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ORIGINAL INVESTIGATION

Heterozygous neuregulin 1 mice are more sensitive to the behavioural effects of Δ^9-tetrahydrocannabinol

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Abstract
Rationale Cannabis use may precipitate schizophrenia especially if the individual has a genetic vulnerability to this mental disorder. Human and animal research indicates that neuregulin 1 (Nrg1) is a susceptibility gene for schizophrenia.
Objectives The aim of this study was to investigate whether dysfunction in the *Nrg1* gene modulates the behavioural effects of Δ^9-tetrahydrocannabinol (THC), the major psychotropic component of cannabis.
Materials and methods Heterozygous *Nrg1* transmembrane-domain knockout mice (*Nrg1* HET) were treated with acute THC (0, 5 or 10 mg/kg i.p.) 30 min before being tested using open field (OF), hole board (HB), light-dark (LD), elevated plus maze (EPM), social interaction (SI) and prepulse inhibition (PPI) tests.
Results Nrg1 HET mice showed differences in baseline behaviour with regard to locomotor activity, exploration and anxiety. More importantly, they were more sensitive to the locomotor suppressant actions of THC compared to wild type-like (WT) mice. In addition, *Nrg1* HET mice expressed a greater THC-induced enhancement in % PPI than WT mice. The effects of THC on anxiety-related behaviour were task-dependent, with *Nrg1* HET mice being more susceptible than WT mice to the anxiogenic effects of THC in LD, but not in the EPM, SI and OF tests.
Conclusions Nrg1 HET mice were more sensitive to the acute effects of THC in an array of different behaviours including those that model symptoms of schizophrenia. It appears that variation in the schizophrenia-related neuregulin 1 gene alters the sensitivity to the behavioural effects of cannabinoids.

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

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Introduction

Part of a clinical diagnosis of schizophrenia is the characterisation of this mental disorder by a range of psychiatric symptoms frequently classified into positive (e.g., hallucinations, delusions), negative (e.g., impaired emotion, social withdrawal) and cognitive symptoms (e.g., impairment of attention, language, memory) (Wong and Van Tol 2003). These symptoms are at least partially based on dopamine

(DA) hyperstimulation and a dysfunction of either glutamate or its *N*-methyl-D-aspartate (NMDA) receptor subtype (Coyle 1996; Wong and Van Tol 2003). According to the neurodevelopmental theory of schizophrenia, such altered neurochemistry is thought to arise due to an interaction between genetic and environmental factors promoting defects of brain circuitry maturation (Duncan et al. 1999; Farber et al. 1995). This results in abnormalities of synaptic connectivity, myelination or structural changes in the cortex of schizophrenia patients.

Genetic factors play an important role in the aetiology of schizophrenia (Cardno and Gottesman 2000; Sullivan et al. 2003). A meta-analysis of genome scans has reported a schizophrenia susceptibility locus on chromosomes 8p12–p23.1. Furthermore, significant associations with a candidate gene on 8p12, neuregulin 1 (*NRG1*), have been reported in Caucasian (Stefansson et al. 2002) and Asian populations (Tang et al. 2004; Yang et al. 2003), although some studies failed to replicate this association (Duan et al. 2005; Iwata et al. 2004). Nevertheless, Nrg1 with its more than 15 isoforms (Falls 2003), has an impact on schizophrenia-related brain processes such as the expression and function of central nervous system (CNS) neurotransmitter receptors for NMDA and gamma-aminobutyric acid (GABA), the activation of glial cells and the regulation of myelin and oligodendrocyte-related gene expression. Furthermore, altered *NRG1* mRNA expression can be found in postmortem dorsolateral prefrontal cortex and hippocampus of schizophrenia patients (Hashimoto et al. 2004; Law et al. 2006).

Several studies have proposed a schizophrenia-related phenotype for transgenic mice heterozygous for transmembrane Nrg1 (Falls 2003): the phenotype includes hyperactivity (reversible with clozapine), deficits in prepulse inhibition and habituation processes and fewer functional NMDA receptors (O'Tuathaigh et al. 2006; Stefansson et al. 2002). Importantly, transmembrane *NRG1* was recently found to associate with schizophrenia (Walss-Bass et al. 2006). Thus, the heterozygous *Nrg1* transmembrane-domain knockout mouse may provide a putative animal model of genetic vulnerability to schizophrenia.

The use of cannabis increases the risk of developing schizophrenia (Linszen et al. 1994), and people with psychotic disorders are more likely to use or have used psychoactive drugs than other psychiatric patients or the general population (Arseneault et al. 2004; Schneier and Siris 1987). It has been hypothesised that chronic (Degenhardt and Hall 2002; Leweke et al. 2004; Linszen et al. 1994) and less robustly acute (Favrat et al. 2005) cannabis consumption has an impact on the aetiology of schizophrenia in subjects with a predisposition for this mental disorder. Importantly, not only cannabis consumption during puberty/adolescence but also a variety of other external/environmental factors such as obstetric complications (Boksa 2004) or nutritional deficiency (Susser and Lin 1992) may unmask schizophrenia in individuals who have a prior vulnerability to the disorder (Degenhardt and Hall 2002; Leweke et al. 2004). This is consistent with the two-hit hypothesis of schizophrenia, which poses that psychotic patients harbour one or various susceptibility genes that are necessary but not sufficient to cause schizophrenia (hit 1). Environmental stressors, such as cannabis abuse, may then help to trigger the onset of schizophrenia by interacting with this genetic vulnerability to the mental disorder (hit 2) (Bayer et al. 1999).

The availability of transgenic mice offers a unique opportunity to systematically investigate such gene–environment interactions, while avoiding the many confounding factors that plague human studies on cannabis-induced psychosis (Henquet et al. 2005). In this study, we examine, whether dysfunction in the *Nrg1* gene modulates the behavioural effects of Δ^9-tetrahydrocannabinol (THC), the major psychotropic component of cannabis, using heterozygous *Nrg1* transmembrane domain knockout (*Nrg1* HET) mice. The effects of different dosages of acutely administered THC on wild type-like and *Nrg1* HET mice were assessed using a multitiered phenotyping strategy focusing on behavioural domains of locomotion, exploration, anxiety and sensorimotor gating.

Materials and methods

Animals

The heterozygous *Nrg1* transmembrane domain knockout mice were provided by Prof. Richard Harvey (Victor Chang Cardiac Research Institute, Sydney Australia) and have been described previously (Stefansson et al. 2002). Test animals were heterozygous $Nrg1^{+/-}$ (*Nrg1* HET) and wild type-like control $Nrg1^{+/+}$ (WT) littermates (backcrossed for 15 generations onto a C57BL6/J background). Genotypes were determined after weaning (postnatal day 21) by tail biopsy and polymerase chain reaction (primers for mutant *Nrg1* mice: Neo173F 5′-ATGAACTGCAGGACGAGG CA-3′ and Neo6301R 5′-GCCACAGTCGATGAATCCA G-3′; primers for wild-type-like control mice: 5′-AACAGC CTGACTGTTAACACC-3′ and 5′-TGCTGTCCATCTGCA CGAGACTA-3′). Male, adult, age-matched (±14 days) test animals of the same genotype were pair-housed in Macrolon cages under a 12:12 h light:dark schedule [light phase: white light (illumination: 80 lx) - dark phase: red light (illumination: <2 lx)] with food and water available ad libitum. Male, adult, age-matched (±7 days), group-housed A/J mice (Animal Resources Centre, Canning Vale, Australia) 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

Δ^9-tetrahydrocannabinol (THC: Sigma-Aldrich, Sydney, Australia) was dissolved in 4% ethanol, 1% Tween 80 and 95% saline (Balerio et al. 2006; Varvel et al. 2006). The drug was administered intraperitoneally (i.p.) in a volume of 10 ml/kg of body weight (BW), 30 min before behavioural testing. Two doses of THC were used in this study, 5 and 10 mg/kg BW (latter one was shown to be CB1 receptor-specific: see e.g., Compton et al. 1996). Relatively high doses of THC were chosen, as cannabis psychosis has been associated with acute administration of high doses of cannabis (Hall and Degenhardt 2000; Johns 2001) and as the C57BL/6J background strain is known for its reduced sensitivity to some of the neurobehavioural effects of THC compared to other mouse strains (Onaivi et al. 1995). Mice were treated with similar volumes of either vehicle (4% ethanol, 1% Tween 80 and 95% saline) or one of the two dosages of THC in a counterbalanced, quasi-randomised design, allowing for within-animal comparison. A washout period of at least 7 days was guaranteed before the animals were retested.

Behavioural testing

All animals were tested at the age of 6–7 months to investigate whether acute THC treatment has a differential effect on the behavioural performance of *Nrg1* HET compared to WT mice. Nrg1 hypomorphic mice exhibit an age-dependent hyperactive phenotype, as mutant animals of 3–4 months of age show similar behaviours as wild type-like control littermates, whereas Nrg1 *HET* mice older than 4 months exhibit an increase in locomotion and exploration compared to WT mice (Karl et al. 2006). A first set of animals (n=10 per genotype) was tested 30 min postinjection in domains such as locomotion, exploration and anxiety using the open field (OF), the hole board (HB) and the elevated plus maze (EPM) tasks, respectively. A second set of mice (n=10–13) was tested 30 min postinjection for anxiety, social behaviours and sensorimotor gating using the light–dark (LD), the social interaction (SI) and the prepulse inhibition (PPI) tests (see Table 1). All behavioural testing was conducted during the light cycle under highly standardized test conditions (Crawley 1999; Karl et al. 2003). For habituation purposes, all test animals were transported to the testing room 1 h before behavioural testing (holding and test room were part of the same facility having therefore identical light, air pressure and temperature conditions). Environmental odours were removed from the different test apparatus by cleaning the equipment after each trial with a 30% ethanol solution.

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

Sets	Genotype (and animal numbers)	Battery of behavioural paradigms (30 min after i.p. injection of either vehicle or THC)
Set 1	WT: n=10; *Nrg1* HET: n=10	Behavioural test battery A OF HB EPM
Set 2	WT: n=10; *Nrg1* HET: n=13	Behavioural test battery B LD SI PPI

A washout period of at least 7 days was guaranteed before the animals were retested in the same battery of behavioural paradigms. Thus, all test animals were observed repeatedly 30 min postinjection in the behavioural test battery A [open field (OF), the hole board (HB) and the elevated plus maze (EPM); set 1] or test battery B [light-dark test (LD), the social interaction test (SI) and the prepulse inhibition paradigm (PPI); set 2]. Two sets of animals were tested over a period of 3 weeks. Mice were treated with either vehicle or one of the two dosages of THC (5 or 10 mg/kg bodyweight) in a counterbalanced, quasi-randomised design, allowing for within-animal comparison.

Open field (OF) Locomotor activity, explorative-like tendencies and anxiety-related behaviours were evaluated by placing the mouse into an infrared photobeam-controlled (x-, y-, and z-axis) open field activity test chamber (MED, Vermont, USA). This paradigm mimics the natural conflict in mice between the tendency to explore a novel environment and to avoid an exposed open area (Crawley 1985; DeFries et al. 1966). The arena (43.2 cm×43.2 cm) was divided into a central and a peripheral zone (MED software coordinates for central zone: 3/3, 3/13, 13/3, 13/13). Animals were tested for 30 min (illumination at floor level: 20 lx), and the animal's total distance travelled (horizontal activity: x- and y-axis) and vertical activity (z-axis) was recorded automatically (software settings: box size: 3; ambulatory trigger: 2; resting delay: 1,000 ms; resolution: 100 ms) in the central and peripheral areas. The ratio of central to total distance travelled and the time spent in the central zone were taken as measures of anxiety (Denenberg 1969).

Hole board (HB) The HB test provides independent measures of locomotor activity and directed exploration (Boissier et al. 1964; van Gaalen and Steckler 2000). Mice were placed into the open field activity test chamber, which was equipped with a hole board floor insert for mice (MED: 16 holes; diameter: 1.6 cm). The infrared photobeams

provided automated measures of the distance travelled, ambulatory frequency and *head-dipping* frequency.

Elevated plus maze (EPM) The EPM represents the natural conflict between the tendency of mice to explore a novel environment and the tendency to avoid a brightly lit, elevated, open area (Montgomery 1958). The grey plus maze had a central platform (6 cm×6 cm), two alternate enclosed arms (35 cm×6 cm; height of enclosing walls 28 cm; dimly illuminated: 10 lx) and two alternate open arms (35 cm×6 cm; without side walls; highly illuminated: 70 lx) with ledges (4 mm×6 mm). The arms' surface was raised 70 cm above the floor. The mouse was placed onto the centre field of the "+" (faced to an enclosed arm) and was allowed to explore the maze for 5 min. Closed arm entries were taken as measures of motor activity, whereas entries in and time spent on open arms and the percentage of open arm entries (open arm entry ratio) were recorded online as a measure of anxiety (Hogg 1996; Pellow et al. 1985). In addition to recording behaviours online by repeatedly trained research staff, the sessions were reanalysed for parameters such as time spent in the different compartments of the EPM. An individual entry was recorded when the animal entered the arm with at least half of its body length.

Light-dark (LD) In the LD test, the time spent in a brightly illuminated zone compared to a dark zone and the occurrence of associated exploratory behaviour (vertical activity) were used to assess anxiety in rodents (Costall et al. 1989; Crawley 1999). The test animals were placed into the open field chamber, which was equipped with a dark box insert for mice (covering half the area of the chamber: MED). An opening located in the centre of the partition connected light (illumination: 20 lx) and dark (illumination: <2 lx) compartments. At the start of the experiment, mice were placed into the lit compartment. The time spent in, entries into and horizontal activity in the differentially illuminated compartments, and vertical activity were recorded during a 10 min test session. The ratio of distance travelled in the light compartment to total distance travelled (distance ratio) and the time mice spent in the light compartment were taken as measures of anxiety.

Social interaction (SI) The SI model is widely used to measure anxiety-like behaviours and to detect anxiogenic- and anxiolytic-like effects of drugs (File 1988; Kask et al. 2001). Test animals were placed together with an A/J standard opponent into the open field activity test chamber (in opposite corners), where they were allowed to explore the arena and each other freely for 10 min. The behaviour of the test mouse was recorded online. Frequency and total duration (so-called active social interaction time) of the active socio-positive behaviours *general sniffing*, *anogenital sniffing, allogrooming*, *following* and *crawling over/under* were recorded.

Prepulse inhibition (PPI) Patients with schizophrenia show impaired sensorimotor gating. PPI is the operational measure of sensorimotor gating, in which a weak pre-stimulus (prepulse) attenuates the startle response (Wang et al. 2003). PPI was tested in two startle chambers (SR-Lab: San Diego Instruments, San Diego, USA). Animals were habituated to the test device for three consecutive days (day 1: 2×2 min; days 2–3: 1×10 min) before being tested 24 h later. The protocol used was adapted from methods developed by Geyer and Swerdlow (1998). Briefly, after a 5 min acclimation period with a 70 dB background noise, the test session began. Each session consisted of 76 trials in a pseudorandomised order: 10×90 dB startle response trials, 18×120 dB startle response trials, 2× prepulse alone trials (prepulse intensities of 74, 78, 82 or 86 dB), 8× PPI response trials (prepulse followed 80 ms later by a 120 dB startle stimulus), and 8× no pulse trials (background noise only). We chose a variable intertrial interval of averaged 15 s (range 10–20 s), prepulse duration of 20 ms and startle duration of 40 ms. Startle response was measured as the average mean amplitude. Percentage of PPI was calculated as [(startle response 120 dB - PPI response)×100/startle response 120 dB]. We also analysed the mean % PPI of all summed prepulse responses for each genotype.

Statistical analysis

One-way analysis of variance (ANOVA) was applied to investigate "baseline" differences in the behavioural performance of vehicle-treated WT and *Nrg1* HET mice. The behavioural response of WT and *Nrg1* HET mice to THC was analysed using two-way ANOVA to investigate the effect of the factors "genotype" and "THC dose" on several behavioural domains. This was followed by one-way ANOVA (factor: "genotype" or "THC dose" split by the corresponding factor) and the Student–Newman–Keuls post hoc test, in which vehicle data were compared to each individual dose of THC (5 and 10 mg/kg) within each genotype. Differences were regarded as statistically significant when $p<0.05$. Results present the *degrees of freedom*, *F*-values and *p*-values of one-way (for baseline comparison) or two-way ANOVA (for "THC dose" effects), while in the figures and tables, the *p*-values of the corresponding post hoc tests are provided. Significant post hoc effects of *Nrg1* HET animals vs WT are indicated by "#" ($^{\#}p<0.05$ and $^{\#\#}p<0.01$), whereas significant effects of acute THC treatment vs vehicle treatment are shown by asterisk ($^{*}p<0.05$ and $^{**}p<0.01$). All data are presented as means ± standard error of the mean (SEM).

Results

Locomotor activity and exploration

The results from the OF, HB and LD are shown in Table 2. One-way ANOVA revealed that vehicle-treated *Nrg1* HET mice exhibited a hyperactive phenotype at baseline as the total distance travelled was significantly increased in the OF [$F(1,18)=21.0$, $p<0.001$] and in the LD [$F(1,21)=8.8$, $p<0.01$] compared to vehicle-treated WT mice. Furthermore, *Nrg1* HET mice crossed significantly more often between the light and the dark compartment of the LD [$F(1,21)=9.6$, $p<0.01$] and entered more often the enclosed arms of the EPM [$F(1,18)=5.1$, $p<0.05$] than WT mice. This hyperactivity was not only evident with regard to locomotion but could be confirmed in explorative-like behaviours as measured by an increased frequency of head dipping in the HB [$F(1,17)=5.1$, $p<0.05$] and of exploration-related vertical activity in the LD test [$F(1,21)=7.1$, $p<0.05$] compared to vehicle-treated WT mice.

THC treatment significantly decreased the total distance travelled in the OF [$F(2,52)=34.6$, $p<0.001$] and the LD test [$F(2,59)=9.9$, $p<0.001$], and the frequency of total crossings [$F(2,59)=11.4$, $p<0.01$] and vertical activity [$F(2,59)=15.4$, $p<0.001$] in the LD test as measured by two-way ANOVA. Interestingly, a differential motor activity- and exploration-suppressing effect of THC was observed between WT and *Nrg1* HET mice as we detected significant genotype by drug interactions for all these parameters: total distance travelled in OF [$F(2,52)=10.3$, $p<0.001$] and LD [$F(2,59)=5.2$, $p<0.01$], frequency of total crossings in LD [$F(2,59)=5.2$, $p<0.01$], and vertical activity [$F(2,59)=4.2$, $p<0.05$] in LD. Thus, there exists a genotype-specific difference in the motor activity-suppressant actions of THC. This conclusion was further supported by post hoc analyses for motor activity/exploration measures conducted within each genotype. In the OF, 10 mg/kg THC significantly reduced the total distance travelled in both genotypes compared to vehicle. However, 5 mg/kg THC significantly inhibited the total distance travelled selectively in the *Nrg1* HET mice (see Table 2). In the LD test, both the 5 and 10 mg/kg doses of THC were effective in reducing the total number of crossings and the frequency of vertical activity of *Nrg1* HET mice. Nevertheless, both cannabinoid doses (5 and 10 mg/kg) were ineffective in reducing these measures in WT mice (see Table 2). Acute THC treatment also decreased the frequency of head dipping [$F(2,50)=44.1$, $p<0.001$] in the HB test as measured by two-way ANOVA. No significant genotype by

Table 2 Locomotor activity, exploration and anxiety-related behaviours

Parameters	WT mice			*Nrg1* HET mice		
	Vehicle	THC 5 mg/kg	THC 10 mg/kg	Vehicle	THC 5 mg/kg	THC 10 mg/kg
Open field						
Total distance [cm] "+"	2,017±121	1,607±131	1,190±194**	3,382±272#	1,358±241**	1,073±200**
Time spent in the centre [s]	294±59	68±21**	31±25**	370±50	134±50**	9±2**
Hole board						
Head dipping fq	15±2	3±1**	1±0.4**	23±3#	5±3**	1±0.5**
Elevated plus maze						
Enclosed arm entries	6±1	9±2	4±1	9±1#	12±3	12±3
Time spent in open arms [s]	16±4	10±5	7±3	30±6#	11±5*	14±6
Open arm entries ratio [%]	36±2	19±5	25±7	38±3	16±6*	24±5
Light–dark						
Total distance [cm] "+"	1,248±166	1,172±186	859±145	1,881±137##	981±156**	911±156**
Total crossings "+"	49±12	46±16	22±11	105±13##	29±12**	18±6**
Vertical activity "+"	35±5	25±10	11±7	63±8#	13±8**	6±4**
Time spent in light zone [s] "+"	72±15	86±32	40±25	147±18##	51±27**	25±11**
Distance travelled in light zone [cm] "+"	274±62	264±87	147±77	542±63##	182±78**	71±35**
Entries into light zone fq "+"	24±6	23±8	11±6	52±6##	14.5±6**	9±3**
Distance ratio [%] "+"	18±4	17±5	12±5	29±2#	13±4**	6±3**
Social interaction						
SI duration [s]	42±5	22±5**	12±2**	44±3	16±2**	9±2**
Sniffing fq	29±2	15±1**	9±1**	32±2	14±2**	7±1**
Anogenital sniffing fq	6±1	2±0.3**	1±0.3**	8±1	2±0.5**	0.4±0.2**

"fq" represent frequencies of any given behaviour; parameters with a significant genotype by THC dose interaction are presented with "+"; significant post hoc effects of *Nrg1* HET animals vs WT control animals at baseline are indicated by "#" or "##" (#$p<0.05$ and ##$p<0.01$), whereas significant effects of acute THC treatment vs vehicle treatment are shown by "*" or "**"(*$p<0.05$ and **$p<0.01$); all data are presented as means ± standard error of the mean (SEM)

dose interaction was observed for this parameter. Both doses of THC (5 and 10 mg/kg) significantly reduced head dipping compared to vehicle in either genotype (Table 2).

Anxiety

The results from the animal models of anxiety are shown in Table 2. Mice were repeatedly tested in the various animal models of anxiety (OF, EPM, LD and SI) according to a within-subjects, counterbalanced, quasi-randomised design with a 1 week interval between testing. To confirm that the effects of THC were consistent with repeated testing over weeks of administration, we analysed the data using two-way ANOVA (factor 1: week of testing and factor 2: THC dose). Our analyses found no significant week of testing by THC dose interaction effects for any parameter recorded in the different anxiety paradigms (data not shown). This highlights that the effects of THC were reproducible irrespective of whether the mice were naïve or pre-exposed to the test apparatus.

In the LD, one-way ANOVA for baseline behaviour revealed an anxiolytic-like phenotype for the vehicle-treated *Nrg1* HET mice. Accordingly, *Nrg1* HET mice spent significantly more time in the light compartment [$F(1,21)=9.0$, $p<0.01$], showed increased locomotion rates [$F(1,21)=8.8$, $p<0.01$] in the area and exhibited an elevated light compartment entry score [$F(1,21)=9.6$, $p<0.01$] compared to vehicle-treated WT mice. Importantly, this anxiolytic-like profile of *Nrg1* HET mice holds when the locomotion-related parameters were corrected for the total distance travelled in this paradigm as highlighted by a significant increase in distance ratio [$F(1,21)=6.8$, $p<0.05$] for *Nrg1* hypomorphs within the aversive light compartment. Furthermore, vehicle-treated *Nrg1* HET mice spent significantly more time on the more aversive open arms of the EPM [$F(1,18)=4.4$, $p=0.05$]. The reduced anxiety observed in these mutant mice appears to be task-dependent as SI performance was not different between vehicle-treated WT and *Nrg1* HET mice.

Two-way ANOVA revealed that THC had a significant anxiogenic-like effect as it reduced the time mice spent on the open arms of the EPM [$F(2,52)=4.7$, $p<0.05$] and the ratio of open arm entries [$F(2,52)=7.7$, $p<0.01$]. Furthermore, THC treatment resulted in a decrease of time spent [$F(2,59)=5.8$, $p<0.01$] and distance travelled [$F(2,59)=9.3$, $p<0.001$] in the light compartment of the LD, of the frequency of entries into the same compartment [$F(2,59)=11.4$, $p<0.001$] and of distance ratio in this area [$F(2,59)=7.7$, $p<0.001$]. We also found a genotype by THC dose interaction for time spent in the light compartment of the LD [$F(2,59)=3.4$, $p<0.05$], for the frequency of entries into [$F(2,59)=5.2$, $p<0.01$] and the distance travelled in [$F(2,59)=4.2$, $p<0.05$] the same area. In addition, there was a trend for a genotype by THC dose interaction for the distance ratio in the light compartment [$F(2,59)=2.9$, $p=0.06$]. Post hoc analysis revealed that THC, at 5 mg/kg, decreased the open arm entry ratio in the EPM for *Nrg1* HET but not WT mice. Furthermore, THC administration, at both doses of 5 and 10 mg/kg, reduced all behaviours measured in the LD in the *Nrg1* HET but not the WT mice (see Table 2).

In the social interaction test, two-way ANOVA revealed no differential effect of THC treatment on *Nrg1* HET and WT mice in any measures derived from this test. THC treatment dose-dependently decreased the total time spent in social interaction [$F(2,59)=51.8$, $p<0.001$], the frequency of sniffing [$F(2,59)=82.2$, $p<0.001$] and the frequency of anogenital sniffing [$F(2,59)=55.8$, $p<0.001$] in a similar fashion in WT and *Nrg1* HET mice. No aggressive interactions were observed between test animals and the opponent A/J mice.

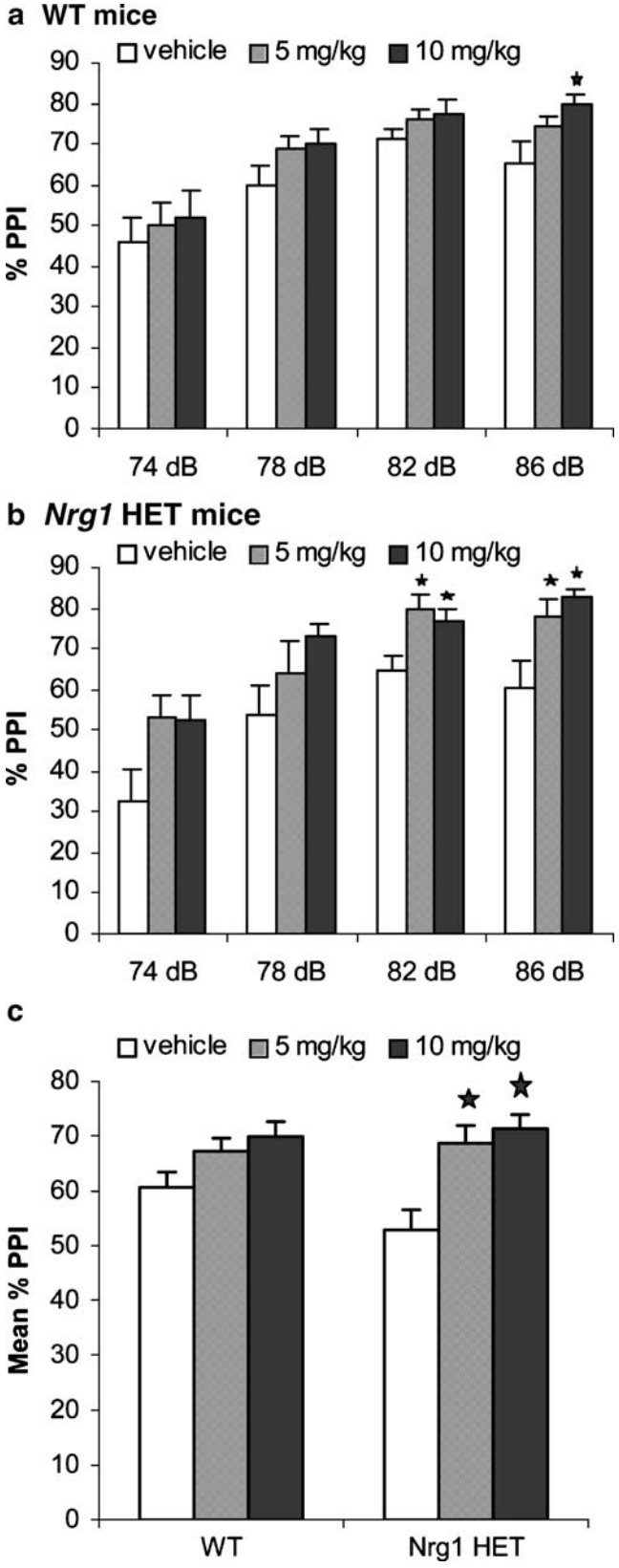
Vehicle-treated *Nrg1* HET mice showed no significant difference to vehicle-treated WT mice in the total time spent in the centre of the arena of the OF. However, two-way ANOVA showed that THC treatment significantly decreased the time spent in the centre of the OF [$F(2,52)=32.7$, $p<0.001$], which is a parameter for anxiety-related behaviour. Post hoc analysis revealed that in both genotypes, acute THC treatment (both 5 and 10 mg/kg) decreased the time spent in the centre of the OF compared to vehicle-treated animals.

Prepulse inhibition

Figure 1a–c presents the data from PPI testing. The prepulse inhibition (measured as % PPI) was elevated for both genotypes with increased prepulse intensities. At baseline, no differences were observed between vehicle-treated WT and *Nrg1* HET mice with regard to % PPI (Fig. 1a,b).

Two-way ANOVA revealed that acute THC administration significantly enhanced % PPI [$F(2,59)=6.4$, $p<0.01$; Fig. 1a,b]. Post hoc analyses indicated that in the *Nrg1* HET mice, the increased % PPI was observed for both THC doses at prepulses of 82 and 86 dB (Fig. 1b), whereas in WT mice, only treatment with 10 mg/kg THC significantly increased % PPI at the 86 dB prepulse (Fig. 1a). When % PPI was calculated as the % PPI of the mean PPI responses across all prepulse intensities, post hoc analysis revealed that both the 5 and 10 mg/kg THC doses increased % PPI in the *Nrg1* HET animals with no corresponding effects being observed in the WT mice (Fig. 1c).

No differences were observed between vehicle-treated *Nrg1* HET and WT mice when measuring their startle response to a 120 dB startle stimulus (Fig. 2). Two-way ANOVA revealed that THC treatment [$F(2,59)=6.1$, $p<0.01$] significantly reduced the startle response. Post hoc analysis revealed that the startle response-inhibiting effect of THC was only evident in *Nrg1* HET after treatment with 10 mg/kg THC and was not observed in the WT animals.



◀ **Fig. 1a–c** Prepulse Inhibition (PPI). The effect of acute THC treatment (30 min after i.p. injection of either vehicle or THC: 5 or 10 mg/kg BW) on % PPI [(startle response 120 dB–PPI response)×100/startle response 120 dB] was measured in WT and *Nrg1* HET mice (n=10–13). Different prepulse stimuli were used (4/8/12/16 dB above 70 dB background noise): **a** % PPI of WT mice, **b** % PPI of *Nrg1* HET mice, and **c** mean of % PPI of all summed prepulse responses for WT and *Nrg1* HET test animals. All data are presented as means ± standard error of the mean (SEM). Significant effects of acute THC treatment vs vehicle treatment are shown by "*" (p<0.05)

Discussion

Vehicle-treated *Nrg1* HET mice expressed a hyperactive, hyper-explorative and anxiolytic-like phenotype compared to WT animals. Importantly, mice hypomorphic for the *Nrg1* gene were more susceptible to THC's locomotor suppressant actions and its stimulating effect in an animal model for sensorimotor gating. In animal models of anxiety, less anxious *Nrg1* HET animals were no more affected by acute THC administration than their WT controls in an array of different models (e.g., social interaction and EPM). However, the *Nrg1* HET mice showed a greater anxiogenic response to THC than WT in the LD test, highlighting that the effects of THC on anxiety in these animals might be task-dependent.

The acute administration of THC decreased spontaneous locomotor activity and exploration in the open field test. Importantly, THC promoted a greater reduction in locomotor and exploratory activity in the *Nrg1* HET mice

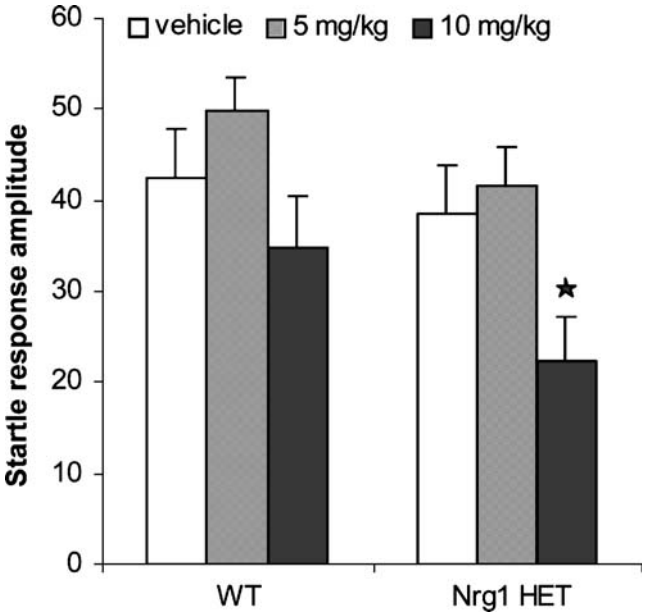


Fig. 2 Startle response. The startle response (averaged startle amplitude) in WT and *Nrg1* HET mice (n=10–13) was measured for a 120-dB startle stimulus 30 min after i.p. injection of vehicle or THC (5 or 10 mg/kg BW). All data are presented as means ± standard error of the mean (SEM). Significant effects of acute THC treatment vs vehicle treatment are shown by "*" (p<0.05)

compared to their WT controls, suggesting that the mutant mice are more susceptible to the well-established locomotor suppressant effects of cannabinoids (Arnold et al. 1998, 2001). It could be argued that this hypersensitivity may simply reflect the *Nrg1* HET mice being relatively hyperactive at baseline, thus, providing more room to delineate the locomotor suppressant effects of THC. However, a floor effect can be ruled out, as locomotor activity of both genotypes is not completely suppressed by the acute THC treatment (see, e.g., total distance in OF—Table 2). One likely explanation for the *Nrg1* HET animals' enhanced sensitivity to the locomotor inhibitory effects of cannabinoids may be based on developmental adaptations during ontogeny in the germline Nrg1 hypomorphic mice (e.g., increased expression of CB1 receptors or greater sensitivity of CB1 receptors to cannabinoid ligands in motor-related regions). The observation that *Nrg1* HET mice are hyperactive, combined with the finding that Nrg1 administration acts to inhibit locomotor behaviour in hamsters (Snodgrass-Belt et al. 2005), indicates that Nrg1 may function as an inhibitory counterbalance to excitatory neural processes mediating locomotor behaviour.

Vehicle-treated *Nrg1* hypomorphic mice exhibit an anxiolytic-like phenotype, which might be influenced by their hyperactivity. However, we detected an increase in the time hypomorphs spent in aversive areas. Thus, the decrease in anxiety is evident within a behavioural parameter, which is relatively unaffected by an unspecific increase in motor activity. These baseline differences exist for spatio-temporal (i.e., LD and EPM) rather than socio-temporal paradigms (i.e., SI). This task-dependent anxiolytic phenotype suggests an impact of Nrg1 on certain explorative/locomotive aspects of anxiety (Rodgers 1997; Rodgers and Johnson 1995). Importantly, anxiety symptoms (and high autonomic arousal) are very variable accompaniments to schizophrenia—often schizophrenia patients are diagnosed with more than one anxiety disorder (Braga et al. 2005; Tibbo et al. 2003; Townsend and Wilson 2005). Obviously, any animal model for candidate genes of schizophrenia models only some, but not all, aspects of the mental disorder. Future research has to clarify if the specific anxiolysis of Nrg1 hypomorphs is related to the comorbidity between anxiety disorders and psychosis reported in schizophrenia or if it is a direct effect of reductions in Nrg1 expression levels.

It has been widely reported that administration of cannabinoids stimulates anxiety-related behaviours in rodents (Onaivi et al. 1990, 1995; Rutkowska et al. 2006). In our study, we observed a task-specific increased susceptibility of *Nrg1* HET mice to the anxiogenic effects of THC compared to control animals. A confounding effect of the increased sensitivity of the *Nrg1* HET mice to the locomotor suppressant effects of THC can be ruled out, as spatiotemporal parameters of other anxiety paradigms (i.e., OF and EPM) were not differentially affected by THC treatment. It is possible that the enhanced sensitivity of the *Nrg1* HET animals to the anxiogenic effects of THC, as measured in the LD test, may reflect a specific anxiogenic-like action of THC on exploration/locomotion-related features of anxiety, which might be expressed to a greater extent in the mutant mice. These features are linked to how an organism adapts and responds to its spatial surrounding rather than to its social environment. Interestingly, both altered habituation to a novel environment in animal models (O'Tuathaigh et al. 2006) and social withdrawal in humans (Dixon et al. 1994) are described within the schizophrenia context. Further investigations should use a battery of different anxiety models screening for conditioned (conflict and other paradigms) and unconditioned responses (exploration, social, antipredator and other) after THC treatment to clarify this task-specificity (Rodgers 1997; Rodgers and Johnson 1995).

Within-subjects designs are commonly utilised in psychopharmacological mutant mice studies due to the difficulty in generating sufficient animal numbers. Importantly, altered anxiety-related behaviour (Espejo 1997; Lee and Rodgers 1990; Nyberg et al. 2003; Rodgers et al. 1996) and modulated sensitivity to the effects of psychotropic drugs upon repeated testing of mice in the same anxiety model (Holmes and Rodgers 1999; Rodgers et al. 1992; Rodgers and Shepherd 1993) are well-known phenomena. For instance, modulators of $GABA_A$ function (e.g., benzodiazepines) have diminished anxiolytic effects when administered to animals with prior test experience of the EPM. This phenomenon is known as "one trial tolerance" (Bertoglio and Carobrez 2002; File et al. 1990; Holmes and Rodgers 1999; Rodgers et al. 1992; Rodgers and Shepherd 1993). Our statistical analyses revealed no such altered effectiveness of THC with repeated testing, as mice displayed consistent anxiogenic responses to THC, whether they were naïve or had prior experience of the test apparatus. However, many studies demonstrating "one trial tolerance" to the effects of benzodiazepines use a 24 h test–retest interval (Albrechet-Souza et al. 2005; Cruz-Morales et al. 2002; File 1990; Holmes and Rodgers 1999). Therefore, it is possible that THC may have distorted effects with a shorter than 7 day intertrial interval, although the selective CB1 receptor antagonist AM 251 also showed consistent anxiety-modulating effects on EPM-naïve or -experienced mice using a 24 h test–retest interval (Rodgers et al. 2005). Interestingly, Bouwknecht et al. (2004) reported a relatively small impact of repeated testing on baseline anxiety behaviour of C57BL/6 mice using a 7 day intertrial interval. Furthermore, they showed consistent effects of the 5HT1A receptor agonist, flesinoxan, with repeated testing in anxiety models. Our results suggest that with a 1 week intertrial period, THC administered according to a within-subjects design provides

consistent behavioural results in various animal models of anxiety.

PPI is a phenomenon, whereby a pre-stimulus reduces the magnitude of the normal startle response to an intense startling stimulus (Graham 1975; Hoffman and Searle 1968). Importantly, motor activity differences (as shown for our animal model) do not to have an impact on the startle reflex to an acoustic startle stimulus (Leng et al. 2004). In the current study, 5 and 10 mg/kg THC exposure significantly increased PPI in the *Nrg1* HET mice, with the corresponding effect of THC not being as pronounced in WT animals. This increased effect of acute THC on PPI in the *Nrg1* HET mice appears to be valid, at least for the 5 mg/kg THC dose, as it was ineffective in reducing the startle response. The observation that 10 mg/kg THC significantly reduced startle in the *Nrg1* HET animals further supports that these mice are more sensitive to sedative actions of cannabinoids than WT animals.

Studies investigating the effects of cannabinoids on PPI in rodents are replete with inconsistencies. The anandamide reuptake and degradation inhibitor, AM404, and the synthetic cannabinoid receptor agonists, WIN 55,212-2 and CP 55,940, have been shown to promote deficits in PPI in some studies (Fernandez-Espejo and Galan-Rodriguez 2004; Mansbach et al. 1996; Martin et al. 2003; Schneider and Koch 2002) but not in others (Bortolato et al. 2005, 2006; Stanley-Cary et al. 2002). Two recent studies have directly examined the effects of THC on PPI (Malone and Taylor 2006; Nagai et al. 2006). Similar to our findings using wild type-like control mice, Malone and Taylor (2006) showed that THC (1 and 3 mg/kg) had no effect on PPI when administered to group-housed control rats. However, isolation-reared rats—suggested as another animal model for schizophrenia—exhibited PPI disruptions after THC treatment. Importantly, a genetic and environmental model should not be treated as identical models for schizophrenia as too little is known about biochemical and behavioural differences between these models. For example, socially isolated rats express increased anxiety-like behaviours (Wright et al. 1991) unlike *Nrg1* HET mice, which display reduced anxiety-related behaviour. Such differences and the fact that these studies were conducted on distinct species may account for the opposing effects of THC on PPI. Furthermore, it is widely accepted that any given animal model for schizophrenia can only represent certain aspects of this mental disorder not its entire complexity (Ellenbroek and Cools 2000; van den Buuse et al. 2005). Therefore, a direct comparison of a singular animal model (i.e., isolation rearing) with a two-hit model (i.e., Nrg1 depletion combined with drug abuse) would be overly simplistic.

Nagai et al. (2006), using almost identical doses of THC (6 and 10 mg/kg) to that employed here, demonstrated CB1 receptor-mediated PPI deficits in mice. The lack of correlation between the findings of Nagai et al. (2006) and our results in wild type-like mice are likely due to strain differences in the effects of cannabinoids as the C57BL6/J background strain used in our study has been shown to be less sensitive to the neurobehavioural effects of THC compared to other inbred strains (Arnold et al. 2001; Onaivi et al. 1995).

Given that chronic and - less robust - acute (Favrat et al. 2005; Linszen et al. 1994) THC exposure is thought to precipitate psychosis in vulnerable individuals (Degenhardt et al. 2003; Hall 1998) and that other known psychosis-promoting agents such as phencyclidine and amphetamine (Geyer et al. 2001; Mansbach and Geyer 1989; Mansbach et al. 1988; Martin et al. 2003) disrupt PPI, the observation that THC exposure increased PPI selectively in *Nrg1* HET animals was unexpected. The self-medication theory is an alternative theory for why people with schizophrenia are more likely to administer drugs of abuse than the general population (Arseneault et al. 2004; Degenhardt et al. 2001; Schneier and Siris 1987; Schofield et al. 2006). According to this theory, schizophrenia patients try to alleviate the symptoms associated with the disorder and/or to counter the side effects of antipsychotic medication. Interestingly, the most widely used drug in the schizophrenia population, nicotine, increases PPI in rats and reverses PPI deficits in schizophrenia patients (Acri et al. 1994; Kumari et al. 2001; Postma et al. 2006). Thus, schizophrenia patients may self-medicate with nicotine to reverse their cognitive deficits. Similarly, our observation of THC-induced enhancement of PPI supports the hypothesis that acute THC may have a partly beneficial action in schizophrenia patients by improving a negative symptom such as attentional dysfunction. Unfortunately, there is no satisfactory animal model for a variety of positive symptoms (i.e., hallucinations, delusions) of schizophrenia; hence, the effects of THC on these core aspects of the mental disorder cannot be concluded upon by the current investigation. However, it is interesting to note that THC, like clozapine, acts to reverse the hyperactive phenotype of *Nrg1* HET mice (Stefansson et al. 2002). On the contrary in human studies, it appears cannabis may acutely exacerbate positive schizophrenia symptoms (Grech et al. 2005; Linszen et al. 1994; van Os et al. 2002). Another study discusses the potential bidirectional interactions between cannabis use and psychosis (Hides et al. 2006).

Given that *Nrg1* HET mice provide a putative animal model of schizophrenia, the current results are consistent with the notion that *acutely* administered THC may reverse this schizophrenia phenotype, decreasing hyperactivity and enhancing attention as measured by PPI. As mice were tested repeatedly, behavioural alterations in *Nrg1* HET mice could be influenced by disrupted habituation and novelty processing-both have been considered central to the cognitive deficits observed in schizophrenia (O'Tuathaigh

et al. 2006). These results may provide an animal model of genetic vulnerability to self-medication using cannabinoids, where users may initiate cannabis use as a means to dampen some symptoms of schizophrenia. However, future investigations are needed to examine the effects of *chronic use* of cannabinoids on the *Nrg1* HET mice, as it is the extended use of cannabis that is more strongly associated with precipitating psychosis and exacerbating symptoms in schizophrenia patients.

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References

Acri JB, Morse DE, Popke EJ, Grunberg NE (1994) Nicotine increases sensory gating measured as inhibition of the acoustic startle reflex in rats. Psychopharmacology (Berl) 114:369–374

Albrechet-Souza L, Oliveira AR, De Luca MC, Tomazini FM, Santos NR, Brandao ML (2005) A comparative study with two types of elevated plus-maze (transparent vs. opaque walls) on the anxiolytic effects of midazolam, one-trial tolerance and fear-induced analgesia. Prog Neuropsychopharmacol Biol Psychiatry 29:571–579

Arnold JC, Topple AN, Hunt GE, McGregor IS (1998) Effects of pre-exposure and co-administration of the cannabinoid receptor agonist CP 55,940 on behavioral sensitization to cocaine. Eur J Pharmacol 354:9–16

Arnold JC, Topple AN, Mallet PE, Hunt GE, McGregor IS (2001) The distribution of cannabinoid-induced Fos expression in rat brain: differences between the Lewis and Wistar strain. Brain Res 921:240–255

Arseneault L, Cannon M, Witton J, Murray RM (2004) Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry 184:110–117

Balerio GN, Aso E, Maldonado R (2006) Role of the cannabinoid system in the effects induced by nicotine on anxiety-like behaviour in mice. Psychopharmacology (Berl) 184:504–513

Bayer TA, Falkai P, Maier W (1999) Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the "two hit hypothesis". J Psychiatr Res 33:543–548

Bertoglio LJ, Carobrez AP (2002) Anxiolytic effects of ethanol and phenobarbital are abolished in test-experienced rats submitted to the elevated plus maze. Pharmacol Biochem Behav 73:963–969

Boissier JR, Simon P, Lwoff JM (1964) Use of a particular mouse reaction (hole board method) for the study of psychotropic drugs. Therapie 19:571–583

Boksa P (2004) Animal models of obstetric complications in relation to schizophrenia. Brain Res Brain Res Rev 45:1–17

Bortolato M, Aru GN, Frau R, Orru M, Luckey GC, Boi G, Gessa GL (2005) The CB receptor agonist WIN 55,212-2 fails to elicit disruption of prepulse inhibition of the startle in Sprague–Dawley rats. Psychopharmacology (Berl) 177:264–271

Bortolato M, Campolongo P, Mangieri RA, Scattoni ML, Frau R, Trezza V, La Rana G, Russo R, Calignano A, Gessa GL, Cuomo V, Piomelli D (2006) Anxiolytic-like properties of the anandamide transport inhibitor AM404. Neuropsychopharmacology 31 (12):2652–2659

Bouwknecht JA, van der Gugten J, Groenink L, Olivier B, Paylor RE (2004) Effects of repeated testing in two inbred strains on flesinoxan dose–response curves in three mouse models for anxiety. Eur J Pharmacol 494:35–44

