Phenotypic effects of repeated psychosocial stress during adolescence in mice mutant for the schizophrenia risk gene neuregulin-1: a putative model of gene × environment interaction

Abbreviated running title: Adolescent stress in neuregulin-1 mutant mice

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Abstract

There is a paucity of animal models by which the contributions of environmental and genetic factors to the pathobiology of psychosis can be investigated. This study examined the individual and combined effects of chronic social stress during adolescence and deletion of the schizophrenia risk gene neuregulin-1 (NRG1) on adult mouse phenotype. Mice were exposed to repeated social defeat stress during adolescence and assessed for exploratory behaviour, working memory, sucrose preference, social behaviour and prepulse inhibition in adulthood. Thereafter, *in vitro* cytokine responses to mitogen stimulation and corticosterone inhibition were assayed in spleen cells, with measurement of cytokine and brain-derived neurotrophic factor (BDNF) mRNA in frontal cortex, hippocampus and striatum. NRG1 mutants exhibited hyperactivity, decreased anxiety, impaired sensorimotor gating and reduced preference for social novelty. The effects of stress on exploratory/anxiety-related parameters, spatial working memory, sucrose preference and basal cytokine levels were modified by NRG1 deletion. Stress also exerted varied effect on spleen cytokine response to concanavalin A and brain cytokine and BDNF mRNA expression in NRG1 mutants. The experience of psychosocial stress during adolescence may trigger further pathobiological features that contribute to the development of schizophrenia, particularly in those with underlying *NRG1* gene abnormalities. This model elaborates the importance of gene × environment interactions in the etiology of schizophrenia.

Key words: Social defeat stress; neuregulin 1; behavioral phenotype; working memory; prepulse inhibition; cytokines; concanavalin A; lipopolysaccharide; gene × environment interaction; schizophrenia

1. Introduction

The pathobiology of schizophrenia is fraught with complexity, hence the development of valid animal models for this disorder presents several challenges. For example, while molecular genetic studies now implicate a number of risk genes (Allen et al., 2008; Gill et al., 2010; Owen et al., 2010), these findings are complemented by a new generation of studies that also implicate specific environmental risk factors (Kelly et al., 2010; Kirkbride et al., 2010; van Os et al., 2010). Therefore, much contemporary theorising focuses on putative gene × environment (G × E) interactions in the development of schizophrenia (van Os et al., 2008; van Winkel et al., 2010; Waddington et al., 2012) and on the potential of studies in mutant mice to inform on these processes (Ayhan et al., 2009; O'Tuathaigh et al., 2011a).

Among such environmental adversities, numerous studies now indicate psychosocial stressors in particular to increase risk for psychosis, especially as a consequence of cumulative exposure (van Winkel et al., 2008; Tessner et al., 2011). Within the domain of psychosocial stressors, social defeat refers to the sense of subordination experienced following an adverse social encounter and has been proposed as a key process linking social adversity with increased risk for schizophrenia (Selten and Cantor-Graae, 2005, 2007). One possible process by which chronic psychosocial stress contributes to the development of schizophrenia is *via* sensitisation of the pro-inflammatory immune response leading to excessive pro-inflammatory cytokine release, a feature which is hypothesised to contribute to the pathophysiology of many psychiatric disorders (Maes, 1994; Fan et al., 2007; Potvin et al., 2008; Watanabe et al., 2010). Indeed, exposure to chronic social defeat is reported to consistently increase pro-inflammatory cytokine responses in mice (Merlot et al., 2004; Powell et al., 2009; Audet et al., 2011). Due to the diversity of

individual responses to environmental stressors, it is thought that the impact of adversities such as social defeat on adult phenotype is dependent not only on the timing and quality of these stressors (Audet et al., 2011; Jones and Fernyhough, 2007, Jacobson-Pick et al., 2011) but also on the existence of underlying genetic vulnerability (van Winkel et al., 2008; Waddington et al., 2012).

Neuregulin-1 (NRG1) regulates various neurodevelopmental processes, including neuronal migration, myelination, synaptic plasticity and neurotransmitter function (Mei and Xiong, 2008). NRG1 is a replicated risk gene for schizophrenia (Bertram, 2008; Gill et al., 2010; Owen et al., 2010) and it has been reported that schizophrenia patients having a NRG1 single nucleotide polymorphism (SNP) express more unusual thoughts during conflict-related interactions, suggesting a potential G × E interaction in relation to NRG1 and psychosocial stress (Keri et al., 2009). Mice with heterozygous deletion of the NRG1 transmembrane (TM)-domain exhibit a schizophrenia-relevant phenotype (Desbonnet et al., 2009; Kirby et al., 2010; O'Tuathaigh et al., 2010a; van den Buuse, 2010), with mild stress in NRG1 mutants paralleled by heightened expression of c-Fos, a marker of neuronal activation, in brain areas relevant to schizophrenia (Boucher et al., 2007); this complements the clinical association between NRG1 risk SNP and vulnerability to psychosocial stress. Furthermore, recent investigations indicate NRG1 mutant rats to show disrupted stress regulation and neuroendocrine reactivity (Taylor et al., 2011).

As the diagnostic symptoms of schizophrenia do not manifest typically until late adolescence or early adulthood, this developmental window may be particularly relevant to disease pathobiology. In this context, we aimed to determine if NRG1 mutation influences long-term effects of chronic psychosocial stress on pro-inflammatory cytokine responses in the brain and other systems (Merlot et al., 2004; Powell et al., 2009; Audet et al., 2011) and whether these

immune effects, in turn, alter kynurenine pathway activity producing excessive kynurenic acid which is known to modulate neurotransmitters involved in cognitive function and schizophrenia (Wonodi and Schwarcz, 2010; Müller et al., 2011).

To clarify aspects of G × E interaction relevant to schizophrenia, this study examines the effects in adulthood of repeated, intermittent social defeat stress (hereafter, chronic social defeat; CSD) during adolescence in NRG1 mutant mice. Phenotypically, it focuses on schizophrenia-related behaviours and pro-inflammatory cytokines in periphery and brain.

2. Methods

2.1 Animals and housing

Heterozygous NRG1 TM-domain mutant mice, generated at the Victor Chang Cardiac Research Institute, University of New South Wales, Darlinghurst, Australia, were bred and genotyped as described previously (Stefansson et al., 2002; O'Tuathaigh et al., 2006). At weaning [postnatal day (P) 21], heterozygous (HET) male NRG1 mutants and wildtype (WT) mice were housed in groups of 3–5 per cage and then housed individually from P30 onwards. Cages were maintained on a standard 12:12 h light:dark cycle [08:00 on; 20:00 off] with *ad libitum* access to food and water. These studies were approved by the Research Ethics Committee of the Royal College of Surgeons in Ireland. They were conducted under licence from the Department of Health and Children in accordance with Irish legislation and the European Communities Council Directive 86/609/EEC for the care and use of experimental

animals, and from the Environmental Protection Agency in relation to the contained use of genetically modified organisms.

2.2 Experimental design

Experiment 1: At P30 mice were allocated to one of four different groups: non-CSD WT (n=10); non-CSD NRG1 HET (n=10); CSD WT (n=10); CSD NRG1 HET (n=10). In adulthood (P60-90), mice were subjected to behavioural assessment: novel open field; spontaneous alternation; sucrose preference; sociability and social novelty preference; social interaction. Thereafter, mice were sacrificed and excised tissue taken for immunological [spleen cytokines and their responsivity to lipopolysaccharide (LPS), conconavalin A (ConA) and corticosterone; brain cytokine mRNA] and neurochemical [cytokine and brain-derived neurotrophic factor (BDNF) mRNA] analyses.

Experiment 2: To minimise disruption associated with excessive repeated behavioural testing (Boucher et al., 2007), separate groups of mice were assessed for prepulse inhibition (PPI) over the same age range: non-CSD WT (n=6); non-CSD NRG1 HET (n=8); CSD WT (n=6); CSD NRG1 HET (n=9).

2.3. Chronic social defeat

The CSD paradigm was based on previously published procedures (Avgustinovich et al., 2005, Berton et al., 2006, Krishnan et al., 2007). Briefly, aggressor CD1 mice (Harlan, UK) were housed singly for a minimum of 3 days in conflict boxes (30 × 30 × 35 cm) to ensure the

establishment of individual territories. Test mice (WT/NRG1 HET) were also housed singly on P30 to reduce group effects and the formation of social structures; this may constitute a short-term, background stressor in all mice, on which CSD is or is not subsequently imposed, and may thus more accurately model 'real world' circumstances in which psychosis emerges. On P35, each test mouse was placed individually into a conflict box and allowed to interact with the aggressive resident CD1 mouse until defeat occurred, subject to a maximum interaction of 10 min; defeat was defined as the mouse standing on its hind legs with limp forepaws, upward angled head and retracted ears (Miczek et al., 1982). Following defeat, a transparent, perforated partition was inserted between the aggressive resident CD1 mouse and the defeated intruder for a 'threat' period of 24 h. In the absence of defeat over 10 min, a new aggressive CD1 resident was used. CSD was repeated for 10 days. On each day, agonistic interactions were performed at the same time (12.00-13.00); to prevent habituation of aggression/threat, defeated test mice were paired with different aggressors on successive days. Control mice were placed into a novel cage for 5 min at the same time for 10 consecutive days (P35-45).

2.4 Behavioural assessments

2.4.1 Novel open field

Exploratory activity in a novel environment was assessed by placing each mouse in the center of an open arena [white perspex sides and base: 30 × 30 × 20 cm] for 10 min. Distance moved and velocity of movement were recorded using Ethovision videotracking (Ethovision®, Noldus, Wageningen, The Netherlands). Using this technology, a central zone and four corner zones were demarcated; time spent in, and frequency of entry into, each zone was measured.

2.4.2 Spontaneous alternation

Spatial working memory was assessed using the continuous variant of the spontaneous alternation procedure, as described previously (O'Tuathaigh et al., 2007). Briefly, mice were placed individually in the center of the Y-maze, consisting of three identical arms (40 × 12.5 × 40 cm), and allowed to explore freely for 10 min. A video camera, mounted centrally above the maze, recorded each session and allowed alternation, distance moved and velocity of movement to be analyzed using Ethovision videotracking (Ethovision®, Noldus, Wageningen, The Netherlands). Spontaneous alternation was defined as successive entries into the three arms, in overlapping triplet sets, with arm choices differing from the previous two choices expressed as a percentage of the total number of arm entries: percent alternation = [number of alternations/(total number of arm entries-2)] × 100.

2.4.3 Sucrose preference

Anhedonia was assessed using the two-bottle free choice form of the sucrose preference procedure, as described previously (Krishnan et al., 2007). Before testing, individually housed mice were first habituated to sucrose by adding a 1% solution to each of two drinking bottles for 24 h. The following day, at PD50, mice were presented with a choice between a bottle containing water and one containing 1% sucrose. Mean sucrose preference [sucrose intake/total intake × 100] was calculated for each mouse.

2.4.4 Sociability and social novelty preference

Sociability and preference for social novelty were assessed in a three-chamber apparatus, as described previously (Nadler et al., 2004; O'Tuathaigh et al., 2007). Briefly, mice were placed in a rectangular apparatus (36 × 20 × 20 cm) divided into three chambers (left and right chambers 13.5 × 20 × 20 cm; center chamber 9 × 20 × 20 cm) by transparent partitions with small circular openings allowing easy access to all compartments. The test was composed of three sequential 10 min trials: 1) habituation (the test mouse was allowed to explore the three empty chambers); 2) sociability (an unfamiliar mouse was placed in an inner mesh wire cage in either the left or right chambers); 3) social novelty preference (a novel mouse was placed into the previously empty inner cage in the chamber, opposite the now familiar mouse). All mice were age- and sex-matched, with each chamber cleaned and lined with fresh bedding between trials. For each of the three stages, behaviours were recorded by a video camera mounted above the apparatus; Ethovision videotracking was used to analyze time spent in each of the chambers.

2.4.5 Social Interaction

Free social interaction between mice in a dyadic encounter was assessed as described previously (O'Tuathaigh et al., 2010a). The test was conducted in the same apparatus used in the social novelty preference test under normal room lighting conditions for a 10 min period. Behaviours analysed included social investigation (anogenital sniffing, sniffing of anogenital region of the conspecific), agonistic behaviours (pinning, the test mouse pins the conspecific to the floor; clawing; aggressive following); walkovers (the mouse places its front paws on the head or back of the conspecific); and non-social exploration such as rearing to wall and free rearing (the mouse is upright with front paws raised).

2.4.6 Prepulse inhibition

Prepulse inhibition (PPI) was assessed as described previously (O'Tuathaigh et al., 2011b). Mice were placed in a startle chamber and PPI was assessed using, in a pseudo-random order, a block of 172 discrete test trials with inter-trial intervals ranging between 5 and 15 s; each block consisted of pulse-alone trials at three different intensities [100, 110 and 120 dB], which were combined with three prepulse signals [4, 8 and 16 dB] to give nine different combinations of prepulse/pulse trials.

2.5 Tissue isolation

Following completion of behavioural assessments [Experiments 1], mice were sacrificed by decapitation. Trunk blood was collected into pre-chilled EDTA-coated tubes (BD Vacutainer, UK). Blood samples were centrifuged (4000 g, 5 min, 4 °C) and plasma was immediately stored at -70 °C until assayed. Brains were dissected on ice, placed in RNAlater solution (Applied Biosystems, UK) and stored at -70°C. Total RNA from the prefrontal cortex (PFC), striatum and hippocampus was isolated for measurement by RT-PCR of IL-1β, IL-6, TNF-α and BDNF mRNA expression in each brain area.

2.6 Corticosterone assay

Total corticosterone in each plasma sample was measured by enzyme immuno-assay (Assay Designs, UK) according to manufacturers' instructions. Due to insufficient plasma volumes, approximately two samples from each group were unavailable for corticosterone assay. Samples were diluted (1:15) with assay buffer provided in the EIA kit. Samples and standards were

assayed in duplicate and averaged. Assay sensitivity was 27 pg/ml and the intra- and inter-assay coefficients of variance were 8.4% and 8.2% respectively. Results are presented as ng/ml.

2.7 Spleen cytokine assays

To assess the effects of CSD and NRG1 deletion on spleen cell cytokine responses to immune mitogens, spleen cells were cultured with lipopolysaccharide (LPS, a B-cell stimulant; Sigma-Aldrich, Ireland) and concanavalin A (ConA, a T-cell stimulant; Sigma-Aldrich, Ireland). The sensitivity of spleen cells to corticosterone-mediated inhibition of cytokine responses to LPS was assessed by incubating cells with corticosterone prior to the addition of LPS, as described previously (Curtin et al., 2009). Spleen culture supernatant samples were analysed for IFN-γ, IL1β, TNF-α, IL6, IL-8 and IL10. For full details on cytokine analyses, see Supplementary methods.

2.8 Brain mRNA analyses

Total RNA from the prefrontal cortex (PFC), striatum and hippocampus was isolated using an Absolutely RNA miniprep kit (Stratagene, USA; Cat. No. 400800), treated with Turbo DNase enzyme (Ambion Ltd, UK; Cat. No. AM1907) to minimize DNA contamination, and 300 ng of total RNA converted to cDNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, UK). Taqman gene expression assays (Applied Biosystems, UK) containing sequence specific primers for TNF-α (Assay ID: Mm00443258_m1), IL1β (Assay ID: Mm00434228_m1), IL6 (Assay ID: Mm00446190_m1) and BDNF (Assay ID: Mm00432069)

were used to achieve target specific amplification. β-actin was used as the endogenous comparator gene. RT-PCR was performed in triplicate on a 7900 Real Time PCR system (Applied Biosystems, UK). Non-CSD controls were used as reference samples and mean ΔCT for the control sample was subtracted from ΔCT for each other experimental samples (ΔΔCT). mRNA expression in each brain area was measured using the formula $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001).

2.9 High performance liquid chromatography

Plasma samples [Experiment 1] were analysed for tryptophan, L-kynurenine and kynurenic acid by isocratic elution with sequential and UV fluorescence detection using HPLC, as described previously (Desbonnet et al., 2008). Briefly, samples (containing 3-Nitro-L-Tyrosine as internal standard) were de-proteinised by adding 20 µl of 4 M perchloric acid to 200 µl of each sample. 20 µl of samples and standards were injected onto the HPLC system, and the chromatograms generated were processed using Waters Empower software. Analytes were identified based on their characteristic retention times and concentrations were calculated using analyte:internal standard peak height ratios, which were measured and compared with standard injections. For some samples, kynurenic acid was not detected, and specific samples were not included in the statistical analysis of kynurenic acid and associated ratios. Results were expressed as nanograms of analyte per milliltre of plasma.

2.10 Statistical analysis

Analysis of variance (ANOVA) was applied to assess group differences across the data, using SPSS 15.0. Repeated measures ANOVA was performed to analyse data for sociability and social novelty preference, startle habituation and %PPI for each pulse intensity. Where the data were not normally distributed, analyses were conducted following square root transformation. Statistically significant effects in each ANOVA were followed with post hoc comparisons, using the Least Significant Difference test. A p value of < 0.05 was considered significant.

3. Results

3.1 Experiment 1

3.1.1 Novel open field

In the open field, NRG1 mutants travelled a greater distance [$F(1,39) = 6.97$, $p < 0.05$]; while CSD was without effect in WT, CSD reduced the heightened activity of unstressed NRG1 mutants to the level of WT [NRG1× CSD: $F(1,39) = 3.23$, $p = 0.08$; $p < 0.05$, stressed vs unstressed NRG1]. A very similar profile was evident for movement velocity (Fig. 1A/B). NRG1 mutants spent less time in corners relative to WT [$F(1,39) = 4.40$, $p < 0.05$]; this profile was unaltered by CSD (Figure 1C). CSD increased excretion of faecal boli [$F(1,39) = 4.17$, $p < 0.05$]; this effect was evident in NRG1 mutants ($p < 0.05$ vs unstressed NRG1 mutants) but not in WT (Fig. 1D).

3.1.2 Spontaneous alternation

CSD reduced % spontaneous alternation [$F(1,39) = 7.51$, $p < 0.01$]; this effect was evident in NRG1 mutants ($p < 0.05$ vs unstressed NRG1 mutants) but not in WT (Fig. 2A).

3.1.3 Sucrose preference

While CSD reduced sucrose preference in WT, CSD was without effect in NRG1 mutants [NRG1 × CSD interaction: $F(1,39) = 4.34$, $p < 0.05$] (Fig. 2B).

3.1.4 Sociability and social novelty preference

The expected preference for spending more time in the chamber containing the unfamiliar mouse relative to the empty chamber (Sociability) was evident across all groups [$F(1,81) = 35.44$, $p < 0.001$]; this profile was unaffected by genotype or CSD (Fig. 3A). The expected switch to spending more time in the [previously empty] chamber containing the novel mouse relative to the chamber containing the now familiar mouse (Social novelty preference) was reduced in NRG1 mutants [chamber: $F(1,81) = 47.82$, $p < 0.001$; chamber × NRG1: $F(1,81) = 3.25$, $p = 0.08$]; while preference for the chamber containing the novel over the now familiar mouse appeared less robust in NRG1 mutants, there was no material effect of CSD (Fig. 3B).

3.1.5 Social Interaction

CSD increased agonistic behaviours [$F(1,40) = 6.27$, $p < 0.05$; particularly in WT ($p <0.05$); Fig. 4B], and walkovers [$F(1,40) = 9.36$, $p < 0.01$; particularly in NRG1 mutants ($p < 0.01$); Fig. 4D]. CSD decreased wall rearing [$F(1,40) = 12.62$, $p < 0.01$; Fig. 4E] and free rearing [$F(1,40) = 8.28$, $p < 0.01$; Fig. 4F]. NRG1 mutants exhibited decreased agonistic behaviours

relative to WT [$F(1,40) = 5.10$, $p < 0.05$; Fig. 4B]. There was no effect of NRG1 mutation on other social behaviours analysed (Fig. 4A/C].

3.1.6 Corticosterone

CSD marginally increased basal corticosterone concentrations in serum samples [$F(1,25) = 12.55$, $p = 0.06$; Fig. 5. NRG1 deletion had no effect on corticosterone.

3.1.7 Spleen cytokine responses to LPS

CSD increased spleen level of all cytokines measured: IL10 [$F(1,32) = 8.12$, $p < 0.01$], IL-1β [$F(1,34) = 7.26$, $p < 0.05$], IL-6 [$F(1,31) = 5.22$, $p < 0.05$], TNF-α [$F(1,27) = 5.73$, $p < 0.05$], IFN-γ [$F(1,31) = 9.79$, $p < 0.01$], IL-8 [$F(1,32) = 4.74$, $p < 0.05$]. NRG1 deletion increased cytokine levels to an extent similar to CSD for IL-10 [$F(1,32) = 6.10$, $p < 0.05$], IL-6 [$F(1,34) = 7.33$, $p < 0.05$] and IFN-γ [$F(1,31) = 8.39$, $p < 0.01$]. These increases in NRG1 mutants were more evident in those that also experienced CSD: IL10, $p < 0.01$; IL6, $p < 0.05$; IFN-γ, $p < 0.01$ vs unstressed NRG1. For IFN-γ, the effect of CSD to increase cytokine levels was greater in NRG1 mutants than in WT [NRG1 × CSD; $F(1,31) = 4.74$, $p < 0.05$] (Table 1).

CSD increased the stimulatory action of LPS in the spleen for each cytokine measured: IL-10 [$F(1,34) = 13.95$, $p < 0.001$], IL-1β [$F(1,34) = 10.07$, $p < 0.01$], IL-6 [$F(1,33) = 15.15$, $p < 0.001$], TNF-α [$F(1,26) = 30.24$, $p < 0.001$], IFN-γ [$F(1,30) = 6.25$, $p < 0.05$], IL-8 [$F(1,32) = 22.61$, $p < 0.001$]. NRG1 deletion did not influence either LPS-induced stimulation of cytokines or the effect of CSD to increase this stimulatory action of LPS (Table 1).

3.1.8 Spleen cytokine responses to ConA

CSD increased spleen level of IL-1β [F(1,34) = 6.20, p <0.05]. NRG1 deletion was without effect on cytokine levels (Table 1).

Neither CSD nor NRG1 deletion influenced the stimulatory action of ConA in the spleen for any cytokine; however, CSD increased ConA-induced stimulation in WT but decreased ConA-induced stimulation in NRG1mutants for IL-1β [NRG1 × CSD: $F(1,35) = 4.81$, $p < 0.05$], IL-6 [NRG1 × CSD: $F(1,35) = 5.68$, $p < 0.05$] and IL-8 [NRG1 × CSD: $F(1,35) = 4.52$, $p < 0.05$] (Table 1).

3.1.9 Spleen cell sensitivity to corticosterone

Corticosterone inhibited LPS-stimulated release of all spleen cytokines: IL-10 [$F(3,102) = 6.12$, $p < 0.001$], IL-1β [$F(3,93) = 15.98$, $p < 0.001$], IL-6 [$F(3,99) = 17.65$, $p < 0.001$], TNF-α [$F(3,96) = 23.75$, $p < 0.001$], IFN-γ [$F(3,102) = 6.12$, $p < 0.01$] and IL-8 [$F(3,99) = 8.23$, $p < 0.001$]. The action of corticosterone to inhibit LPS-stimulated cytokine release was disrupted by exposure to CSD for IL-10 [$F(1,34) = 6.10$, $p < 0.05$], IL-1β [$F(1,33) = 7.12$, $p < 0.05$] and IL-8 [$F(1,31) = 5.34$, $p < 0.05$]. For IL-10, the action of 0.005 μM corticosterone to inhibit LPS-stimulated cytokine release was reversed to facilitation in unstressed NRG1 mutants but restored to inhibition in NRG1 mutants exposed to CSD [NRG1: $F(1,36) = 4.56$, $p < 0.05$; CSD: $F(1,36) = 5.95$, $p = 0.02$; NRG1× CSD: $F(1,36) = 3.39$, $p = 0.07$; non-CSD NRG1, p <0.01 vs CSD NRG1 and non-CSD WT].

3.1.10 Brain mRNA expression

The effects of CSD on regional brain expression of mRNAs for IL-1β, TNF-α and BDNF differed between WT and NRG1 mutants: in PFC, CSD was without effect on IL-1β in WT but

decreased expression in NRG1 mutants [NRG1 × CSD: (F1,37) = 5.47, $p < 0.05$]; in striatum, CSD (a) reduced IL-1β in WT but increased expression in NRG1 mutants [NRG1 × CSD: $F(1,30) = 4.77$, $p < 0.05$] and (b) increased BDNF in WT but decreased expression in NRG1 mutants [NRG1 × CSD: $F(1,34) = 5.56$, $p < 0.05$]; in hippocampus, CSD was without effect on TNF-α in WT but increased expression in NRG1 mutants [NRG1 × CSD: $F(1,36) = 5.24$, $p < 0.05$] (Table 2).

3.1.9 Tryptophan, L-kynurenine and kynurenic acid

There were no effects of CSD or NRG1 deletion on tryptophan, L-kynurenine and kynurenic acid concentrations in plasma (Table 3).

3.2 Experiment 2

3.2.1 Prepulse inhibition

Assessment of startle responsivity before and after PPI trial blocks showed habituation at a pulse-alone intensity of 100dB, with startle amplitude consistently lower in NRG1 mutants than in WT [trial blocks: $F(1,25) = 7.19$, $p < 0.02$; NRG1: $F(1,25) = 7.28$, $p < 0.02$] but unaffected by CSD (Fig. 6A); similar profiles were evident at pulse-alone intensities of 110dB and 120dB (data not shown).

%PPI increased with prepulse intensity [4, 8 and 16dB] for a pulse intensity of 110dB [$F(2,52) = 4.12$, $p <0.05$] but less so for 100 or 120dB (Fig. 6B-D). %PPI was reduced in NRG1 mutants relative to WT at each pulse intensity [100dB: $F(1,26) = 4.53$, $p < 0.05$; 110dB: $F(1,26) = 6.10$, $p < 0.05$; 120dB: $F(1,26) = 4.62$, $p < 0.05$]. Separate ANOVAs for each prepulse-pulse

condition revealed %PPI to be decreased in NRG1 mutants relative to WT for all pulse intensities when preceded by a prepulse of 16dB ($p < 0.05$), and for 110dB pulses when preceded by prepulses of 4dB ($p < 0.05$) and 8dB ($p < 0.01$). These profiles were unaltered by exposure to CSD.

4. Discussion

Results indicate that the sensory CSD paradigm in adolescent WT mice induces behavioural and immunological alterations that persist into adulthood. Specifically, adolescent mice exposed to CSD exhibit anhedonia, increased agonistic behaviours and reduced explorative rearing in a novel environment, and a heightened cytokine response to LPS, supporting previous findings (Mays, 2010; Paul et al., 2011; Audet et al., 2011; Bowens et al., 2011). Consistent with the literature on NRG1 transmembrane (TM)-domain mutants, exploratory locomotor activity in a novel environment was elevated (Desbonnet et al., 2009; Kirby et al., 2010; van den Buuse et al., 2009). This phenotype was no longer apparent in NRG1 mutants exposed to CSD during adolescence, indicating an interaction between environmental exposure and genotype. While NRG1 mutants also spent less time in the more 'secure' corner zones, this was not influenced by CSD. As this effect was independent of locomotor activity, being present in both hyperactive non-CSD and non-hyperactive CSD NRG1 mutants, it may reflect reduced anxiety; indeed, anxiolytic-like effects have been reported in NRG1 mutants when tested in the elevated plus-maze and light-dark box (Boucher et al., 2007). However, that CSD increased excretion of faecal boli in NRG1 mutants but not in WT suggests that NRG1 mutants, while showing lower anxiety in the open field, may be more susceptible to the effects of CSD on autonomic responsivity.

Intact spontaneous alternation behaviour in NRG1 mutants, an index of spatial working memory that is a core feature of cognitive impairment in psychotic illness (Reichenberg and Harvey, 2007; Bora et al., 2009), is in accordance with previous reports of minimal effects on cognition (O'Tuathaigh et al., 2007; Duffy et al., 2010). Although CSD had no effect on spontaneous alternation in WT, some disruption has recently been noted in normal mice following a longer period of repeated social defeat (Yu et al., 2011; Wang et al., 2011). Therefore, disruption of spontaneous alternation by CSD in NRG1 mutants suggests that deletion of NRG1 may shorten the duration of CSD required for disruption of spatial working memory. Impaired performance in this test in CSD NRG1 mutants could be accounted for by altered BDNF expression in the striatum. During early development BDNF has been demonstrated to protect against neural tissue loss and spatial memory deficits in adulthood (Almli et al., 2000). Reductions in plasma BDNF (Buckley et al., 2007; Favalli et al., 2012) and brain BDNF expression (Weickert et al., 2003; Wong et al., 2010; Favalli et al., 2012) have been reported in schizophrenia, and changes in region-specific BDNF expression have been linked with susceptibility to social defeat stress (Krishnan et al., 2007; Berton et al., 2006), particularly in adolescence (Coppens et al., 2011). Furthermore, there is cellular evidence that NRG1 can interact with BDNF in regulating neuronal processes (Mei and Xiong, 2008; Kalkman, 2009; Balu and Coyle, 2011).

Sucrose preference is widely used as a measure of anhedonia in rodents and is considered a central feature of both psychotic illness and depression (Winograd-Gurvich et al., 2006). While CSD reduced sucrose preference in WT, as reported recently in normal mice (Yu et al., 2011), this effect of CSD was absent in NRG1 mutants, indicating an interaction between environmental exposure and genotype.

In accordance with previous findings (O'Tuathaigh et al., 2007, 2008), NRG1 mice displayed unaltered sociability and increased aggressive behaviours in a dyadic social interaction scenario. Although preference for social novelty was present in NRG1 mutants, this effect appeared less robust than that observed in WT. Additionally, NRG1 mutants exhibited disruption to PPI, as reported in some (Stefansson et al., 2002; Boucher et al., 2010) but not all studies; PPI findings may be particularly sensitive to conditions and stimulus parameters in NRG1 mutants (see Karl et al., 2011). PPI is a measure showing strong cross-species homology, replicable impairment in schizophrenia and disruption in normal human subjects with NRG1 gene polymorphisms (Swerdlow et al., 2008; Braff, 2010; Roussos et al., 2010), hence it now constitutes an important animal model for this disorder. The fact that these profiles for social behaviour and PPI appear to be unaltered by CSD suggests the involvement of mechanisms distinct from those subserving novelty-induced exploration, spontaneous alternation and sucrose preference.

At a cellular level, there is increasing evidence that immune function in schizophrenia is abnormal and that chronic psychosocial stress produces a complex release of several pro-inflammatory cytokines (Merlot et al., 2004; Powell et al., 2009; Audet et al., 2011); the most consistent findings in schizophrenia patients include increases in IL-6 and IL-2, suggestive of an ongoing inflammatory process (Potvin et al., 2008). Both CSD and NRG1 deletion increased basal levels of IL10, IL-6 and IFN-γ in spleen cell cultures, with increases in NRG1 mutants more evident in those exposed to CSD. In particular, CSD-induced increases in IFN-γ levels were greater in NRG1 mutants than in WT. Although basal pro-inflammatory cytokines are elevated, the L-kynurenine pathway of tryptophan metabolism, dysregulation of which potentially contributes to the pathophysiology of schizophrenia (Wonodi and Schwarcz, 2010; Müller et al., 2011), is not systemically up-regulated by IFN-γ induction of the enzyme

indolamine 2,3-dioxegenase in NRG1 mutants. However, this does not preclude the possibility that central measures of L-kynurenine and kynurenic acid are altered by NRG1 deletion and may play a part in NRG1 effects on NMDA receptor systems and cognitive deficits (O'Tuathaigh et al., 2007; Duffy et al., 2010; Chesworth et al., 2012).

Consistent with previous findings, CSD increased LPS-induced stimulation of spleen cytokines (Merlot et al., 2004; Powell et al., 2009); this was unaltered by NRG1 deletion. Our data suggest that sensitization of spleen immune cells to LPS due to CSD was not mediated by corticosterone disinhibition, as has been demonstrated in other social defeat models (Avitsuur et al., 2002; Bailey et al., 2004). It is possible that this sensitised response to CSD may involve alternative mechanisms, such as reduction in β-adrenoceptor-mediated inhibition of cytokine release (Curtin et al., 2009).

Although NRG1 deletion was without effect on LPS-induced cytokine release, it did disrupt the effect of CSD on ConA-induced stimulation of IL-1β, IL-6 and IL-8, which is specific for T-lymphocytes. As T-cell pools have been shown to increase following CSD in mice, it is possible that NRG1 mutation influences the proportion of T-cells in the spleen, and hence their response to CSD (Avitsur, 2002; Mays et al., 2010). Additionally, there was an increase in cytokine response to LPS following pre-incubation with the lowest concentration of corticosterone in NRG1 mutants not exposed to CSD. This may be due to an imbalance of spleen mineralocorticoid:glucocorticoid receptor ratio: firstly, while mineralocorticoid receptors respond to low concentrations of glucocorticoids, higher concentrations are needed for additional activation of glucocorticoid receptors (Joels et al., 1994); secondly, NRG1 mutant rats that express reduced type II NRG1 protein exhibit abnormalities of the neuroendocrine stress system, including disruption of the critical balance between mineralocorticoid and glucocorticoid

receptors in the hippocampus and a related increase in basal corticosterone concentrations (Taylor et al., 2010).

Recent studies indicate that NRG1 plays an important regulatory and neuroprotective role in the peripheral and central immune system (Xu et al., 2005; Marballi et al., 2010). In the present study, NRG1 mutation also influenced the effects of CSD on brain cytokine and BDNF mRNA expression, in a manner that differed between brain regions: in NRG1 mutants CSD decreased IL-1β in PFC, increased IL-1β and decreased BDNF in striatum and increased TNF-α expression in the hippocampus. Evidence indicates that NRG1-ErbB receptor signalling reduces Akt phosphorylation of glycogen synthase kinase (GSK)-3β (Wen et al., 2008) to result in reduced NF-κB-mediated induction of pro-inflammatory cytokines (Hazeki et al., 2007). As CSD has been shown to exert long-term effects on signalling in the Akt/GSK-3β pathway (Böer et al., 2008; Krishnan et al., 2008), the effects of CSD on GSK-3β/NF-κB regulation of cytokine expression may be disrupted by NRG1 deletion. Interestingly, decreased NRG1-induced activation of the Akt /GSK-3β pathway has been reported recently in first episode schizophrenia (Keri et al., 2010).

