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HETEROZYGOUS NEUREGULIN 1 MICE DISPLAY GREATER BASELINE AND Δ^9-TETRAHYDROCANNABINOL-INDUCED c-Fos EXPRESSION

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Abstract—Cannabis use may increase the risk of developing schizophrenia by precipitating the disorder in genetically vulnerable individuals. Neuregulin 1 (*NRG1*) is a schizophrenia susceptibility gene and mutant mice heterozygous for the transmembrane domain of this gene (*Nrg1* HET mice) exhibit a schizophrenia-related phenotype. We have recently shown that *Nrg1* HET mice are more sensitive to the behavioral effects of the main psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (THC). In the present study, we examined the effects of THC (10 mg/kg i.p.) on neuronal activity in *Nrg1* HET mice and wild type-like (WT) mice using c-Fos immunohistochemistry. In the lateral septum, THC selectively increased c-Fos expression in *Nrg1* HET mice with no corresponding effect being observed in WT mice. In addition, THC promoted a greater increase in c-Fos expression in *Nrg1* HET mice than WT mice in the central nucleus of the amygdala, the bed nucleus of the stria terminalis and the paraventricular nucleus of the hypothalamus. Consistent with *Nrg1* HET mice exhibiting a schizophrenia-related phenotype, these mice expressed greater drug-free levels of c-Fos in two regions thought to be involved in schizophrenia, the shell of the nucleus accumbens and the lateral septum. Interestingly, the effects of genotype on c-Fos expression, drug-free or following THC exposure, were only observed when animals experienced behavioral testing prior to perfusion. This suggests an interaction with stress was necessary for the promotion of these effects. These data provide neurobiological correlates for the enhanced behavioral sensitivity of *Nrg1* HET mice to THC and reinforce the existence of cannabinoid-neuregulin 1 interactions in the CNS. This research may enhance our understanding of how genetic factors increase individual vulnerability to schizophrenia and cannabis-induced psychosis.

Key words: cannabinoid, c-Fos, schizophrenia, susceptibility gene, transgenic mice.

Schizophrenia is a chronic and disabling mental disorder that affects 1% of the world's population. Drugs of abuse such as cannabis increase the risk of developing schizophrenia (Linszen et al., 1994; Arseneault et al., 2004). Cannabis consumption is associated with an earlier onset of psychotic symptoms, an exacerbation of the disorder, poor treatment outcome and an increased likelihood of psychotic relapse (Degenhardt and Hall, 2002). The observation that many cannabis users do not develop schizophrenia has led to the hypothesis that cannabinoid exposure is a component cause that might unmask schizophrenia in people that have a predisposition to the disorder. Indeed, the cause of schizophrenia is likely to be multifactorial involving the interaction of susceptibility genes with environmental risk factors such as cannabis use (Bayer et al., 1999; Arseneault et al., 2004).

Human and animal studies have reported that neuregulin 1 (*NRG1*), a gene from chromosome 8p, is a candidate gene for schizophrenia (Stefansson et al., 2002; Walss-Bass et al., 2006). Nrg1 is a ligand for ErbB receptor tyrosine kinases which when bound may affect schizophrenia-related neurodevelopmental processes such as the expression and function of CNS neurotransmitter receptors, myelination, axon guidance, neuronal migration and glial differentiation (Falls, 2003). Several studies have shown that mutant mice heterozygous for the transmembrane domain of *Nrg1* (*Nrg1* HET mice) exhibit a schizophrenia-related behavioral phenotype. This includes sensorimotor gating deficits, hyperlocomotion and an increased sensitivity to environmental factors (Stefansson et al., 2002; O'Tuathaigh et al., 2006; Boucher et al., 2007; Karl et al., 2007). In addition, consistent with the neurodevelopmental theory of schizophrenia, these mice display an age-dependent phenotype and a hypofunctional glutamatergic system with a deficit in NMDA receptor expression (Stefansson et al., 2002; Karl et al., 2007). Taken together, *Nrg1* HET mice provide one of the most promising animal models of genetic vulnerability to schizophrenia.

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Abbreviations: ANOVA, analysis of variance; BNST, bed nucleus of the stria terminalis; CEA, central nucleus of the amygdala; CPU, caudate putamen; LH, lateral hypothalamus; LSV, ventrolateral septum; NAC, nucleus accumbens core; NAS, nucleus accumbens shell; *NRG1*, neuregulin 1; *Nrg1* HET, heterozygous neuregulin 1; PAG, periaqueductal gray; PB, phosphate buffer; PBH, phosphate-buffered horse serum; PFA, paraformaldehyde; PFC, prefrontal cortex; PPI, prepulse inhibition; PV, paraventricular nucleus of the thalamus; PVN, paraventricular nucleus of the hypothalamus; THC, Δ^9-tetrahydrocannabinol; VEH, vehicle; VMH, ventromedial hypothalamus; VTA, ventral tegmental area; WT, wild type-like.

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We have recently shown that *Nrg1* HET mice also have increased sensitivity to the acute behavioral effects of the main psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (THC) (Boucher et al., 2007). These mice were more sensitive to the locomotor suppressant and anxiogenic effects of THC than wild type-like (WT) mice. Further, THC selectively facilitated sensorimotor gating as measured by prepulse inhibition (PPI) in *Nrg1* HET mice. This prior study provided the first evidence that heterozygous deletion of a schizophrenia susceptibility gene alters the behavioral effects of cannabinoids. Here we examine the neurobiological underpinnings of such a phenomenon by measuring THC-induced neuronal activation in *Nrg1* HET and WT mice using c-Fos immunohistochemistry.

EXPERIMENTAL PROCEDURES

Animals

Male adult (18–19 weeks) heterozygous *Nrg1* transmembrane domain mice generated from a C57BL/6 background strain were provided by Prof. Richard Harvey (Victor Chang Cardiac Research Institute, Sydney, Australia) as previously described (Boucher et al., 2007; Karl et al., 2007). Animals were pair-housed under a 12 h light/dark schedule with food and water available *ad libitum*. 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." Every effort was made to minimize the number of animals used and their suffering.

Drug and experimental procedures

As previously described by Boucher et al. (2007), THC (Sigma-Aldrich, Sydney, NSW, Australia) was dissolved in 4% ethanol, 1% Tween 80, and 95% saline. THC or vehicle (VEH) was administered intraperitoneally (i.p.) at 10 mg/kg in a volume of 10 ml/kg of body weight. Thirty minutes after the injection, mice were tested in various paradigms of motor coordination (wire hang, pole, beam walk and rotarod tests), explorative (open field and hole-board tests) and anxiety-related behaviors (light–dark test and elevated plus maze). Animals were habituated to these behavioral tests on two consecutive days prior to the administration of THC to limit the impact of novelty-induced c-Fos expression. Data for these results are not reported due to an insufficient number of animals being tested to provide meaningful results. Immediately following behavioral testing and approximately 90 min after injection with either VEH or THC (10 mg/kg), mice were anesthetized with halothane and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde (PFA). To assess the influence of behavioral testing on c-Fos expression, another group of animals was treated with VEH or THC (10 mg/kg) 90 min before the perfusion, however none of these animals were tested in any behavioral paradigms. We then analyzed c-Fos expression using the same protocol and analyzed the regions of interest where a differential effect between *Nrg1* HET and WT mice was observed in animals that were behaviorally tested.

Immunohistochemistry

The brains were removed and postfixed overnight in fresh PFA at 4 °C. For cryoprotection, brains were incubated in 15% sucrose until they sank (approximately 1–2 days). The brains were then placed in 30% sucrose for approximately 3 days, until they sank again. Following this, the brains were placed in the cryostat for slow freezing for 1 h at −11–17 °C and sliced at 40 μm. Tissues were stored in freezing solution (ethanediol/glycerol) at −15–20 °C until the c-Fos staining was performed. For immunohistochemistry, free floating sections were washed in phosphate buffer (PB) before being sequentially incubated in 3% hydrogen peroxide for 30 min and 3% normal horse serum for 30 min. Sections were then stained with the c-Fos primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA, rabbit polyclonal; reacts with c-Fos p62 of mouse and non–cross-reactive with FosB, Fra-1 or Fra-2) diluted 1:10,000 in phosphate-buffered horse serum (PBH) (0.1% bovine serum albumin, 0.2% Triton X-100, 2% normal horse serum) for 3 days at 4 °C. Sections were washed in PB and then incubated for 1 h in biotinylated anti-rabbit IgG secondary antibody (Vector Laboratories, Burlingame, CA, USA) diluted 1:500 in PBH. Sections were washed again before being incubated in ExtrAvidin peroxidase (Sigma, diluted 1:1000 in PBH) for 2 h. After three washes in PB, peroxidase activity was visualized by placing the tissue in nickel diaminobenzidine (0.05% diaminobenzidine tetrahydrochloride, 0.4% ammonium chloride, 2% nickel ammonium sulfate, 20% D-glucose) and glucose oxidase (Sigma, 1:1000) and the reaction was terminated after 10 min by washing in PB. Sections were stored in PB at 4 °C before being mounted onto gelatinized slides, dehydrated, xylene cleared and coverslipped. Fos immunoreactive cells (black and dark brown) were quantified by a rater blind to treatment with reference to the mouse brain atlas of Paxinos and Franklin (2000) as previously described (McGregor et al., 1998; Arnold et al., 2001). Digitized images were produced by a Leica DC500 camera using a 10× objective attached to an Olympus BX51 light microscope. Images were acquired using Leica IM1000 Image Manager (version 4) software. The only post-production enhancement was reduction of color to black and white and the adjustment of brightness and contrast equally for all images using Adobe Photoshop CS2 (version 9.0).

Data analysis

Data for c-Fos immunoreactivity were analyzed using two-way analysis of variance (ANOVA). The two factors were genotype (WT and *Nrg1* HET mice) and treatment (VEH and THC). When a significant two-way ANOVA was found, within group comparisons were made using Tukey's post hoc test (where the factors of genotype or treatment were split by the corresponding factor). These post hoc comparisons are shown in Table 1 and Table 2. To assess the effects of behavioral testing on c-Fos expression, the regions of interest were also analyzed by two-way ANOVA in animals not previously tested in behavioral paradigms. These data were additionally analyzed using a three-way ANOVA where the factors were genotype (WT and *Nrg1* HET mice), treatment (VEH and THC) and behavior (testing or no testing). A significant level of $P<0.05$ was chosen for all comparisons.

RESULTS

VEH-treated *Nrg1* HET express greater c-Fos levels in the LSV and NAS

c-Fos expression in behaviorally tested animals for all reported regions is shown in Table 1. The comparison of baseline genotype differences in behaviorally tested animals showed that VEH-treated *Nrg1* HET mice expressed significantly more c-Fos than VEH-treated WT mice in the ventrolateral septum (LSV) and in the nucleus accumbens shell (NAS) (Fig. 1). This was highlighted by two-way ANOVA with a significant overall effect of genotype in the LSV [$F(1,12)=40.18$, $P<0.01$] and in the NAS [$F(1,12)=8.9$, $P<0.05$]. Consistent with this post hoc analysis using Tukey's test showed an increased c-Fos expression in VEH-treated *Nrg1* HET compared with WT mice in the LSV ($P<0.05$) and

Table 1. Quantification of c-Fos expression in WT and *Nrg1* HET mice after behavioral testing

Region	Bregma	WT		*Nrg1* HET	
		VEH	THC	VEH	THC
Frontal regions					
PFC, medial	+1.98	24.5±7	16±1	23±6	28±6
NAS	+0.98	12±1	12±2	21±3*	22.5±5
NAC	+0.98	1.5±1	1±0.5	1±0.6	1.5±1
CPU, medial	+0.98	8±4	1±1	4±2	12±7
CPU, central	+0.98	1±1	0.5±0.5	1±0.5	1±1
CPU, dorsolateral	+0.98	0±0	1±1	1±0.5	0.25±0.25
LSV	+0.98	39±8	36±9	65±4*	99±6##
BNST, dorsolateral	+0.26	2±0.4	37±13#	2±1	52±7###
Hypothalamus					
PVN	−0.94	19±8	53.5±8#	30±4	69±3###
Lateral	−1.58	8±2	10.5±3	10±3	13±3
Ventromedial	−1.58	0.5±0.3	3.5±2	2.5±1	3±1
Thalamus					
PV	−0.94	27±4	68.5±16#	28±8	70±15#
Amygdala					
CEA	−1.34	1.5±1	39±14#	2±1	48±10##
Hippocampus					
CA1	−1.58	1±0.5	0±0	1±1	0±0
CA3	−1.58	2±1	0.25±0.25	1±1	0.5±0.3
Midbrain					
VTA	−3.28–3.40	6±3	7.5±2	7.5±2	11±1
PAG, ventrolateral	−4.60	10±3	11.5±2	10.5±2	18±5

Number of Fos-labeled cells in various brain regions of behaviorally tested WT and *Nrg1* HET mice following VEH or THC (10 mg/kg) (n=4 per group). Data are presented as means±S.E.M.

* $P<0.05$, significant effects of vehicle-treated *Nrg1* HET compared to WT mice.

# $P<0.05$, significant effects of THC compared to vehicle in *Nrg1* HET and WT mice.

$P<0.01$, significant effects of THC compared to vehicle in *Nrg1* HET and WT mice.

$P<0.001$, significant effects of THC compared to vehicle in *Nrg1* HET and WT mice.

in the NAS ($P<0.05$). A trend for an increased baseline c-Fos expression was also detected in the paraventricular nucleus of the hypothalamus (PVN) [$F(1,12)=4.49$, $P=0.056$].

THC-induced c-Fos expression in *Nrg1* HET and WT mice not tested in behavioral paradigms prior to perfusion is shown in Table 2. Interestingly, VEH-treated *Nrg1* HET mice did not express more c-Fos than VEH-treated WT mice, as shown by two-way ANOVA with no effect of genotype being observed in any region examined including the LSV and the NAS. Moreover, three-way ANOVA comparing the effect of THC on mice that were behaviorally tested or not prior to c-Fos analysis showed a genotype by behavioral testing interaction in the LSV [$F(1,19)=19.02$, $P<0.001$] and a trend in the NAS [$F(1,19)=3.63$, $P=0.07$]. These results highlight that the selective increase in c-Fos in the LSV and NAS of VEH-treated *Nrg1* HET mice was only apparent when animals were behaviorally tested prior to the perfusion.

Nrg1 HET but not WT mice display THC-induced c-Fos expression in the LSV

When examining the effect of THC treatment in behaviorally tested animals we observed that, THC selectively in-

Table 2. Quantification of c-Fos expression in WT and Nrg1 HET mice without prior behavioral testing

Region	Bregma	WT		*Nrg1* HET	
		VEH	THC	VEH	THC
NAS	+0.98	7±3	5±2	4±0	7±7
LSV	+0.98	14±6	40±3#	18.5±6.5	41±4#
BNST, dorsolateral	+0.26	0±0	13±3#	1±1	34±7#
PVN	−0.94	6±2	52±24	7±0	57±20
CEA	−1.34	0±0	28±5##	9±2	40±8

Number of Fos-labeled cells in brain regions of non-behaviorally tested WT and *Nrg1* HET mice following VEH or THC (10 mg/kg) (n=2–3 per group). Data are presented as means±S.E.M.

# $P<0.05$, significant effects of THC compared to vehicle in *Nrg1* HET and WT mice.

$P<0.01$, significant effects of THC compared to vehicle in *Nrg1* HET and WT mice.

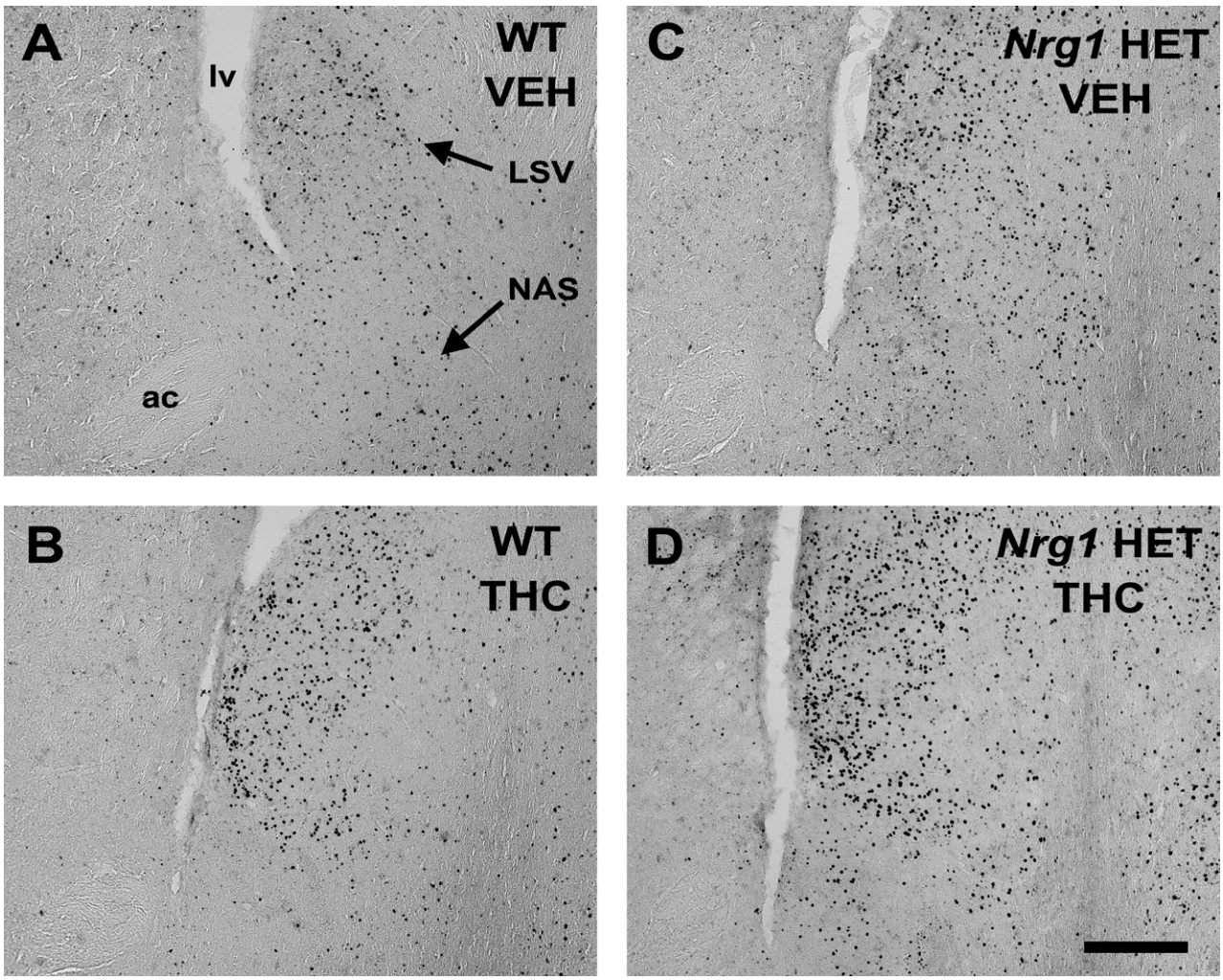

Fig. 1. Fos-labeled neurons within the LSV and the NAS in representative sections from WT mice treated with (A) VEH or (B) THC (10 mg/kg) and *Nrg1* HET mice treated with (C) VEH or (D) THC (10 mg/kg). The lateral ventricle (lv) and anterior commissure (ac) are also indicated. Scale bar=300 μm.

creased c-Fos expression in *Nrg1* HET mice with no corresponding effect being observed in WT mice in the LSV (see Table 1 and Fig. 1). This was highlighted by two-way ANOVA with a strong trend in treatment [$F(1,12)=4.72$, $P=0.05$] and a significant genotype by treatment interaction [$F(1,12)=6.95$, $P<0.05$]. Consistent with this post hoc analysis of the LSV showed THC selectively increased c-Fos expression compared with VEH only in *Nrg1* HET mice ($P<0.01$) but not in WT mice.

Two-way ANOVA of THC-induced c-Fos expression in animals not exposed to behavioral tests prior to the perfusion (see Table 2) revealed no effect of genotype and no genotype by treatment interaction in the LSV. In non-behaviorally tested animals, THC increased c-Fos expression in the LSV as supported by an effect of the treatment [$F(1,7)=26.15$, $P<0.01$]. However, no differential effect was observed as analyzed by Tukey's post hoc test where the THC-induced increase in c-Fos expression was similar for both WT and *Nrg1* HET mice (Ps<0.05 for both groups). Interestingly, when examining the effect of behavior on the results observed in the LSV, three-way ANOVA revealed a genotype by treatment by behavioral testing interaction [$F(1,19)=4.62$, $P<0.05$], highlighting that the increased sensitivity of *Nrg1* HET mice to THC-induced c-Fos in the LSV is dependent on behavioral testing.

The effects of THC on c-Fos expression in other brain regions

In behaviorally tested animals, THC increased c-Fos expression in both *Nrg1* HET and WT mice according to two-way ANOVA with a significant overall effect of treatment in the paraventricular nucleus of the thalamus (PV) [$F(1,12)=12.78$, $P<0.01$], the central nucleus of the amygdala (CEA) [$F(1,12)=24.01$, $P<0.01$], the dorsolateral part of the bed nucleus of the stria terminalis (BNST) [$F(1,12)=33.62$, $P<0.01$] and the PVN [$F(1,12)=34.49$, $P<0.01$] (see Table 1). Although no genotype by treatment interactions were observed in these regions, post hoc analysis revealed that THC exerted a greater magnitude of effect on

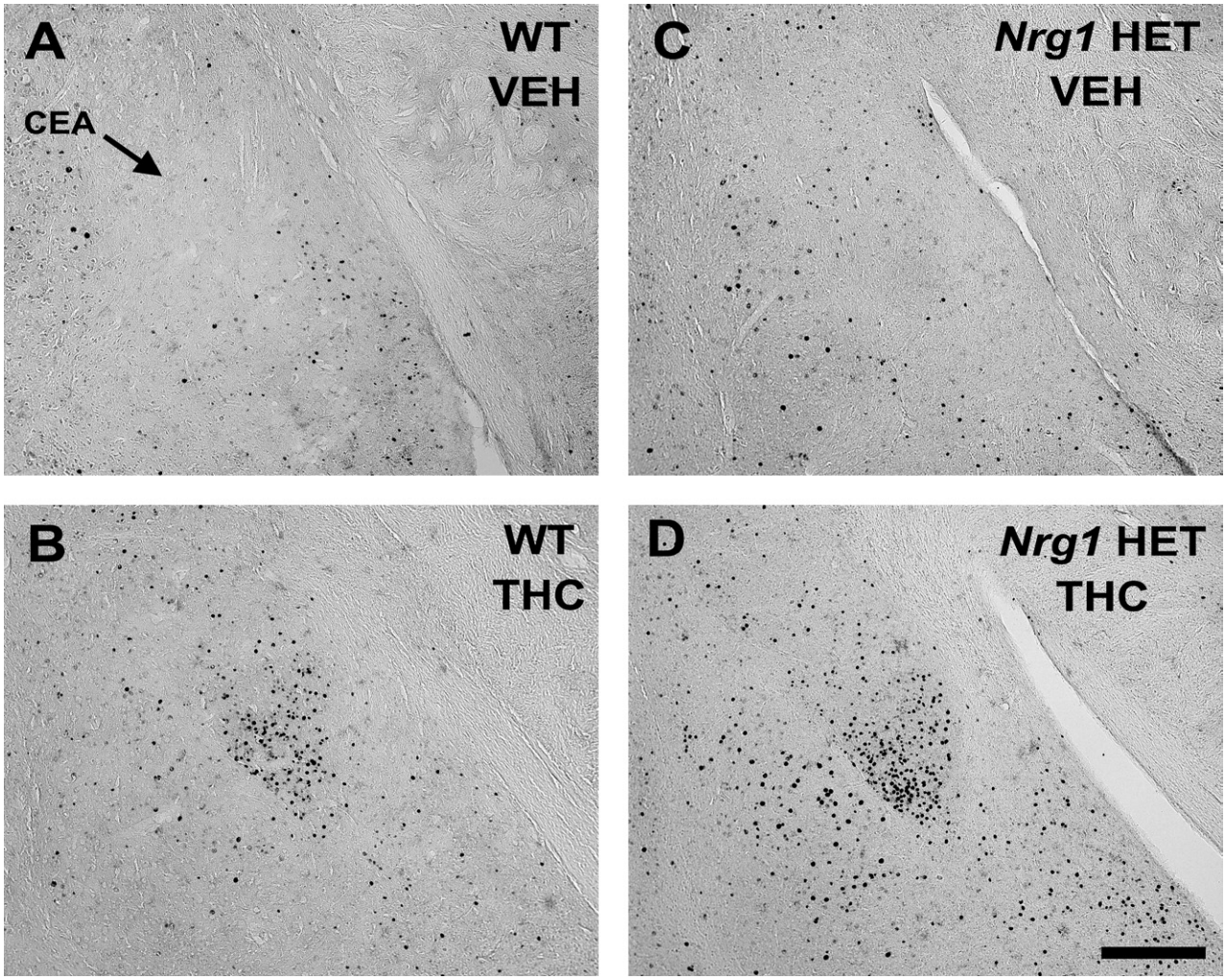

Fig. 2. Fos-labeled neurons within the CEA in representative sections from WT mice treated with (A) VEH or (B) THC (10 mg/kg) and *Nrg1* HET mice treated with (C) VEH or (D) THC (10 mg/kg). Scale bar=300 μm.

the *Nrg1* HET mice in the CEA (Fig. 2), dorsolateral BNST (Fig. 3) and PVN (Fig. 4) ($P<0.01$, $P<0.001$ and $P<0.001$ respectively) compared with WT mice (Ps<0.05).

Interestingly, in animals not behaviorally tested this increased magnitude of effect of THC on *Nrg1* HET compared with WT mice in these regions was not observed. According to two-way ANOVA, THC increased c-Fos expression as supported by an effect of the treatment in the CEA [$F(1,7)=28.39$, $P<0.01$], BNST [$F(1,7)=23.54$, $P<0.01$] and PVN [$F(1,7)=7.41$, $P<0.05$]. However, Tukey's post hoc test showed that in animals that have not been behaviorally tested, a greater magnitude of effect of THC in *Nrg1* HET compared with WT mice was not observed in any region (see Table 2). These results show that the increased magnitude of effects of THC on c-Fos expression in the CEA, BNST and PVN is also dependent on behavioral testing.

Other brain regions were also analyzed for c-Fos expression in behaviorally tested animals (Table 1), with THC having no effect on either *Nrg1* HET or WT mice in the medial prefrontal cortex (PFC), the NAS, the core of the nucleus accumbens (NAC), the caudate putamen (CPU), the lateral and ventromedial hypothalamus (LH and VMH, respectively), the CA1 and CA3 subregions of the hippocampus, the ventral tegmental area (VTA) and the ventrolateral periaqueductal gray (PAG).

DISCUSSION

The main finding of the present study is that THC selectively induced c-Fos in the LSV of *Nrg1* HET mice with no such effect being observed in WT mice. Interestingly, this differential effect of genotype was dependent upon behavioral experimentation as this effect was not observed when animals were exposed to THC and left in their home cage prior to perfusion. Consonant with prior research THC increased c-Fos levels according to the classic expression pattern for cannabinoids. That is, THC increased c-Fos-labeled neurons in the PV, CEA, dorsolateral BNST and PVN in both genotypes. Interestingly, the magnitude of effect of THC-induced c-Fos expression was greater in *Nrg1* HET than WT mice in the CEA, dorsolateral BNST

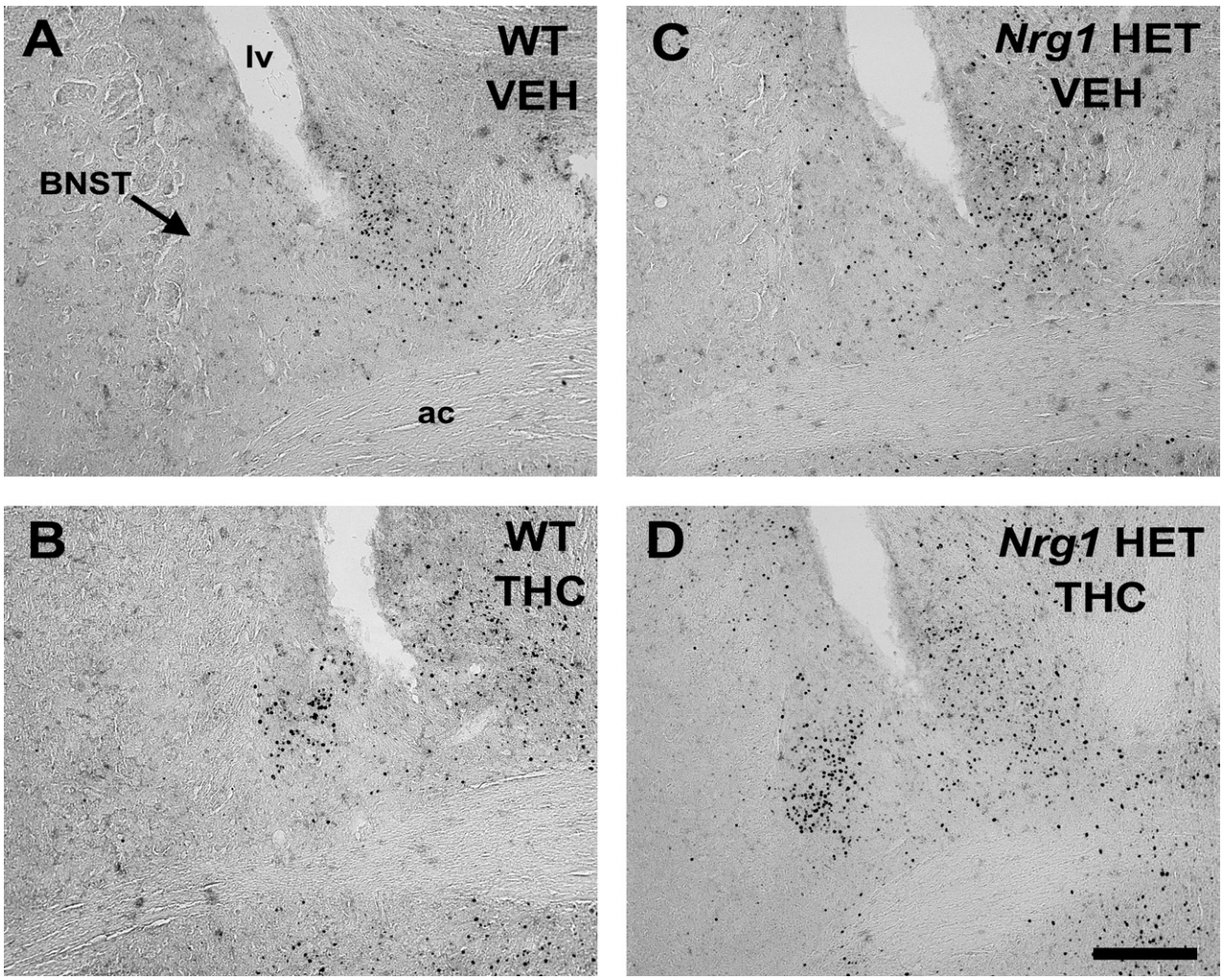

Fig. 3. Fos-labeled neurons within the dorsolateral part of the BNST in representative sections from WT mice treated with (A) VEH or (B) THC (10 mg/kg) and *Nrg1* HET mice treated with (C) VEH or (D) THC (10 mg/kg). The lateral ventricle (lv) and anterior commissure (ac) are also indicated. Scale bar=300 μm.

and PVN only in behaviorally tested animals. Supporting the notion that *Nrg1* HET mice display a schizophrenia-related phenotype, these mice showed augmented baseline c-Fos expression in both the NAS and the LSV compared with WT mice, although, once again, this effect was reliant on behavioral assessment with no genotype effect being observed in these regions in testing naïve animals.

Here we demonstrate for the first time that behaviorally tested *Nrg1* HET mice have higher baseline c-Fos expression in the NAS and LSV. As the lateral septum projects to the NAS (Risold and Swanson, 1997; Sheehan et al., 2004), enhanced c-Fos expression in these regions may indicate an abnormal LSV–NAS pathway in *Nrg1* HET mice. Given that both these areas are implicated in the etiology of schizophrenia, the current results provide further support for the notion that *Nrg1* HET mice display a schizophrenia-related phenotype (Stefansson et al., 2002; Boucher et al., 2007; Karl et al., 2007). Human studies show that excessive dopamine release in schizophrenia patients subserves the positive symptoms of the disorder and explains the effectiveness of typical antipsychotic agents that antagonize dopamine receptors (Carlsson, 1988; Egan and Weinberger, 1997). Augmented dopamine release is likely responsible for the increased c-Fos levels observed in the NAS of *Nrg1* HET mice as this region predominantly releases this neurotransmitter. This also offers a parsimonious explanation for the marked motor and exploratory hyperactivity exhibited by *Nrg1* HET mice (Stefansson et al., 2002; Boucher et al., 2007; Karl et al., 2007).

Increased c-Fos expression in the LSV and NAS of *Nrg1* HET mice compared with WT mice in behaviorally tested animals may also provide a neuronal correlate for PPI deficits observed in *Nrg1* HET mice first reported by Stefansson et al. (2002). While we did not initially repeat this finding (Boucher et al., 2007) we have recently replicated such an effect using a modified PPI design (unpublished observations). Schizophrenia patients display deficits in PPI consistent with attentional dysfunction being a symptom of the disorder. Importantly, a polymorphism in

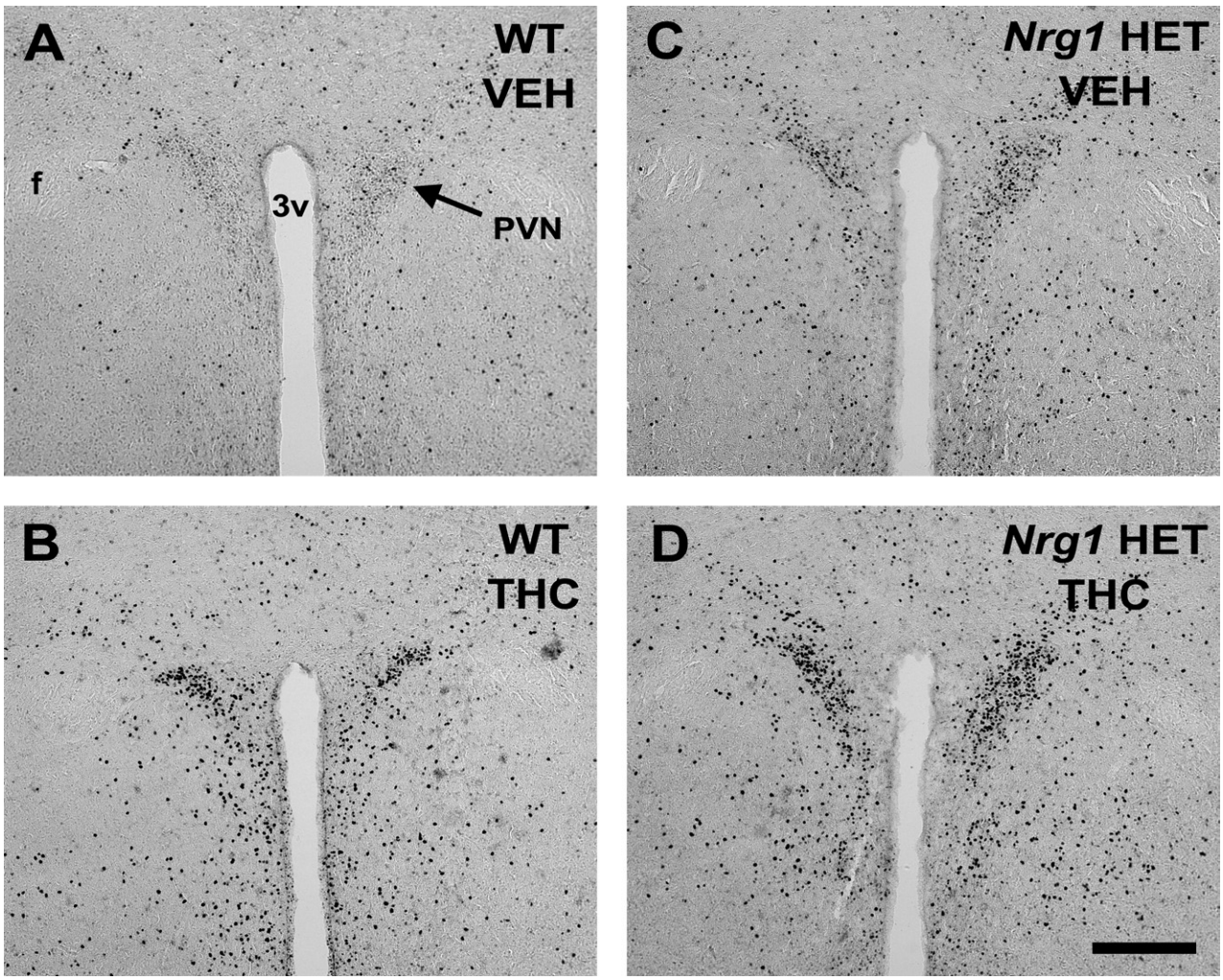

Fig. 4. Fos-labeled neurons within the PVN in representative sections from WT mice treated with (A) VEH or (B) THC (10 mg/kg) and *Nrg1* HET mice treated with (C) VEH or (D) THC (10 mg/kg). The third ventricle (3v) and the fornix (f) are also indicated. Scale bar=300 μm.

the *NRG1* gene was recently associated with PPI deficits in both healthy and schizophrenic patients (Hong et al., 2007). Further, elevated dopamine levels in the NAS are well established in mediating PPI deficits and the septum also appears to mediate sensory gating phenomena (Koch, 1996; van Luijtelaar et al., 2001). Given that the LSV and NAS are responsive to stress (Abercrombie et al., 1989; Sheehan et al., 2004), it is possible that reduced Nrg1 levels interact with the stress of behavioral testing promoting overactivity in these brain sites which might subserve the PPI deficits observed in *Nrg1* HET mice.

THC administration strongly increased c-Fos expression in the PV, CEA, dorsolateral BNST and PVN. These results are consistent with studies examining the effects of cannabinoids on c-Fos expression in both mice (Valjent et al., 2002) and rats (McGregor et al., 1998; Arnold et al., 2001). However, no effect of THC on c-Fos expression was observed in the PFC, NAS, NAC, CPU, LH, VMH, CA1, CA3, VTA and PAG. Unlike our study, Valjent et al. (2002) demonstrated THC-induced c-Fos expression in the NAS, NAC, CPU and VMH, while Derkinderen et al. (2003) reported that THC promoted c-Fos in the CA1 and CA3. Strain differences in the effectiveness of cannabinoids might explain why we did not replicate these findings in our study as we used a distinct strain to that used by Valjent et al. (2002) and Derkinderen et al. (2003) who both used CD-1 mice. Here the C57BL/6 background strain was utilized which has been previously shown to be subsensitive to the actions of cannabinoids (Onaivi et al., 1995). Genetic variation in the responsiveness of animals to cannabinoids is well-documented, for example, Arnold et al. (2001) demonstrated that Lewis rats display less cannabinoid-induced c-Fos compared with Wistar rats.