Braga RJ, Mendlowicz MV, Marrocos RP, Figueira IL (2005) Anxiety disorders in outpatients with schizophrenia: prevalence and impact on the subjective quality of life. J Psychiatr Res 39:409–414

Cardno AG, Gottesman II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97:12–17

Compton DR, Aceto MD, Lowe J, Martin BR (1996) In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta 9-tetrahydrocannabinol-induced responses and apparent agonist activity. J Pharmacol Exp Ther 277:586–594

Costall B, Jones BJ, Kelly ME, Naylor RJ, Tomkins DM (1989) Exploration of mice in a black and white test box: validation as a model of anxiety. Pharmacol Biochem Behav 32:777–785

Coyle JT (1996) The glutamatergic dysfunction hypothesis for schizophrenia. Harv Rev Psychiatry 3:241–253

Crawley JN (1985) Exploratory behavior models of anxiety in mice. Neurosci Biobehav Rev 9:37–44

Crawley JN (1999) Behavioral phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. Brain Res 835:18–26

Cruz-Morales SE, Santos NR, Brandao ML (2002) One-trial tolerance to midazolam is due to enhancement of fear and reduction of anxiolytic-sensitive behaviors in the elevated plus-maze retest in the rat. Pharmacol Biochem Behav 72:973–978

DeFries JC, Hegmann JP, Weir MW (1966) Open-field behavior in mice: evidence for a major gene effect mediated by the visual system. Science 154:1577–1579

Degenhardt L, Hall W (2002) Cannabis and psychosis. Curr Psychiatry Rep 4:191–196

Degenhardt L, Hall W, Lynskey M (2001) Alcohol, cannabis and tobacco use among Australians: a comparison of their associations with other drug use and use disorders, affective and anxiety disorders, and psychosis. Addiction 96:1603–1614

Degenhardt L, Hall W, Lynskey M (2003) Testing hypotheses about the relationship between cannabis use and psychosis. Drug Alcohol Depend 71:37–48

Denenberg VH (1969) Open-field behavior in the rat: what does it mean? Ann N Y Acad Sci 159:852–859

Dixon AK, Huber C, Lowe DA (1994) Clozapine promotes approach-oriented behavior in male mice. J Clin Psychiatry 55:4–7

Duan J, Martinez M, Sanders AR, Hou C, Krasner AJ, Schwartz DB, Gejman PV (2005) Neuregulin 1 (NRG1) and schizophrenia: analysis of a US family sample and the evidence in the balance. Psychol Med 35:1599–1610

Duncan GE, Sheitman BB, Lieberman JA (1999) An integrated view of pathophysiological models of schizophrenia. Brain Res Brain Res Rev 29:250–264

Ellenbroek BA, Cools AR (2000) Animal models for the negative symptoms of schizophrenia. Behav Pharmacol 11:223–233

Espejo EF (1997) Effects of weekly or daily exposure to the elevated plus-maze in male mice. Behav Brain Res 87:233–238

Falls DL (2003) Neuregulins: functions, forms, and signaling strategies. Exp Cell Res 284:14–30

Farber NB, Wozniak DF, Price MT, Labruyere J, Huss J, St Peter H, Olney JW (1995) Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: potential relevance to schizophrenia? Biol Psychiatry 38:788–796

Favrat B, Menetrey A, Augsburger M, Rothuizen LE, Appenzeller M, Buclin T, Pin M, Mangin P, Giroud C (2005) Two cases of "cannabis acute psychosis" following the administration of oral cannabis. BMC Psychiatry 5:17

Fernandez-Espejo E, Galan-Rodriguez B (2004) Sensorimotor gating in mice is disrupted after AM404, an anandamide reuptake and degradation inhibitor. Psychopharmacology (Berl) 175:220–224

File SE (1988) How good is social interaction as a test of anxiety? In: Simon P, Soubrié P, Wildlocher D (eds) Animal models of psychiatric disorders. Selected models of anxiety, depression and psychosis. Karger, Basel, München, Paris, London, New York, New Delhi, Singapore, Tokyo, and Sydney, pp 151–166

File SE (1990) One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze. Psychopharmacology (Berl) 100:281–282

File SE, Mabbutt PS, Hitchcott PK (1990) Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. Psychopharmacology (Berl) 102:98–101

Geyer MA, Swerdlow NR (1998) Measurement of startle response, prepulse inhibition, and habituation. In: Crawley JN, Skolnick P (eds) Current protocols in neuroscience, New York, pp 8.7.1–8.7.15

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 156:117–154

Graham FK (1975) Presidential Address, 1974. The more or less startling effects of weak prestimulation. Psychophysiology 12:238–248

Grech A, Van Os J, Jones PB, Lewis SW, Murray RM (2005) Cannabis use and outcome of recent onset psychosis. Eur Psychiatr 20:349–353

Hall W (1998) Cannabis use and psychosis. Drug Alcohol Rev 17:433–444

Hall W, Degenhardt L (2000) Cannabis use and psychosis: a review of clinical and epidemiological evidence. Aust N Z J Psychiatry 34:26–34

Hashimoto R, Straub RE, Weickert CS, Hyde TM, Kleinman JE, Weinberger DR (2004) Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. Mol Psychiatry 9:299–307

Henquet C, Murray R, Linszen D, van Os J (2005) The environment and schizophrenia: the role of cannabis use. Schizophr Bull 31:608–612

Hides L, Dawe S, Kavanagh DJ, Young RM (2006) Psychotic symptom and cannabis relapse in recent-onset psychosis. Prospective study. Br J Psychiatry 189:137–143

Hoffman HS, Searle JL (1968) Acoustic and temporal factors in the evocation of startle. J Acoust Soc Am 43:269–282

Hogg S (1996) A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav 54:21–30

Holmes A, Rodgers RJ (1999) Influence of spatial and temporal manipulations on the anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated plus-maze. Neurosci Biobehav Rev 23:971–980

Iwata N, Suzuki T, Ikeda M, Kitajima T, Yamanouchi Y, Inada T, Ozaki N (2004) No association with the neuregulin 1 haplotype to Japanese schizophrenia. Mol Psychiatry 9:126–127

Johns A (2001) Psychiatric effects of cannabis. Br J Psychiatry 178:116–122

Karl T, Pabst R, von Horsten S (2003) Behavioral phenotyping of mice in pharmacological and toxicological research. Exp Toxicol Pathol 55:69–83

Karl T, Duffy L, Scimone A, Harvey RP, Schofield P (2006) Altered motor activity, exploration, and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. Genes Brain Behav (in press)

Kask A, Nguyen HP, Pabst R, von Horsten S (2001) Factors influencing behavior of group-housed male rats in the social interaction test: focus on cohort removal. Physiol Behav 74:277–282

Kumari V, Soni W, Sharma T (2001) Influence of cigarette smoking on prepulse inhibition of the acoustic startle response in schizophrenia. Hum Psychopharmacol 16:321–326

Law AJ, Lipska BK, Weickert CS, Hyde TM, Straub RE, Hashimoto R, Harrison PJ, Kleinman JE, Weinberger DR (2006) Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5′ SNPs associated with the disease. Proc Natl Acad Sci USA 103:6747–6752

Lee C, Rodgers RJ (1990) Antinociceptive effects of elevated plus-maze exposure: influence of opiate receptor manipulations. Psychopharmacology (Berl) 102:507–513

Leng A, Yee BK, Feldon J, Ferger B (2004) Acoustic startle response, prepulse inhibition, and spontaneous locomotor activity in MPTP-treated mice. Behav Brain Res 154:449–456

Leweke FM, Gerth CW, Klosterkotter J (2004) Cannabis-associated psychosis: current status of research. CNS Drugs 18:895–910

Linszen DH, Dingemans PM, Lenior ME (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. Arch Gen Psychiatry 51:273–279

Malone DT, Taylor DA (2006) The effect of delta9-tetrahydrocannabinol on sensorimotor gating in socially isolated rats. Behav Brain Res 166:101–109

Mansbach RS, Geyer MA (1989) Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. Neuropsychopharmacology 2:299–308

