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Research report

Sex-specific neuroendocrine and behavioral phenotypes in hypomorphic Type II Neuregulin 1 rats

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ABSTRACT

Neuregulin 1 (NRG1) is an important growth factor involved in the development and plasticity of the central nervous system. Since its identification as a susceptibility gene for schizophrenia, several transgenic mouse models have been employed to elucidate the role NRG1 may play in the pathogenesis of psychiatric disease. Unfortunately very few studies have included females, despite the fact that some work suggests that the consequences of disrupted NRG1 expression may be sex-specific. Here, we used *Nrg1* hypomorphic (*Nrg1*^{Tn}) Fischer rats to demonstrate sex-specific changes in neuroendocrine and behavioral phenotypes as a consequence of reduced Type II NRG1 expression. We have previously shown that male *Nrg1*^{Tn} rats have increased basal corticosterone levels, and fail to habituate to an open field despite normal overall levels of locomotor activity. The current studies show that, in contrast, female *Nrg1*^{Tn} rats exhibit enhanced suppression of corticosterone levels following an acute stress, reduced locomotor activity, and enhanced habituation to novel environments. Furthermore, we also show that female, but not male, *Nrg1*^{Tn} rats have impaired prepulse inhibition. Finally, we provide evidence that sex-specific changes are not likely attributable to major disruptions in the hypothalamic-pituitary-gonadal axis, as measures of pubertal onset, estrous cyclicity, and reproductive capacity were unaltered in female *Nrg1*^{Tn} rats. Our results provide further support for both the involvement of NRG1 in the control of hypothalamic-pituitary-adrenal axis function and the sex-specific nature of this relationship.

1. Introduction

Neuregulin 1 (NRG1) is a growth factor that plays many roles in central nervous system development and function. In both humans and rats, the *Nrg1* gene is highly complex and is alternatively spliced into six types of proteins, all of which contain and signal via an epidermal growth factor (EGF) domain [1–3]. The effects of NRG1 are mediated by the ErbB tyrosine kinase receptor family, particularly ErbB4 [1]. NRG1 and ErbB4 signaling has been implicated in synapse formation, radial neuron migration, synaptic plasticity, neurotransmitter receptor expression and the hormonal control of puberty [1–4]. Furthermore, NRG1 is expressed in many regions of the brain, including the hippocampus (HPC), prefrontal cortex (PFC) and paraventricular nucleus (PVN) of the hypothalamus [5,6]. Additionally, *NRG1* has repeatedly been identified as a susceptibility gene for schizophrenia and is also associated with bipolar disorder [3,7–9]. This strong association with schizophrenia has prompted investigation of the functional role of NRG1 and ErbB4 with mutant animal models.

Recently, we have shown that NRG1 may also be involved in regulating hypothalamic-pituitary-adrenal (HPA) axis activity [6]. The HPA axis facilitates adaptation to both internal and environmental stressors. Adrenal glucocorticoid (GC) hormones (such as corticosterone in the rat) are the principal mediators of this adaptive response. GCs influence the activity of neurons in the brain by binding to the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), which regulate neuronal gene transcription and metabolism and can modulate complex behaviors [10–13]. In hypomorphic Type II NRG1 (*Nrg1*^{Tn}) male rats, we found disruptions in basal and acute stress recovery corticosterone (CORT) secretions as well as differential changes in expression of GR receptors in the brain [6]. The involvement of NRG1 in the regulation of the HPA axis may be relevant in its role as a schizophrenia susceptibility gene as stressful events, alterations in GC secretion or GR and MR function are associated with multiple psychiatric disorders [10,14–19].

Several *Nrg1* knock out (KO) mouse lines have been behaviorally characterized and exhibit different phenotypes depending on the region of the gene knocked out. In these mice, mutations have typically been targeted to 3′ regions of the NRG1 gene, including the transmembrane (TM) domain [7,20,21], the EGF domain [22] and

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immunoglobulin-like (Ig) domain [23]. However, it is the 5′ region of *NRG1* in humans that contains the most risk haplotype associations with schizophrenia and bipolar disorder [3,7,8]. The 5′ region codes for two isoforms of NRG1, Type IV and Type II, though Type IV may not be expressed at a detectable level in the adult rat brain [24,25]. In heterozygous *Nrg1* TM domain KO mice, hyperactivity, failure to habituate and impaired prepulse inhibition (PPI) were reported [7,21,26,27]. While heterozygous *Nrg1* EGF domain KO mice also demonstrate hyperactivity, they show a facilitated ability to habituate to a novel environment [28], whereas heterozygous *Nrg1* Ig domain KO mice do not exhibit a hyperactive phenotype [23]. Mice over-expressing Type I NRG1 or with decreased expression of Type III NRG1 also demonstrate PPI deficits [29,30]. We have shown that male rats homozygous for the *Nrg1*^{Tn} mutation are not hyperactive and fail to habituate to an open field [6].

