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THE INTERACTION OF DISRUPTED TYPE II NEUREGULIN 1 AND CHRONIC ADOLESCENT STRESS ON ADULT ANXIETY- AND FEAR-RELATED BEHAVIORS

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Abstract—The incidence of anxiety, mood, substance abuse disorders and schizophrenia increases during adolescence. Epidemiological evidence confirms that exposure to stress during sensitive periods of development can create vulnerabilities that put genetically predisposed individuals at increased risk for psychiatric disorders. *Neuregulin 1* (*NRG1*) is a frequently identified schizophrenia susceptibility gene that has also been associated with the psychotic features of bipolar disorder. Previously, we established that Type II NRG1 is expressed in the hypothalamic–pituitary–adrenal (HPA) axis neurocircuitry. We also found, using a line of *Nrg1* hypomorphic rats (*Nrg1*^{Tn}), that genetic disruption of Type II NRG1 results in altered HPA axis function and environmental reactivity. The present studies used the *Nrg1*^{Tn} rats to test whether Type II NRG1 gene disruption and chronic stress exposure during adolescence interact to alter adult anxiety- and fear-related behaviors. Male and female *Nrg1*^{Tn} and wild-type rats were exposed to chronic variable stress (CVS) during mid-adolescence and then tested for anxiety-like behavior, cued fear conditioning and basal corticosterone secretion in adulthood. The disruption of Type II NRG1 alone significantly impacts rat anxiety-related behavior by reversing normal sex-related differences and impairs the ability to acquire cued fear conditioning. Sex-specific interactions between genotype and adolescent stress also were identified such that CVS-treated wild-type females exhibited a slight reduction in anxiety-like behavior and basal corticosterone, while CVS-treated *Nrg1*^{Tn} females exhibited a significant increase in cued fear extinction. These studies confirm the importance of Type II NRG1 in anxiety and fear behaviors and point to adolescence as a time when stressful experiences can shape adult behavior and HPA axis function.

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Abbreviations: AMG, amygdala; ANOVA, analysis of variance; CON, control; CORT, corticosterone; CVS, chronic variable stress; EDTA, ethylenediaminetetraacetic acid; EPM, elevated plus maze; GC, glucocorticoid; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; KO, knock out; MR, mineralocorticoid receptor; NRG1, neuregulin 1; OTR, open-to-total ratio; TM, transmembrane; WT, wild type.

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Key words: neuregulin, HPA axis, sex difference, elevated plus maze, fear conditioning, reactivity.

INTRODUCTION

The hypothalamic–pituitary–adrenal (HPA) axis facilitates adaptation to environmental challenges and the adrenal glucocorticoid (GC) hormones (corticosterone in the rat, cortisol in humans) are the principal mediators of this adaptive response. When stress is repeatedly encountered, the chronic elevation of GCs can result in dysregulation of the HPA axis and altered limbic structure and function (McLaughlin et al., 2009). The brain regions most vulnerable to chronic stress are the hippocampus, amygdala and prefrontal cortex (McEwen, 2007). Interestingly, in both humans and rodents, these regions also show significant maturation during adolescence (Huttenlocher, 1979; Spear, 2000; Romeo and Sisk, 2001; Giedd, 2004, 2008; Koshibu et al., 2004; Zehr et al., 2006; Markham et al., 2007).

Adolescence is a critically important period for the development of normal adult behaviors and disturbance of adolescent development contributes to the etiology of several psychiatric disorders (Walker, 2002; Andersen and Teicher, 2008; Paus et al., 2008; Walker et al., 2008; Ernst et al., 2009). Many aspects of normal brain function mature during adolescence including neurotransmitter activity and receptor expression, synapse development and pruning, and myelination (Teicher et al., 1995; Andersen et al., 2000; Lee et al., 2003; Markham et al., 2007). The neural pathways involved in coordinating stress responses also continue development during adolescence (Spear, 2000; Andersen, 2003; Casey et al., 2008) and there may also be modified HPA axis responses to acute and chronic stress during this time (Romeo and Sisk, 2001; Romeo et al., 2006).

Chronic stress exposure during adolescence can produce long-lasting behavioral and neuroendocrine effects, depending on the timing of exposure, the sex of the animal and the type of stressors used (McCormick et al., 2010; Romeo, 2010). Genetic vulnerabilities may be an additional factor that interacts with adolescent stress exposure to alter adult outcomes relevant to psychiatric disorders. Disruptions of particular genes

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may create a vulnerability to adolescent stress and ultimately alter the developmental trajectory of the adolescent brain. Since the risk for many psychiatric disorders (i.e. anxiety and mood disorders, schizophrenia and substance abuse) increases during adolescence (Hankin et al., 1998; Spear, 2000; Walker, 2002; Andersen, 2003; Costello et al., 2003; Dahl, 2004; Patton and Viner, 2007; Paus et al., 2008), it is critical to assess interactions between genetic susceptibility and stress exposure during this period of maturation and potential vulnerability.

The specific susceptibility genes that mediate stress interactions within psychiatric populations have not been clearly identified. One candidate may be the *Neuregulin 1* (*NRG1*) gene, which has been repeatedly associated with schizophrenia and bipolar disorder (Stefansson et al., 2002, 2003; Harrison and Law, 2006; Prata et al., 2009; Greenwood et al., 2011, 2012). In humans and rats, the *NRG1* gene is highly complex and can be alternatively spliced into six types (I–VI) of proteins, based on N-terminal structural and functional differences (Falls, 2003; Esper et al., 2006; Liu et al., 2011). NRG1 signaling via its preferential tyrosine kinase receptor, ErbB4, plays many roles in neural development, including radial neuron migration, axon guidance, myelination, oligodendrocyte development and synapse formation (Falls, 2003; Mei and Xiong, 2008). In addition, NRG1 is important for adult neural function, including the regulation of the serotonergic system, NMDA, $GABA_A$ and α7 nicotinic receptors, modulation of long-term potentiation, transcriptional regulation and hormonal control of puberty (Falls, 2003; Prevot et al., 2003; Harrison and Law, 2006; Dean et al., 2008).

While gene–environment interactions account for much of the pathology associated with psychiatric disorders (Bayer et al., 1999; Caspi et al., 2003; Howes et al., 2004; Mittal et al., 2008; van Os et al., 2008; Aguilera et al., 2009; Jaaro-Peled et al., 2009; Keri et al., 2009) these interactions have not been extensively investigated during adolescence. However, there are reported gene–environment interactions between *NRG1* and stress in adult humans. In patients with schizophrenia, a single nucleotide polymorphism in the 5′ region of *NRG1* interacts with psychosocial stress to affect reactivity to expressed emotion (Keri et al., 2009). *NRG1* genotype also interacts with job strain to increase risk of heart disease (Hintsanen et al., 2007). Studies in genetically modified animals also demonstrate gene by environment interactions. For example, in heterozygous *Nrg1* transmembrane (TM) domain knock-out (KO) mice, environmental enrichment during adulthood increases exploratory behavior, while adolescent stress exposure appears to have the opposite effect on adult exploratory behavior (Karl et al., 2007; Desbonnet et al., 2012). These studies suggest that disruption of *Nrg1* may interact with chronic adolescent stress on other behaviors relevant to psychiatric disorders.

Previously, we confirmed that a specific isoform of NRG1, