interestingly, *allogrooming* and *following* are viewed as more 'playful' forms of social interaction in rats (Homberg et al. 2007), raising the possibility that in female mice, the *Nrg1* mutation may exert protective effects on neural pathways governing these forms of behaviour that are altered by THC in WT mice.

A core feature of schizophrenia is the deficits in cognitive domains such as working memory (Elvevag and Goldberg 2000) and visual (Brebion et al. 2009; Chen et al. 2009) and auditory discrimination (Javitt et al. 2000). We used novel object recognition and spontaneous alternation in the Y maze to investigate working memory in THC-treated female *Nrg1* HET mice. There were no differences between genotypes or in the effects of THC treatment on these cognition-related behaviours. Object exploration times were very low, which might be attributable to the sedative properties of THC treatment but perhaps also, in the vehicle-treated mice, to avoidance of the objects (Misslin and Ropartz 1981). Rodents tend to spend more time exploring novel objects when the environment is familiar (Powell et al. 2004), and habituation to the arena on days prior to testing may have increased object exploration, although in other NOR paradigms involving habituation, we have observed a similarly low level of exploration in female *Nrg1* HET mice [Spencer, Karl et al. (unpublished data)]. Intact spatial learning in the Barnes maze and spontaneous alternation in both male and female *Nrg1* HET mice were previously reported in addition to an elevated escape latency in male *Nrg1* HET mice (O'Tuathaigh et al. 2007), and further investigation of cognitive behavioural domains other than spatial working memory will illuminate this aspect of the *Nrg1* HET phenotype.

PPI is a measure of sensorimotor gating that is a viable endophenotype with which to investigate the genetics of schizophrenia in animal models (Powell et al. 2009). In the present study, PPI was enhanced in female *Nrg1* HET mice when tested with a protocol incorporating a variable ISI, but was unchanged in a fixed ISI protocol identical to that used in (Boucher et al. 2007a). The length and variability of ISI can impact PPI, as shown in previous studies in which male *Nrg1* HET mice showed disrupted (Stefansson et al. 2002) or unaltered (Boucher et al. 2007a; van den Buuse et al. 2009) PPI, dependent on the PPI protocol used. Importantly, independently of the PPI protocol used, THC did not affect PPI in female mice in the present study, in contrast to its enhancement of PPI in male *Nrg1* HET mice (Boucher et al. 2007a). In the fixed ISI protocol, THC (5 mg/kg) reduced the startle response in female WT mice, an effect also in contrast to the results from the same PPI protocol in the male study (Boucher et al. 2007a). Overall, the present data and previous findings in *Nrg1* HET mice suggest that the effects of THC on PPI may differ depending on sex and genotype and that baseline PPI is dependent on protocol and other parametric differences. Interestingly, sex-specific differences are reported for PPI in humans: healthy women show lower PPI than men, and female schizophrenia patients display fewer PPI deficits than males with the disorder (Kumari et al. 2004).

Overall, the baseline phenotype of female *Nrg1* HET mice in the present study is consistent with previous studies in the domains of hyperactivity and anxiolytic-like behaviour (Boucher et al. 2007a; Karl et al. 2007). THC does not augment this phenotype and the female *Nrg1* HET mice do not show enhanced susceptibility to any of its behavioural effects (as observed for male mutants). In fact, female *Nrg1* HET mice appear to be resistant to some social withdrawal-like effects of THC. The emerging picture, therefore, is that the sensitivity to the behavioural effects of the cannabis constituent THC manifests differently in female and male *Nrg1* HET mice. These results are interesting in light of evidence for sex differences in the function of schizophrenia-

related genes and effects of cannabis. For example, increased anxiety-like behaviour can be observed in female, but not male, homozygous COMT mutant mice (Gogos et al. 1998) and there is a sex-specific association of a variation in the COMT gene with psychiatric phenotypes such as anxiety (Harrison and Tunbridge 2007). Sex-dependent effects of cannabis are evident in the proclivity to self-administration of cannabinoid agonists and the likelihood of adverse effects on emotion and cognition in rodents, and it has been suggested that oestrogen may play a role in these differences (McGregor and Arnold 2007). It is intriguing to speculate on the influence of sex hormones on neuregulin-mediated neuronal migration and myelination (Mei and Xiong 2008), which might underlie observed sex differences in cerebral grey and white matter volume during childhood and adolescence (De Bellis et al. 2001). Such changes in morphological brain development could underlie the baseline- and THC-induced behavioural differences observed in female and male *Nrg1* HET mice. Thus, direct sex comparison of markers of neurodevelopment after THC challenge is a compelling avenue for further experimentation.

In summary, female *Nrg1* HET mice show similar or reduced sensitivity to the acute effects of THC compared with WT controls, in a different pattern to that observed in a previous study in male *Nrg1* HET mice. We also show for the first time that female *Nrg1* HET mice exhibit a protocol-dependent enhanced sensorimotor gating (PPI). These data provide further evidence for the interaction of the cannabinoid system and neuregulin 1 signalling, which will be the subject of future studies investigating mechanisms related to the observed behaviours. Furthermore, this study indicates that it is possible that manipulation of schizophrenia risk genes has sex-specific effects on how cannabis impacts on schizophrenia-related behavioural domains.

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