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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

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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.

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; Selten et al., 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;

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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 (Kéri 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., 2010; 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 TM-domain NRG1 mutant mice generated at the Victor Chang Cardiac Research Institute, University of New South Wales, Darlinghurst, Australia, were maintained on a C57BL6 background [14 backcrosses] (Stefansson et al., 2002). Heterozygous (HET) and wildtype (WT) mutants were generated from heterozygous breeding pairs and genotyped using polymerase chain reaction analysis (O'Tuathaigh et al., 2006). Only male mice were used in these experiments. 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 postnatal day 30 (P30) until the day of sacrifice. 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

2.2.1. 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, mice were subjected to behavioural assessment: sucrose preference (P50); novel open field (P60); spontaneous alternation (P65); sociability and social novelty preference (P70–75); social interaction (P80–85). 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.

2.2.2. 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 centre 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