Mansbach RS, Geyer MA, Braff DL (1988) Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology (Berl) 94:507–514

Mansbach RS, Rovetti CC, Winston EN, Lowe JA 3rd (1996) Effects of the cannabinoid CB1 receptor antagonist SR141716A on the behavior of pigeons and rats. Psychopharmacology (Berl) 124:315–322

Martin RS, Secchi RL, Sung E, Lemaire M, Bonhaus DW, Hedley LR, Lowe DA (2003) Effects of cannabinoid receptor ligands on psychosis-relevant behavior models in the rat. Psychopharmacology (Berl) 165:128–135

Montgomery KC (1958) The relation between fear induced by novel stimulation and exploratory behavior. J Comp Physiol Psychol 48:254–260

Nagai H, Egashira N, Sano K, Ogata A, Mizuki A, Mishima K, Iwasaki K, Shoyama Y, Nishimura R, Fujiwara M (2006) Antipsychotics improve delta9-tetrahydrocannabinol-induced impairment of the prepulse inhibition of the startle reflex in mice. Pharmacol Biochem Behav 84:330–336

Nyberg JM, Vekovischeva O, Sandnabba NK (2003) Anxiety profiles of mice selectively bred for intermale aggression. Behav Genet 33:503–511

Onaivi ES, Green MR, Martin BR (1990) Pharmacological characterization of cannabinoids in the elevated plus maze. J Pharmacol Exp Ther 253:1002–1009

Onaivi ES, Chakrabarti A, Gwebu ET, Chaudhuri G (1995) Neurobehavioral effects of delta 9-THC and cannabinoid (CB1) receptor gene expression in mice. Behav Brain Res 72:115–125

O'Tuathaigh CM, O'Sullivan GJ, Kinsella A, Harvey RP, Tighe O, Croke DT, Waddington JL (2006) Sexually dimorphic changes in

the exploratory and habituation profiles of heterozygous neuregulin-1 knockout mice. Neuroreport 17:79–83
Pellow S, Chopin P, File SE, Briley M (1985) Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–167
Postma P, Gray JA, Sharma T, Geyer M, Mehrotra R, Das M, Zachariah E, Hines M, Williams SC, Kumari V (2006) A behavioural and functional neuroimaging investigation into the effects of nicotine on sensorimotor gating in healthy subjects and persons with schizophrenia. Psychopharmacology (Berl) 184:589–599
Rodgers RJ (1997) Animal models of 'anxiety': where next? Behav Pharmacol 8:477–496; discussion 497–504
Rodgers RJ, Johnson NJ (1995) Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol Biochem Behav 52:297–303
Rodgers RJ, Shepherd JK (1993) Influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze and light/dark tests of anxiety in mice. Psychopharmacology (Berl) 113:237–242
Rodgers RJ, Lee C, Shepherd JK (1992) Effects of diazepam on behavioural and antinociceptive responses to the elevated plus-maze in male mice depend upon treatment regimen and prior maze experience. Psychopharmacology (Berl) 106:102–110
Rodgers RJ, Johnson NJ, Cole JC, Dewar CV, Kidd GR, Kimpson PH (1996) Plus-maze retest profile in mice: importance of initial stages of trail 1 and response to post-trail cholinergic receptor blockade. Pharmacol Biochem Behav 54:41–50
Rodgers RJ, Evans PM, Murphy A (2005) Anxiogenic profile of AM-251, a selective cannabinoid CB1 receptor antagonist, in plus-maze-naive and plus-maze-experienced mice. Behav Pharmacol 16:405–413
Rutkowska M, Jamontt J, Gliniak H (2006) Effects of cannabinoids on the anxiety-like response in mice. Pharmacol Rep 58:200–206
Schneider M, Koch M (2002) The cannabinoid agonist WIN 55,212-2 reduces sensorimotor gating and recognition memory in rats. Behav Pharmacol 13:29–37
Schneier FR, Siris SG (1987) A review of psychoactive substance use and abuse in schizophrenia. Patterns of drug choice. J Nerv Ment Dis 175:641–652
Schofield D, Tennant C, Nash L, Degenhardt L, Cornish A, Hobbs C, Brennan G (2006) Reasons for cannabis use in psychosis. Aust N Z J Psychiatry 40:570–574
Snodgrass-Belt P, Gilbert JL, Davis FC (2005) Central administration of transforming growth factor-alpha and neuregulin-1 suppress active behaviors and cause weight loss in hamsters. Brain Res 1038:171–182
Stanley-Cary CC, Harris C, Martin-Iverson MT (2002) Differing effects of the cannabinoid agonist, CP 55,940, in an alcohol or Tween 80 solvent, on prepulse inhibition of the acoustic startle reflex in the rat. Behav Pharmacol 13:15–28
Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, Brynjolfsson J, Gunnarsdottir S, Ivarsson O, Chou TT, Hjaltason O, Birgisdottir B, Jonsson H, Gudnadottir VG, Gudmundsdottir E, Bjornsson A, Ingvarsson B, Ingason A, Sigfusson S, Hardardottir H, Harvey RP, Lai D, Zhou M, Brunner D, Mutel V, Gonzalo A, Lemke G, Sainz J, Johannesson G, Andresson T, Gudbjartsson D, Manolescu A, Frigge ML, Gurney ME, Kong A, Gulcher JR, Petursson H, Stefansson K (2002) Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 71:877–892
Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60:1187–1192
Susser ES, Lin SP (1992) Schizophrenia after prenatal exposure to the Dutch Hunger Winter of 1944–1945. Arch Gen Psychiatry 49:983–988
Tang JX, Chen WY, He G, Zhou J, Gu NF, Feng GY, He L (2004) Polymorphisms within 5′ end of the neuregulin 1 gene are genetically associated with schizophrenia in the Chinese population. Mol Psychiatry 9:11–12
Tibbo P, Swainson J, Chue P, LeMelledo JM (2003) Prevalence and relationship to delusions and hallucinations of anxiety disorders in schizophrenia. Depress Anxiety 17:65–72
Townsend MH, Wilson MS (2005) Comorbid anxiety disorders and divalproex sodium use among partial hospital patients with psychotic disorders. Compr Psychiatry 46:368–370
van den Buuse M, Garner B, Gogos A, Kusljic S (2005) Importance of animal models in schizophrenia research. Aust N Z J Psychiatry 39:550–557
van Gaalen MM, Steckler T (2000) Behavioural analysis of four mouse strains in an anxiety test battery. Behav Brain Res 115:95–106
van Os J, Bak M, Hanssen M, Bijl RV, de Graaf R, Verdoux H (2002) Cannabis use and psychosis: a longitudinal population-based study. Am J Epidemiol 156:319–327
Varvel SA, Wiley JL, Yang R, Bridgen DT, Long K, Lichtman AH, Martin BR (2006) Interactions between THC and cannabidiol in mouse models of cannabinoid activity. Psychopharmacology (Berl) 186:226–234
Walss-Bass C, Liu W, Lew DF, Villegas R, Montero P, Dassori A, Leach RJ, Almasy L, Escamilla M, Raventos H (2006) A novel missense mutation in the transmembrane domain of neuregulin 1 is associated with schizophrenia. Biol Psychiatry 60(6):548–553
Wang JH, Short J, Ledent C, Lawrence AJ, van den Buuse M (2003) Reduced startle habituation and prepulse inhibition in mice lacking the adenosine A2A receptor. Behav Brain Res 143:201–207
Wong AH, Van Tol HH (2003) Schizophrenia: from phenomenology to neurobiology. Neurosci Biobehav Rev 27:269–306
Wright IK, Upton N, Marsden CA (1991) Resocialisation of isolation-reared rats does not alter their anxiogenic profile on the elevated X-maze model of anxiety. Physiol Behav 50:1129–1132
Yang JZ, Si TM, Ruan Y, Ling YS, Han YH, Wang XL, Zhou M, Zhang HY, Kong QM, Liu C, Zhang DR, Yu YQ, Liu SZ, Ju GZ, Shu L, Ma DL, Zhang D (2003) Association study of neuregulin 1 gene with schizophrenia. Mol Psychiatry 8:706–709