To conclude, in addition to confirming the involvement of NRG1 in schizophrenia-relevant phenotypes such as hyperactivity in response to novelty, disruption to sensorimotor gating and impairment in social novelty preference, together with anxiety, the present study establishes a role for psychosocial stress during adolescence and interactions between this environmental exposure and NRG1 mutation in the emergence of schizophrenia-relevant phenotypes in adulthood; these effects are summarised in Table 4. In particular, we report for the first time that exposure to adolescent CSD in NRG1 mutants results in elevated basal cytokine concentrations in spleen, disparate cytokine responses to *in vitro* stimulation with ConA, and regionally-specific

alterations in the expression of IL-1β, TNF-α and BDNF mRNA in brain. While the functional significance of altered cytokine expression in the absence of any direct inflammatory stimulus remains to be clarified, the present findings elaborate evidence for immune system imbalance in the pathobiology of schizophrenia and the adverse effects associated with chronically elevated pro-inflammatory states (Maes, 1994; Garcia-Bueno et al., 2008; Potvin et al., 2008; Hansel et al., 2010; Watanabe et al., 2010). Specifically, they implicate (i) the immune system as a potentially important link between NRG1 and the pathobiology of schizophrenia, and (ii) the critical nature of early environmental exposures such as repeated adolescent stressors to the emergence of psychosis phenotypes in those with underlying NRG1 gene abnormalities.

Conflict of Interest Statement

All authors declare no conflict of interest.

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Fig. 1. Effects of chronic social defeat (CSD) and non-stressed control (C) procedures during adolescence in wildtype (WT) and NRG1 heterozygous mice (HET) on distance travelled (A), velocity (B), time spent in the corners (C) and faecal boli [FB] (D) during a 10 min exposure to a novel open field. Data are expressed as means ± SEM. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs corresponding WT; $*p < 0.05$ vs corresponding non-stressed.

Fig. 2. Effects of chronic social defeat (CSD) and non-stressed control (C) procedures during adolescence in wildtype (WT) and NRG1 heterozygous mice (HET) on % alternation in the 10 min spontaneous alternation test (A) and % preference for sucrose over water (B). Data are expressed as means ± SEM. $*p<0.05$, $**p<0.01$ vs corresponding non-stressed group.

Fig. 3. Effects of chronic social defeat (CSD) and non-stressed control (C) procedures during adolescence in wild-type (WT) and NRG1 heterozygous mice (HET) on chamber times during assessment for sociability (A) and preference for social novelty (B). Data are expressed as means ± SEM. $*p < 0.05$, $**p < 0.01$ $***p < 0.001$ vs alternate chamber.

Fig. 4. Effects of chronic social defeat (CSD) and non-stressed control (C) procedures during adolescence in wild-type (WT) and NRG1 heterozygous mice (HET) on social investigation (A), agonistic behaviours (B); number of attacks(C), walkovers (D); wall rears (E) and free rears (F) during a 10 minute social interaction test. Data are expressed as means ± SEM.

Fig. 5. Effects of chronic social defeat (CSD) and non-stressed control (C) procedures during adolescence in wild-type (WT) and NRG1 heterozygous mice (HET) on basal plasma conticosterone concentrations (ng/ml). Data are expressed as means ± SEM.

Fig. 6. Effects of chronic social defeat (CSD) and non-stressed control (C) procedures during adolescence in wild-type (WT) and NRG1 heterozygous mice (HET) on startle responses to pre- and post-test 100dB pulses (A) and % prepulse inhibition at 100dB (B), 110dB (C), and 120dB (D) pulse intensities. Data are expressed as means ± SEM. Effect of genotype, $*p < 0.05$, $**p < 0.01$; effect of startle habituation, $^{\#}p < 0.05$.

Table 1. Cytokine concentrations (pg/ml) in spleen cell culture supernatant of adult wildtype (WT) and NRG1 heterozygous mice (HET) exposed to chronic social defeat (CSD) or non-stressed control (C) procedures following stimulation with either lipopolysaccharide (LPS; 1μg/ml, 37°C, 24h), concanavalin A (ConA; 5μg/ml, 37°C, 72h) or culture medium alone. Data are expressed as means ± SEM. Columns on the right depict main effects of 2-way ANOVA for unstimulated and stimulated conditions. Significant *p* values are highlighted. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$vs corresponding non-stressed group.

Table 2. Cytokine mRNA expression (2^{-ddCT}) in brain regions of adult wildtype (WT) and NRG1 heterozygous mice (HET) exposed to either chronic social defeat (CSD) or non-stressed control (C) procedures during adolescence. Data are expressed as means ± SEM. Columns on the right depict main effects of 2-way ANOVA. Significant *p*-values are highlighted. $*p < 0.05$ vs corresponding non-stressed group.

Table 3. Plasma concentrations (ng/ml) of L-kynurenine, tryptophan, kynurenic acid and kynurenine/tryptophan and kynurenic acid/kynurenine ratios in adult wildtype (WT) and heterozygous NRG1 (HET) mutant mice exposed to either chronic social defeat (CSD) or non-stressed control (C) procedures during adolescence. Data are expressed as means ± SEM.

Table 4. Summary of effects of chronic social defeat (CSD) and non-stressed control (C) procedures during adolescence in wildtype (WT) and NRG1 heterozygous (HET) mice: = no significant difference relative to unstressed WT; ↓ reduced relative to unstressed WT; ↑ increased relative to unstressed WT. [a]all cytokines assayed except IL-8; [b]cytokines increased include IL-1β, TNF-α and IL-8, [c]cytokines increased include IL-10, IL-6, TNF-α and IL-8; [d]CSD increased ConA-induced stimulation in WT but decreased ConA-induced stimulation in NRG1 for IL-1β IL-6 and IL-8.

Figure 1

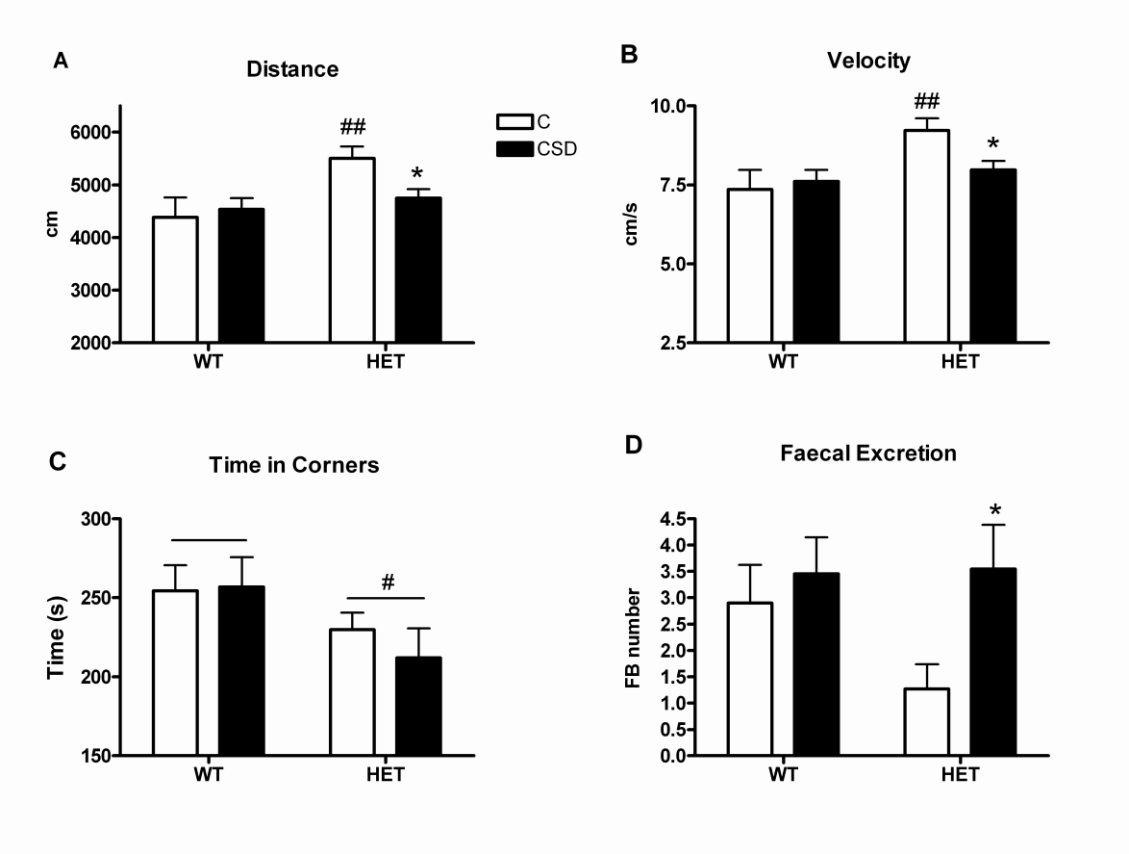
A
Distance
C
CSD
cm
6000
5000
4000
3000
2000
##
*
WT
HET
B
Velocity
cm/s
10.0
7.5
5.0
2.5
##
*
WT
HET
C
Time in Corners
Time (s)
300
250
200
150
#
WT
HET
D
Faecal Excretion
FB number
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
WT
HET

Figure 2

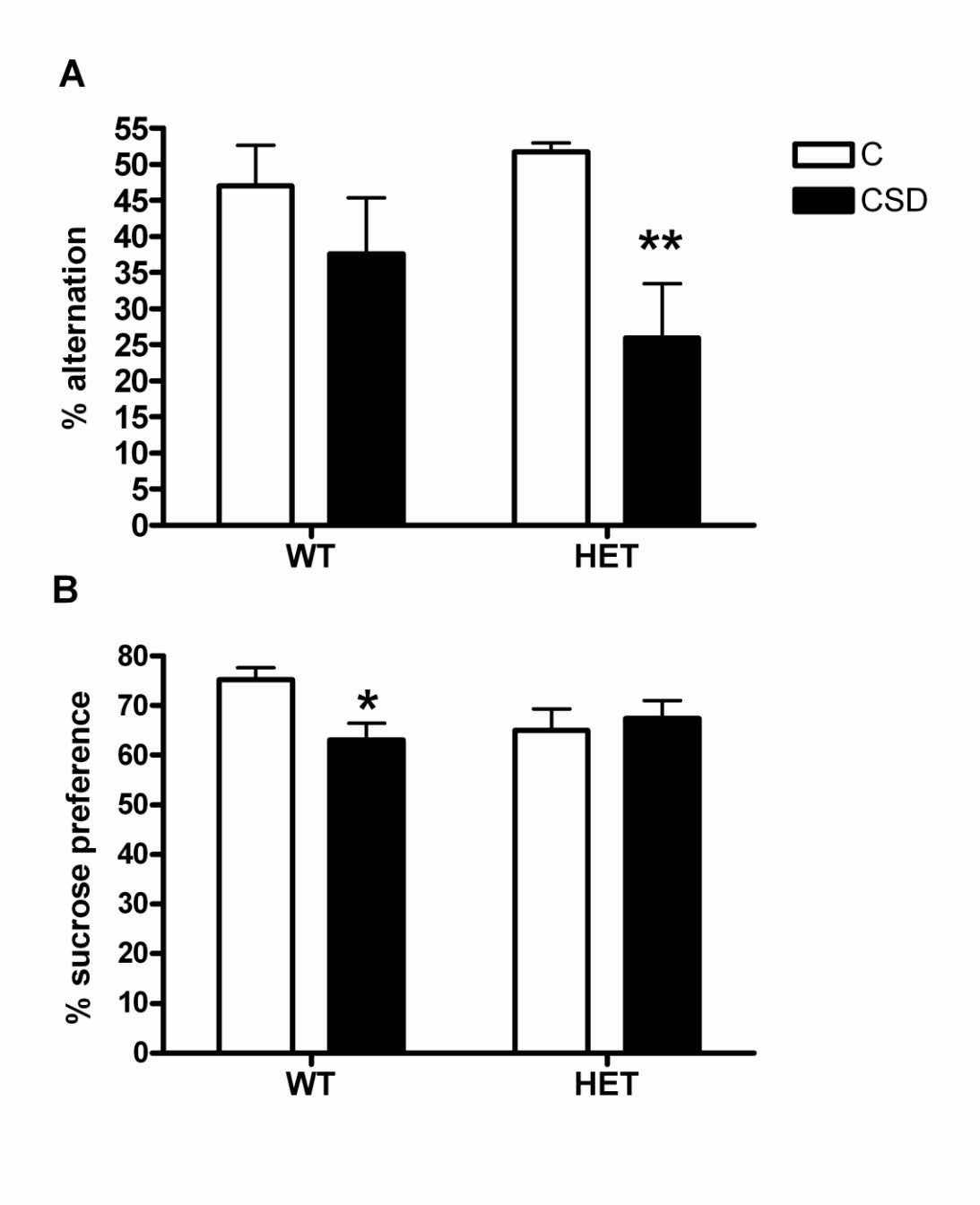
A
% alternation
55
50
45
40
35
30
25
20
15
10
5
0
WT
HET
**
C
CSD
B
% sucrose preference
80
70
60
50
40
30
20
10
0
WT
HET
*

Figure 3

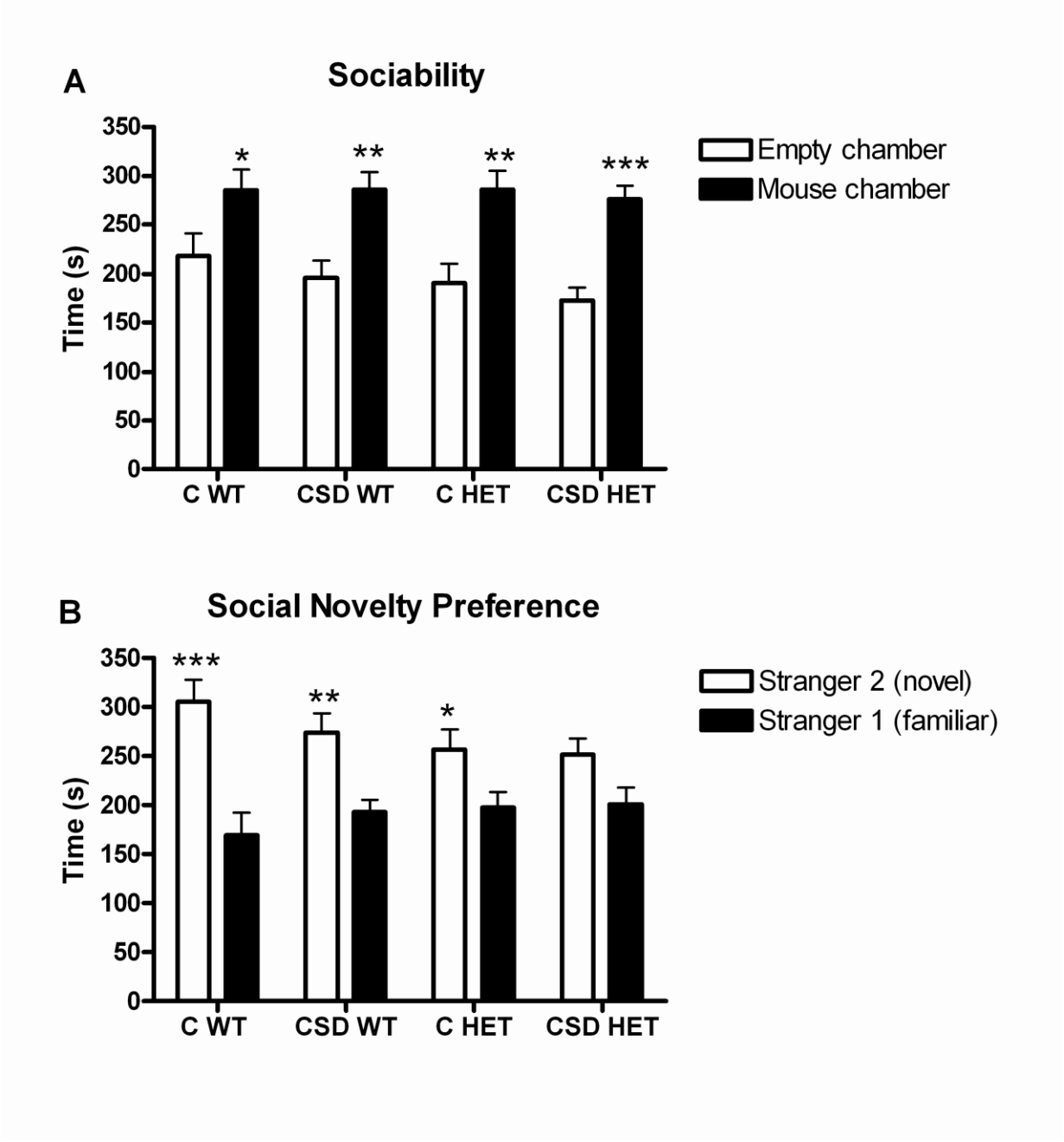
A
Sociability
Time (s)
350
300
250
200
150
100
50
0
*
**
**
***
C WT
CSD WT
C HET
CSD HET
Empty chamber
Mouse chamber
B
Social Novelty Preference
Time (s)
350
300
250
200
150
100
50
0
***
**
*
C WT
CSD WT
C HET
CSD HET
Stranger 2 (novel)
Stranger 1 (familiar)

