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The response of neuregulin 1 mutant mice to acute restraint stress

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ABSTRACT

Stress plays a role in the development and severity of psychotic symptoms and there may be a genetic component to stress vulnerability in schizophrenia. Using an established mouse model for schizophrenia, we investigated the behavioural and endocrine response of *Nrg1* transmembrane domain mutant mice (*Nrg1* HET) and wild type-like (WT) littermates to acute restraint stress. Animals were screened at 3–4 months and 6–7 months of age (before and after onset of hyperlocomotion) for open field behaviour and serum corticosterone levels. In younger mice, stress reduced locomotive and explorative measures and increased anxiety-like behaviour regardless of genotype. Older *Nrg1* mutants were less susceptible to the effects of stress on anxiety-related behaviours. All mice responded to restraint stress with robust increases in serum corticosterone. Importantly, the stress-induced increase in corticosterone was more pronounced in *Nrg1* mutant than WT mice at the younger but not the older age. Our results suggest that transmembrane domain Nrg1 has only a moderate effect on the acute stress response of mice. The behavioural differences detected between WT and *Nrg1* HET mice at the older age were evident without parallel modifications to the glucocorticoid system.

1. Introduction

Neuregulin 1 (*NRG1*) has been associated with an increased risk to develop schizophrenia ([27,35]; meta-analyses: [23,26,30]). The protein is involved in axon guidance, myelination, and synapse formation. Alternative promoter usage results in numerous splice variant types and more than 30 isoforms [11,21]. The isoform variants most commonly expressed in the brain contain a transmembrane domain [10,11,35].

Studies suggest stress plays a role in the development and severity of psychotic symptoms. Indeed, stress can precipitate symptom onset [7] and trigger relapse in schizophrenia patients [13]. Importantly, there may be a genetic component to stress vulnerability in schizophrenia, because (1) schizophrenia patients handle negative life events more poorly than healthy subjects [12] and have an underlying vulnerability to stress [34], (2) first degree relatives of schizophrenia patients demonstrate increased stress sensitivity [24], and (3) a *NRG1* polymorphism interacts with psychosocial stress to affect reactivity to expressed emotions [18].

The heterozygous *Nrg1* transmembrane domain mutant mouse (*Nrg1* HET) is an animal model of schizophrenia providing face, construct and predictive validity. *Nrg1* HETs display an age-dependent hyperactivity (reversible by clozapine [15,27]). Additionally, mutant mice exhibit an anxiolytic-like and cognitive phenotype [6,9,15]. Interestingly, *Nrg1* modulates the effects of the psychoactive cannabis constituent Δ^9-tetrahydrocannabinol on stress-related brain circuitry in mice [3,4] and is expressed in brain regions controlling stress reactivity [5]. Furthermore, observations from animal caretakers suggest that *Nrg1* mutant mice are more susceptible to transport stress.

The current study aimed to evaluate the behavioural and endocrine (*i.e.* serum corticosterone) response of *Nrg1* mutants and WT littermates to acute restraint stress. Test animals were screened before and after onset of the schizophrenia-relevant hyperlocomotive phenotype for baseline and stress-induced open field behaviour. This paradigm has produced the most consistent schizophrenia-relevant *Nrg1* phenotype in the past and enables the analysis of anxiety-related behaviours [15].

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2. Material and methods

2.1. Animals

Test animals were male *Nrg1* HET and WT littermates (C57BL6/JArc background) [27], as they have shown more pronounced phenotypes than females [4,6,9,20]. Age-matched test mice (±7 days) were used at the age of 3–4 months (8 *Nrg1* HETs, 11 WTs) and the age of 6–7 months (10 *Nrg1* HETs, 17 WTs). Test mice were pair-housed in cages equipped with an igloo (Bioserv, Frenchtown, USA) and a metal ring (3 cm diameter) in the cage lid. Mice were kept under a 12:12 h light:dark schedule (red light permanently present: illumination < 5 lx). Research and animal care procedures were approved by the University of New South Wales Animal Care and Ethics Committee and were in accordance with the EC Directive 86/609/EEC for animal experiments.

2.2. Open field (OF)

Baseline OF testing was conducted to confirm the age-dependent hyperlocomotive phenotype of *Nrg1* mutants [15–17]. The response to restraint stress was investigated after an inter-test interval of at least seven days. Mice were placed in OF activity chambers (Med Associates Inc., St Albans, USA) for 30 min. Distance travelled and vertical activity (as an indirect measure of *rearing*) as well as *resting* in central and peripheral zones was recorded [15,16]. The ratio of the distance travelled in the center relative to the total distance travelled ('distance ratio') and the time spent in the center ('center time') were taken as measures of anxiety [8].

2.3. Corticosterone analysis

Mice were taken from the home cage 60 min after onset of the light phase and placed in restraint tubes for 15 min (Broome Rodent Restrainer: Harvard Apparatus, Holliston, USA). Following a 5 min recovery period, mice were tested in the OF. Blood samples were taken prior to and following restraint stress within 3 min from the tail vein. Samples were stored on ice until centrifugation (13,000 rpm for 5 min). Serum corticosterone was analysed using a radioimmunoassay kit from ICN Biomedicals (Costa Mesa, USA) [25].