THC-induced c-Fos expression in the PV, CEA, dorsolateral BNST and PVN is likely to reflect cannabinoid action on an integrated circuit that mediates emotional, endocrine and behavioral responses to sensory information. The PV sends highly processed sensory information to the BNST and CEA (Moga et al., 1995). The BNST appears to be a rostral extension of the CEA and these areas share reciprocal connections consistent with their intimate role in mediating anxiety-related behavior (Swanson and Petrovich,

1998). Further, the CEA indirectly connects with the PVN via a relay through the BNST (Prewitt and Herman, 1998; Dong et al., 2001). Thus, THC-induced activation of a PV–CEA–BNST–PVN "stress circuit" likely subserves the well-characterized effects of cannabinoids on anxiety-related behavior and the hypothalamo-pituitary–adrenal (HPA) axis (Onaivi et al., 1995; Viveros et al., 2005; Boucher et al., 2007).

The observation that heterozygous deletion of the *Nrg1* gene renders animals more sensitive to the effects of THC in the LSV provides a neurobiological correlate for our recent data showing *Nrg1* HET mice are more sensitive to the behavioral effects of THC. Boucher et al. (2007) showed THC facilitated PPI in *Nrg1* HET but not in WT mice. This is interesting as drugs which modulate PPI in animal studies, whether they be pro-psychotic agents that impair PPI (e.g. amphetamine, phencyclidine) (Dulawa and Geyer, 1996), or anti-psychotic agents that facilitate PPI (e.g. haloperidol, clozapine) (Ouagazzal et al., 2001) all increase the expression of c-Fos in the lateral septum (Sumner et al., 2004). The lateral septum is also likely involved in the "stress circuit" outlined above sharing reciprocal connections with the PV, BNST, CEA and the PVN (Moga et al., 1995; Risold and Swanson, 1997; Sheehan et al., 2004). Further, the LSV is thought to mediate stress- and anxiety-related behavior (Dielenberg et al., 2001; Sheehan et al., 2004). Therefore, the selective effect of THC on c-Fos expression in the LSV of *Nrg1* HET mice, combined with the observation that THC exerted an increased magnitude of effect on c-Fos expression in the CEA, BNST and PVN only in behaviorally tested *Nrg1* HET mice suggests that depletion of *Nrg1* interacts with the stress of experimentation to enhance the sensitivity of an LSV–CEA–BNST–PVN circuit to the actions of THC. Such a circuit might also underlie the enhanced behavioral effects of THC on *Nrg1* HET mice including increased THC-induced anxiety-related behavior as measured in the light–dark emergence test (Boucher et al., 2007).

The enhanced sensitivity of *Nrg1* HET mice to THC-induced c-Fos expression was only observed in animals exposed to behavioral testing. Behavioral testing involves manipulations likely to stress animals such as handling and removal from the home cage. Indeed, it has been shown that rats exposed to a battery of behavioral tests exhibit higher levels of the stress hormone corticosterone compared with rats with no prior behavioral experience (Uphouse et al., 1983). Interestingly in our study, the brain regions where THC exerted greater effects upon *Nrg1* HET mice are stress-related, i.e. the LSV, CEA, BNST and PVN. The interaction between neuregulins, stress and cannabinoids is interesting given studies highlighting that such neurochemical systems independently impact upon stress systems. For example, a recent human study showed a single nucleotide polymorphism in *NRG1* interacts with environmental stress in the form of job strain to enhance the development of atherosclerosis (Hintsanen et al., 2007). Further, THC exposure increases corticosterone levels (Weidenfeld et al., 1994) and deletion of CB1 receptors decreases basal release of this hormone (Uriguen et al., 2004). Furthermore, a synergistic interaction occurs between cannabinoids and stress exposure on c-Fos expression in the CEA (Patel et al., 2005). This is consistent with human evidence reporting that the stress of mild oral surgery precipitates adverse emotional reactions in cannabis-intoxicated patients (Gregg et al., 1976).

The robust increased sensitivity of behaviorally tested *Nrg1* HET mice to THC induced c-Fos expression further supports the view that interactions occur between neuregulin and cannabinoid systems. Such an interaction may occur in this region as neuregulins, ErbB3, ErbB4 and CB1 receptors are localized in the septum (Pinkas-Kramarski et al., 1994, 1997; Tsou et al., 1998; Steiner et al., 1999). The cellular and molecular explanation for such an interaction in the CNS is unknown. Cancer research has shown that crosstalk exists between G protein–coupled receptors and ErbB receptors mediated by two known mechanisms: 1) G protein receptor–promoted metalloproteinase cleavage of membrane-tethered ErbB ligands or 2) G protein receptor activation of Src family kinases and phosphorylation of ErbB receptors (Yarden and Sliwkowski, 2001). Interestingly, cannabinoids have been shown to activate metalloproteinases (Rosch et al., 2006) and Src (Berghuis et al., 2005; He et al., 2005). Furthermore, cannabinoids induce cancer cell proliferation by metalloproteinase-mediated transactivation of the ErbB1 receptor (Hart et al., 2004).

CONCLUSION

Here we present neurobiological evidence that behaviorally tested *Nrg1* HET mice are more sensitive to the effects of THC. This was most robustly observed in the LSV where THC promoted c-Fos expression selectively in *Nrg1* HET mice with no corresponding effect being observed in WT mice. This further supports our prior behavioral research showing that interactions occur between cannabinoid and neuregulin systems. Consistent with *Nrg1* HET mice exhibiting a schizophrenia-related phenotype, these animals displayed greater baseline c-Fos expression in two regions implicated in the etiology of schizophrenia, the NAS and the LSV. The effects of genotype on c-Fos expression at baseline or following THC exposure were only observed when animals experienced behavioral testing prior to perfusion. This suggests an interaction with stress was necessary in the promotion of these effects. Taken together, these data demonstrate that heterozygous deletion of *Nrg1*—a schizophrenia susceptibility gene—alters the sensitivity of animals to the neurobehavioral effects of the main psychoactive constituent of cannabis, THC under conditions of stress. This research enhances our understanding of how genetic factors may increase an individual's vulnerability to schizophrenia and cannabis-induced psychosis.

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REFERENCES

Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ (1989) Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J Neurochem 52:1655–1658.

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.

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.

Berghuis P, Dobszay MB, Wang X, Spano S, Ledda F, Sousa KM, Schulte G, Ernfors P, Mackie K, Paratcha G, Hurd YL, Harkany T (2005) Endocannabinoids regulate interneuron migration and morphogenesis by transactivating the TrkB receptor. Proc Natl Acad Sci U S A 102:19115–19120.

Boucher AA, Arnold JC, Duffy L, Schofield PR, Micheau J, Karl T (2007) Heterozygous neuregulin 1 mice are more sensitive to the behavioural effects of delta(9)-tetrahydrocannabinol. Psychopharmacology (Berl) 192:325–336.