As is typical of animal research, relatively few studies of *Nrg1* mutant mice have been conducted with females. However, those that did include females found several sex-specific differences in exploration of the environment, spatial cognition and anxiety behavior caused by disruption of NRG1 [21,26,29]. Thus it is feasible that NRG1 may play a role in regulating certain behaviors in a sex-specific manner. Additionally, NRG1–ErbB4 signaling in hypothalamic astrocytes is required for normal female sexual development [4]. Mice expressing dominant-negative ErbB4 receptors displayed delayed sexual maturation and diminished reproductive capacity in adulthood as a result of the inability of hypothalamic astrocytes to respond to NRG1 [4]. It is currently unknown whether disruptions to NRG1 itself result in altered female sexual development or specific measures of adult reproductive capability. However, evidence from several heterozygous *Nrg1* mutant mouse lines supports that *Nrg1* mutant mice are viable and fertile [7,22,26].

The primary goal of these studies was to test the hypothesis that NRG1 plays a role in regulating sexually dimorphic neuroendocrine and behavioral responses to the environment. To accomplish this goal, female *Nrg1*^{Tn} rats were used to determine the effects of disrupted Type II NRG1 on pubertal onset, adult reproductive capability, basal and stress induced CORT secretion and GR and MR expression. In the present study, open field locomotor activity and habituation were also examined in females, while acoustic startle and PPI were examined in both males in females. In addition, potential differences in these phenotypes between male and female *Nrg1*^{Tn} rats were explored.

2. Methods and materials

2.1. Animals

Random cycling female and male (acoustic startle and PPI studies only) Fischer 344 wild type (WT) and homozygous *Nrg1* mutant (*Nrg1*^{Tn}) rats were used in the present studies. This model was developed and obtained from the PhysGen Program in Genomic Applications (http://pga.mcw.edu/) at the Medical College of Wisconsin. In these animals, the mRNA and protein corresponding to Type II NRG1 in the brain are disrupted by the random insertion of the Sleeping Beauty transposon into the first intron of the *Nrg1* gene [6]. All animals were housed at the Maryland Psychiatric Research Center in a temperature- and light-controlled (lights on 06.00–20.00 h) facility. Water and chow (Harlan Teklad, Frederick, MD) were available *ad libitum*. All procedures conformed to the guidelines for animal research established by the National Institutes of Health, and were approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee. Separate groups of animals were used for each set of reproductive measurements, acute stress and Western blotting and each behavioral experiment.

2.2. Female pubertal onset and adult reproductive function

2.2.1. Pubertal onset

Starting on postnatal day 29, female WT and *Nrg1*^{Tn} rats (n = 8–9/genotype) were inspected daily for inperforation of the vaginal membrane (i.e. vaginal opening) as a marker for the onset of puberty. In female rats, ovulation and the hormonal changes that accompany the onset of puberty are usually associated with vaginal opening [31].

2.2.2. Adult reproductive function

Adult (~65 days of age) female WT and *Nrg1*^{Tn} rats (n = 10–13/genotype) were used for determination of estrous cyclicity. Using the lavage method [32,33], vaginal samples were taken once a day for 15 days between 11.00 h and 14.00 h. Samples were observed under a light microscope at 100× magnification. The proportion of cell types was used to determine estrous cycle phases as follows: proestrus consisted of primarily round nucleated epithelial cells, estrus was characterized by predominantly cornified epithelial cells, and diestrus I and II were characterized by a predominance of leukocytes [32,33].

To determine adult reproductive capacity, the interval between initial exposure to a male breeder and the birth of the first litter was recorded in a group of nulliparous female WT and *Nrg1*^{Tn} rats (n = 10–12/genotype). In addition, litter size, percent survival from birth to weaning and percent male pups for these litters were recorded and compared between genotypes.

2.3. Neuroendocrine measurements

2.3.1. Acute restraint stress

Adolescent (43–45 days of age) and adult (76–79 days of age) female WT and *Nrg1*^{Tn} (n = 43–46/genotype) rats were moved from the vivarium to a temperature- and light-controlled behavioral testing room for acclimation. Approximately 65% of these females (and subsequent plasma samples) were run simultaneously with the male rats described in our previous publication [6] and additional animals were added at a later time. Animals were handled and exposed to acute restraint stress as previously described [6]. Two weeks after being moved to the behavioral testing room, animals were randomly divided into three groups and each group was euthanized by decapitation at a different time point: before (basal time point, n = 16–19/genotype), at the end of (peak time point, n = 13/genotype) or 120 min after (recovery time point, n = 14/genotype) exposure to an acute restraint stress session. Acute restraint stress consisted of placing the animals in cylindrical plastic restraint tubes for 30 min according to our previously published protocol [34]. Trunk blood was collected into tubes containing 10% EDTA and plasma was isolated by centrifugation. Brains and pituitary glands were rapidly removed from the skull and frozen on powdered dry ice. Both brains and plasma were stored at −80 °C until use. Plasma levels of CORT were determined by radioimmunoassay (RIA) according to protocols provided by the manufacturer (MP Biomedicals, Orangeburg, NY).

2.3.2. Western blotting

Western blots were performed as previously described [6] with the following modifications. Whole brains and pituitary glands (n = 11/genotype) were extracted from the skull of adult female WT and *Nrg1*^{Tn} rats at the basal time point of the acute stress experiment (Section 2.3.1) to examine GR and MR expression in the dorsal HPC (n = 8/genotype), PVN (n = 8/genotype) and amygdala (AMG) (n = 8/genotype). Brain regions were identified using the Paxinos and Watson brain atlas [35]. Brain tissue samples from the HPC, PVN and AMG were isolated from 1 mm frozen coronal sections using a 1 mm rat brain punch (Stoelting, Wood Dale, IL). Bilateral