2.4. Statistical analysis

Results were analysed as published previously [15,16] by three-way repeated measures (RM) analysis of variance (ANOVA) using the within factor 'restraint stress' and 'age' and the between factor 'genotype'. This was followed by lower-degree ANOVAs (*i.e.* two-way and one-way ANOVAs) split by corresponding factors where appropriate. One-way ANOVAs were also chosen for Δ (Restraint–Baseline). Analyses were conducted using Statview Version 5.0. Differences were regarded as statistically significant if $p < .05$. All data are presented as means ± standard error of the mean (SEM) or Δ mean (Restraint–Baseline).

3. Results

3.1. Open field behaviour

Nrg1 mutant mice exhibited hyperlocomotion compared to WT mice ($p = .001$). However, looking at age effects separately, OF locomotion was only significantly different between *Nrg1* HET mice and WT littermates at the age of 6–7 months ($p = .003$; Fig. 1B), whereas total distance travelled of young *Nrg1* HET mice was not significantly increased ($p > .05$; Fig. 1A). Exposure to acute stress had no significant impact on the hyperlocomotive phenotype of older *Nrg1* mutant mice (no interaction of 'restraint stress' with 'genotype'). Furthermore, 'genotype' had an impact on resting time ($p = .03$) and distance travelled in the periphery ($p = .003$). Two-way ANOVAs for the older cohort revealed that mutants exhibited a decrease in resting time ($p = .02$; significant at baseline) and increase in locomotion in the periphery ($p = .02$; significant post restraint stress) compared to WTs (Table 1).

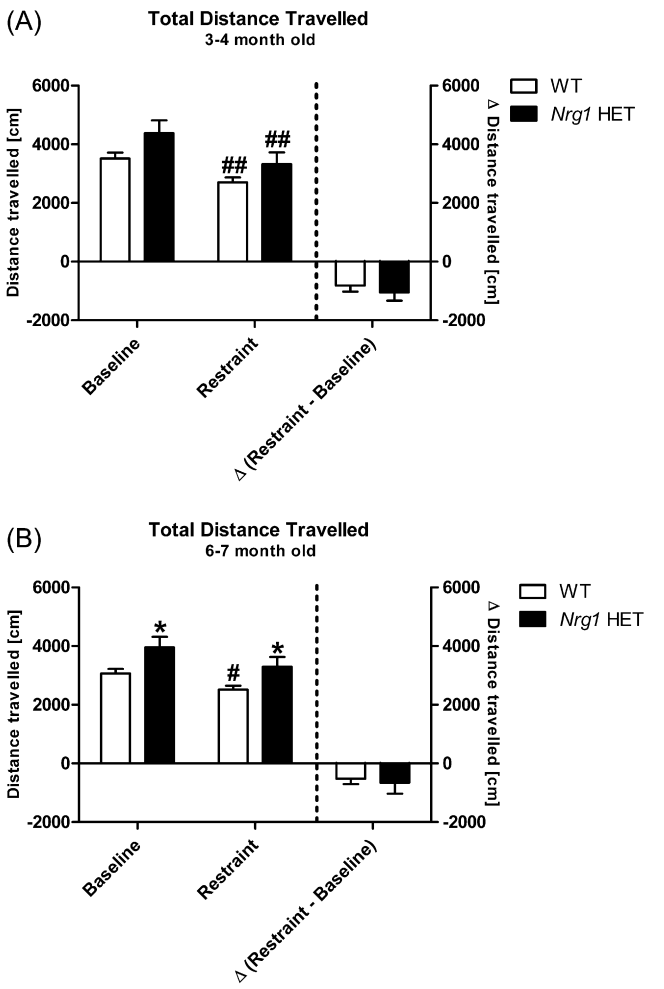

Fig. 1. Open field locomotion. Total distance travelled [cm] is shown in (A) 3–4-month old mice and (B) 6–7-month old mice at baseline and following acute restraint stress. Significant one-way ANOVA effects of stress *versus* baseline of the corresponding genotype are indicated by '#' ($^{\#}p < .05$, $^{\#\#}p < .01$) whereas significant effects of *Nrg1 versus* WT mice of the corresponding stress group are indicated by asterisks ($^{*}p < .05$).

Acute restraint stress had a significant impact on the behavioural performance of mice. Stress inhibited locomotive and explorative behaviours and increased resting time. Three-way ANOVAs detected an overall effect of stress on total distance travelled ($p < .001$), distance travelled in the periphery ($p < .001$), *rearing* frequency ($p < .001$), and resting time ($p = .001$) (Fig. 1 and Table 1). This impact of stress on OF behaviour was evident in both test cohorts: mice at the age of 3–4 months showed a significant stress response in total and peripheral distance travelled (both $p < .001$), frequency of *rearing* ($p = .002$) and resting time ($p < .001$) (Fig. 1A and Table 1). Similarly, mice in the 6–7 month old test group exhibited stress-induced changes to distance travelled ($p = .003$), distance travelled in the periphery ($p = .003$), frequency of *rearing* ($p < .001$), and resting time ($p = .004$) (Fig. 1B and Table 1).