Figure 4

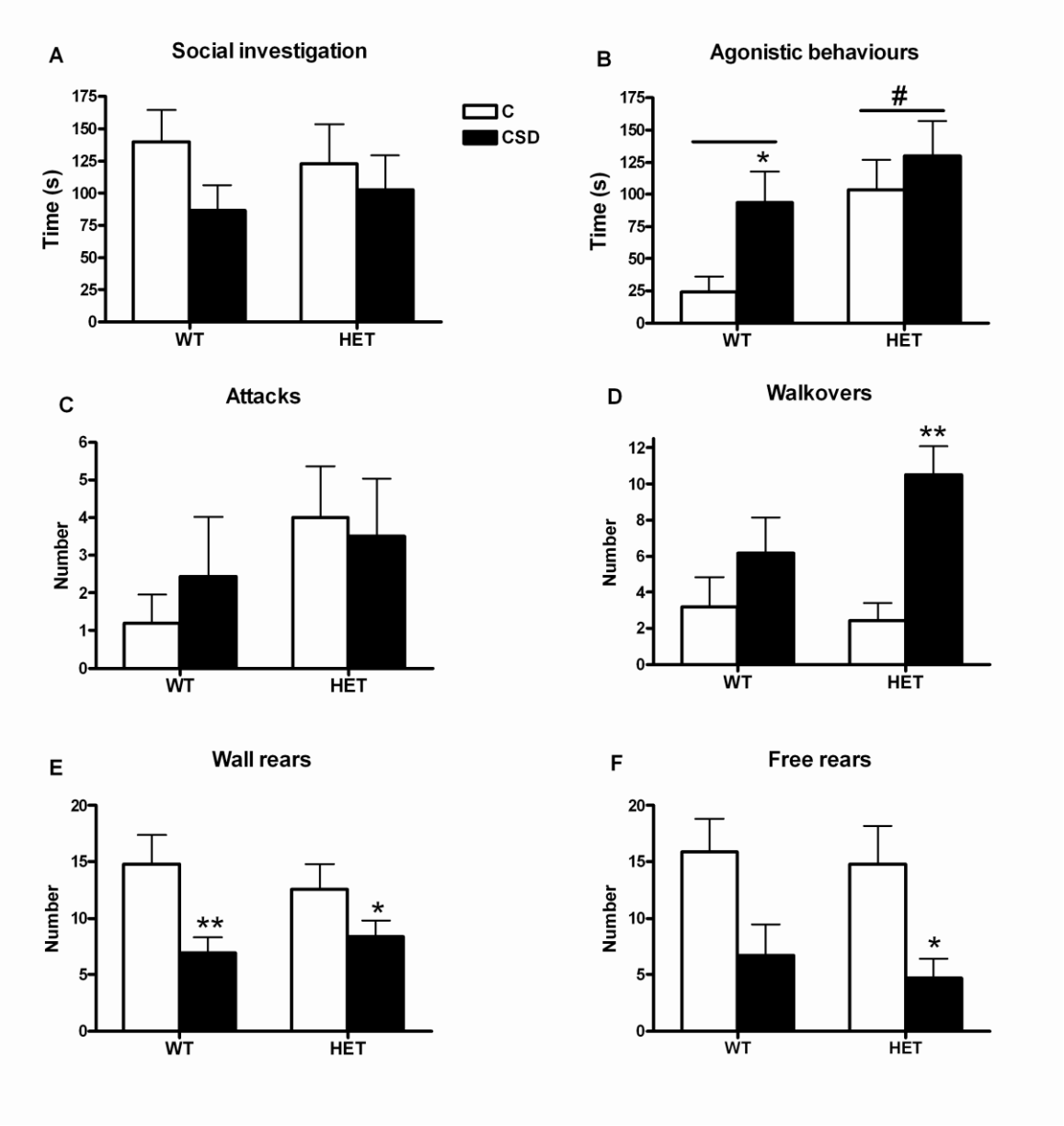
A
Social investigation
Time (s)
C
CSD
WT
HET
B
Agonistic behaviours
Time (s)
*
#
WT
HET
C
Attacks
Number
WT
HET
D
Walkovers
Number
**
WT
HET
E
Wall rears
Number
**
*
WT
HET
F
Free rears
Number
*
WT
HET

Figure 5

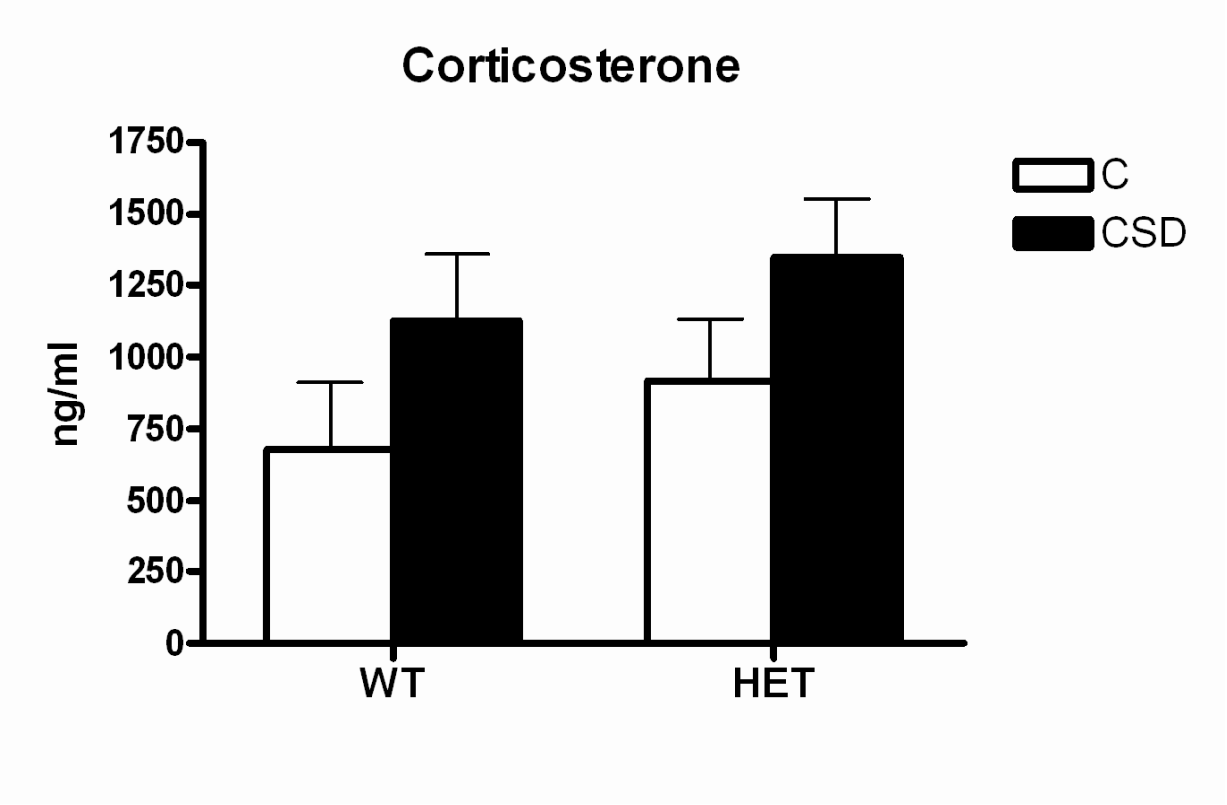
Corticosterone
1750
1500
1250
1000
750
500
250
0
ng/ml
C
CSD
WT
HET

Figure 6

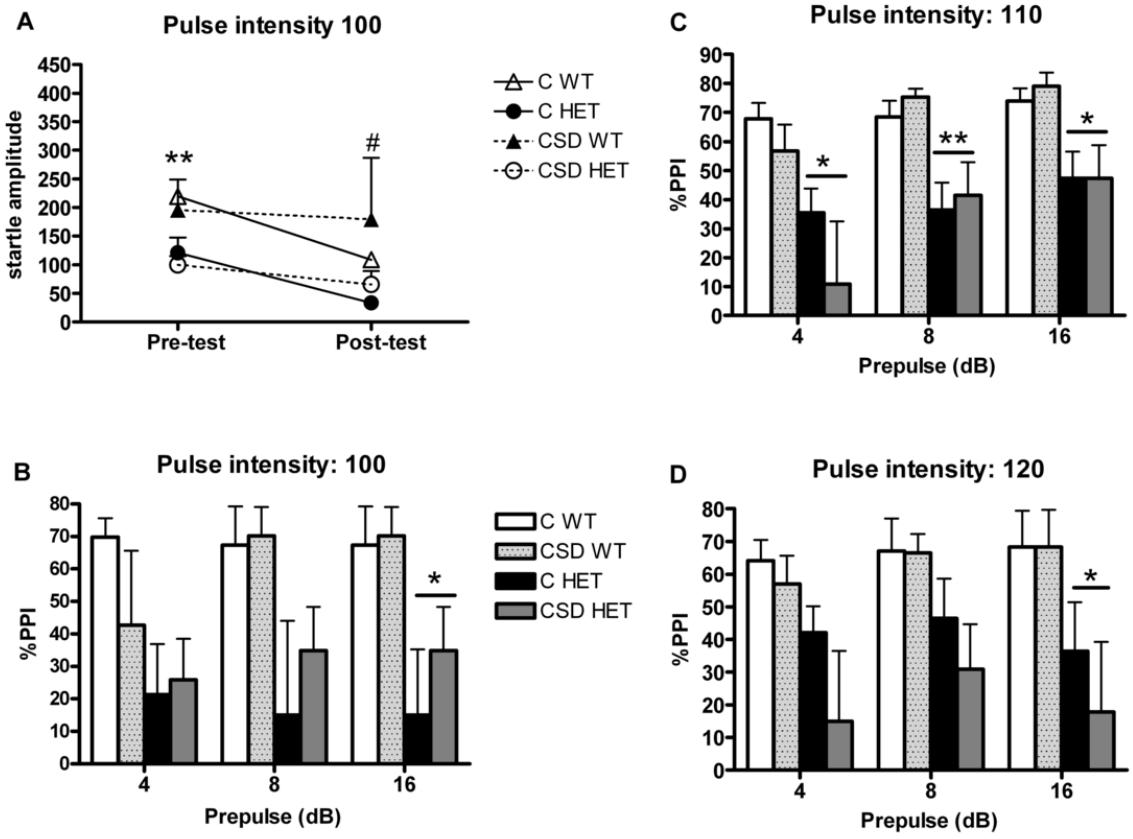
A
Pulse intensity 100
startle amplitude
Pre-test
Post-test
**
#
C WT
C HET
CSD WT
CSD HET
C
Pulse intensity: 110
%PPI
Prepulse (dB)
B
Pulse intensity: 100
D
Pulse intensity: 120