Carlsson A (1988) The current status of the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1:179–186.

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

Derkinderen P, Valjent E, Toutant M, Corvol JC, Enslen H, Ledent C, Trzaskos J, Caboche J, Girault JA (2003) Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. J Neurosci 23:2371–2382.

Dielenberg RA, Hunt GE, McGregor IS (2001) "When a rat smells a cat": the distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. Neuroscience 104:1085–1097.

Dong HW, Petrovich GD, Swanson LW (2001) Topography of projections from amygdala to bed nuclei of the stria terminalis. Brain Res Brain Res Rev 38:192–246.

Dulawa SC, Geyer MA (1996) Psychopharmacology of prepulse inhibition in mice. Chin J Physiol 39:139–146.

Egan MF, Weinberger DR (1997) Neurobiology of schizophrenia. Curr Opin Neurobiol 7:701–707.

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

Gregg JM, Small EW, Moore R, Raft D, Toomey TC (1976) Emotional response to intravenous delta9tetrahydrocannabinol during oral surgery. J Oral Surg 34:301–313.

Hart S, Fischer OM, Ullrich A (2004) Cannabinoids induce cancer cell proliferation via tumor necrosis factor alpha-converting enzyme (TACE/ADAM17)-mediated transactivation of the epidermal growth factor receptor. Cancer Res 64:1943–1950.

He JC, Gomes I, Nguyen T, Jayaram G, Ram PT, Devi LA, Iyengar R (2005) The G alpha(o/i)-coupled cannabinoid receptor-mediated neurite outgrowth involves Rap regulation of Src and Stat3. J Biol Chem 280:33426–33434.

Hintsanen M, Elovainio M, Puttonen S, Kivimaki M, Raitakari OT, Lehtimaki T, Rontu R, Juonala M, Kahonen M, Viikari J, Keltikangas-Jarvinen L (2007) Neuregulin-1 genotype moderates the association between job strain and early atherosclerosis in young men. Ann Behav Med 33:148–155.

Hong LE, Wonodi I, Stine OC, Mitchell BD, Thaker GK (2007) Evidence of missense mutations on the neuregulin 1 gene affecting function of prepulse inhibition. Biol Psychiatry, in press.

Karl T, Duffy L, Scimone A, Harvey RP, Schofield PR (2007) Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. Genes Brain Behav, in press.

Koch M (1996) The septohippocampal system is involved in prepulse inhibition of the acoustic startle response in rats. Behav Neurosci 110:468–477.

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

McGregor IS, Arnold JC, Weber MF, Topple AN, Hunt GE (1998) A comparison of delta 9-THC and anandamide induced c-fos expression in the rat forebrain. Brain Res 802:19–26.

Moga MM, Weis RP, Moore RY (1995) Efferent projections of the paraventricular thalamic nucleus in the rat. J Comp Neurol 359: 221–238.

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.

Ouagazzal AM, Jenck F, Moreau JL (2001) Drug-induced potentiation of prepulse inhibition of acoustic startle reflex in mice: a model for detecting antipsychotic activity? Psychopharmacology (Berl) 156: 273–283.

Patel S, Cravatt BF, Hillard CJ (2005) Synergistic interactions between cannabinoids and environmental stress in the activation of the central amygdala. Neuropsychopharmacology 30:497–507.

Paxinos G, Franklin KBJ (2000) The mouse brain in stereotaxic coordinates, 2nd edition X. Burlington, MA: Academic Press.

Pinkas-Kramarski R, Eilam R, Alroy I, Levkowitz G, Lonai P, Yarden Y (1997) Differential expression of NDF/neuregulin receptors ErbB-3 and ErbB-4 and involvement in inhibition of neuronal differentiation. Oncogene 15:2803–2815.

Pinkas-Kramarski R, Eilam R, Spiegler O, Lavi S, Liu N, Chang D, Wen D, Schwartz M, Yarden Y (1994) Brain neurons and glial cells express Neu differentiation factor/heregulin: a survival factor for astrocytes. Proc Natl Acad Sci U S A 91:9387–9391.

Prewitt CM, Herman JP (1998) Anatomical interactions between the central amygdaloid nucleus and the hypothalamic paraventricular nucleus of the rat: a dual tract-tracing analysis. J Chem Neuroanat 15:173–185.

Risold PY, Swanson LW (1997) Connections of the rat lateral septal complex. Brain Res Brain Res Rev 24:115–195.

Rosch S, Ramer R, Brune K, Hinz B (2006) R(+)-methanandamide and other cannabinoids induce the expression of cyclooxygenase-2 and matrix metalloproteinases in human nonpigmented ciliary epithelial cells. J Pharmacol Exp Ther 316:1219–1228.

Sheehan TP, Chambers RA, Russell DS (2004) Regulation of affect by the lateral septum: implications for neuropsychiatry. Brain Res Brain Res Rev 46:71–117.

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.

Steiner H, Blum M, Kitai ST, Fedi P (1999) Differential expression of ErbB3 and ErbB4 neuregulin receptors in dopamine neurons and forebrain areas of the adult rat. Exp Neurol 159:494–503.

Sumner BE, Cruise LA, Slattery DA, Hill DR, Shahid M, Henry B (2004) Testing the validity of c-fos expression profiling to aid the therapeutic classification of psychoactive drugs. Psychopharmacology (Berl) 171:306–321.

Swanson LW, Petrovich GD (1998) What is the amygdala? Trends Neurosci 21:323–331.

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83:393–411.

Uphouse L, Tilson H, Mitchell CL (1983) Long-term effects of behavioral testing on serum hormones and brain weight. Life Sci 33: 1395–1400.

Uriguen L, Perez-Rial S, Ledent C, Palomo T, Manzanares J (2004) Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. Neuropharmacology 46:966–973.

Valjent E, Mitchell JM, Besson MJ, Caboche J, Maldonado R (2002) Behavioural and biochemical evidence for interactions between delta 9-tetrahydrocannabinol and nicotine. Br J Pharmacol 135: 564–578.

van Luijtelaar G, Fabene PF, de Bruin N, Jongema C, Ellenbroek BA, Veening JG (2001) Neural correlates of sensory gating in the rat: decreased Fos induction in the lateral septum. Brain Res Bull 54:145–151.

Viveros MP, Marco EM, File SE (2005) Endocannabinoid system and stress and anxiety responses. Pharmacol Biochem Behav 81:331–342.

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: 548–553.

Weidenfeld J, Feldman S, Mechoulam R (1994) Effect of the brain constituent anandamide, a cannabinoid receptor agonist, on the hypothalamo-pituitary-adrenal axis in the rat. Neuroendocrinology 59:110–112.

Yarden Y, Sliwkowski MX (2001) Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2:127–137.

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