Table 1

	Unstimulated				Stimulated				*p*-value					
	Non-stressed		Stressed		Non-stressed		Stressed		Unstim			Stim		
	WT (n=10)	HET (n=10)	WT (n=10)	HET (n=10)	WT (n=10)	HET (n=10)	WT (n=10)	HET (n=10)	NRG1	Stress	NRG1 × Stress	NRG1	Stress	NRG1 × Stress
LPS														
IL-10	10.0 ± 3.0	13.5 ± 3.8	14.9±2.1	29.8 ± 5.5**	157.5 ± 46.7	137.9 ± 48.1	286.3 ± 30.3	342.3± 62.8**	0.019	0.008	0.134	0.691	0.0007	0.397
IL-1β	2.0 ± 0.6	1.5 ± 0.5	2.8 ± 0.6	4.2 ± 0.9*	7.8 ± 2.0	6.4 ± 2.1	20.9 ± 5.7*	18.2 ± 4.8	0.473	0.011	0.169	0.597	0.003	0.87
IL-6	7.1 ± 3.0	11.5 ± 3.9	9.8 ± 1.5	26.9 ± 6.3*	334.9 ± 105	256.1 ± 87.8	647.3 ± 112	719.6 ±98***	0.011	0.029	0.122	0.600	0.0005	0.104
TNF-α	0.6 ± 0.3	0.9 ± 0.3	0.9 ± 0.2	1.5 ± 0.5*	107.4 ± 34.5	65.9 ± 23.2	275.5 ± 47.8**	261.5±21.3***	0.528	0.024	0.104	0.409	0.0001	0.68
IFN-γ	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	1.3 ± 0.35**	33.2 ± 11.9	29.5 ± 14.1	165.6 ± 72.6	204.7 ± 98.5	0.007	0.004	0.037	0.676	0.018	0.631
mKC	3.8 ± 1.7	6 ± 2.4	6.5 ± 1.2	12.4 ± 2.9	113.5 ± 35.6	110.8 ± 39.8	266.1 ± 30.2**	288.4 ± 28.9**	0.059	0.037	0.388	0.779	0.0001	0.720
ConA														
IL-10	11.1 ± 1.6	13.1 ±2.7	8.9 ± 1.7	10.9 ± 3.0	243.2 ± 70.7	429.3 ± 134.2	485.3 ± 174.3	276.5 ± 87.4	0.553	0.523	0.808	0.927	0.720	0.119
IL-1β	1.5 ± 0.2	1.6 ± 0.2	0.9 ± 0.2	1.0 ± 0.1	5.5 ± 1.0	6.3 ± 0.9	6.6 ± 1.1	4.6 ± 0.6	0.663	0.017	0.990	0.800	0.892	0.035
IL-6	10.7 ± 1.1	11.5 ± 1.2	9.0 ± 1.0	9.2 ± 1.2	400.9 ± 74.1	690.1 ± 111.4	599.7 ± 106.5	448.9 ± 64.0	0.638	0.078	0.807	0.459	0.820	0.023
TNF-α	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.2	154.2 ± 44.4	236.8 ± 51.5	269.2 ± 83.1	183.5 ± 39.1	0.693	0.758	0.798	0.978	0.593	0.149
IFN-γ	0.3 ± 0.0	0.3 ± 0.0	0.3± 0.0	0.2 ± 0.0	941.3 ± 423.7	1128.3± 807.5	1806.2 ± 807.5	655.8 ±188.2	0.714	0.198	0.663	0.361	0.700	0.222
mKC	6.5 ± 2.3	8.8 ± 1.7	7.3 ± 1.4	6.5 ± 2.8	37.3 ± 8.3	45.1 ± 6.6	46.1 ± 7	29.2 ± 4.1	0.530	0.723	0.480	0.672	0.431	0.041

Table 2

Brain mRNA							
	WT		HET		*P*-VALUE		
	C (n=10)	CSD (n=10)	C (n=10)	CSD (n=10)	NRG1	CSD	NRG1 × CSD
Prefrontal cortex							
IL-1β	0.92 ± 0.10	0.99 ± 0.10	1.20 ± 0.2	0.66 ± 0.06*	0.819	0.077	0.025
IL-6	1.07 ± 0.11	1.26 ± 0.11	1.17 ± 0.19	1.09 ± 0.07	0.798	0.680	0.273
TNF-α	1.26 ± 0.29	0.89 ± 0.14	0.94 ± 0.15	0.72 ± 0.08	0.156	0.097	0.669
BDNF	1.04 ± 0.10	1.21 ± 0.18	1.01 ± 0.08	1.01 ± 0.09	0.35	0.486	0.508
Hippocampus							
IL-1β	1.17 ± 0.27	0.32 ± 0.30	1.2 ± 0.19	1.80 ± 0.91	0.630	0.474	0.666
IL-6	1.13 ± 0.31	1.24 ± 0.23	1.17 ± 0.22	1.36 ± 0.27	0.784	0.585	0.889
TNF-α	0.71 ± 0.18	0.88 ± 0.16	1.67 ± 0.24	0.8 ± 0.2*	0.066	0.136	0.033
BDNF	1.11 ± 0.20	1.53 ± 0.17	1.39 ± 0.14	1.51 ± 0.2	0.075	0.633	0.822
Striatum							
IL-1β	1.17 ± 0.2	0.80 ± 0.14	0.78 ± 0.07	1.28 ± 0.28	0.834	0.737	0.037
IL-6	1.08 ± 0.19	2.0 ± 0.64	1.50 ± 0.41	2.15 ± 0.37	0.522	0.085	0.750
TNF-α	1.13 ± 0.22	1.73.2 ± 0.71	1.43 ± 0.39	1.59 ± 0.32	0.866	0.404	0.626
BDNF	1.08 ± 0.12	1.98 ± 0.61	1.36 ± 0.27	0.42 ± 0.11	0.113	0.956	0.024

Table 3

	WT		HET	
	C	**CSD**	**C**	**CSD**
L-kynurenine	214 ± 64	218 ± 64	208 ± 37	314 ± 81
Tryptophan	10568± 1407	11029 ± 1329	10050 ± 825	11543 ± 807
Kynurenic Acid (KA)	2.9 ± 0.8	6.1 ± 1.4	13.4 ± 4.6	10.3 ± 5.5
kynurenine/Trypophan ratio	0.02 ± 0.003	0.02 ± 0.003	0.02 ± 0.003	0.03 ± 0.007
KA/kynurenine ratio	0.02 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.01

Table 4

Phenotype	Unstressed		CSD stress	
	WT	Het	WT	Het
Behaviour				
Exploratory activity	=	↑	=	=
Time in open field corners	=	↓	=	↓
Spontaneous alternation	=	=	=	↓
Sociability	=	=	=	=
Social novelty preference	=	↓	=	↓
Agonistic behaviour	=	↑	↑	=
Walkovers	=	=	=	↑
Rearing	=	=	↓	↓
Sucrose preference	=	=	↓	=
Prepulse inhibition	=	↓	=	↓
Cytokines				
Basal spleen cytokine	=	=	=	↑[a]
Cytokine response to LPS	=	=	↑[b]	↑[c]
Cytokine response to ConA	=	=	↑[d]	↓[d]
PFC IL-1β mRNA	=	=	=	↓
Striatum IL-1β mRNA	=	=	↓	↑
Hippocampus TNF-α mRNA	=	=	=	↑
BDNF				
Striatum BDNF mRNA	=	=	↑	↓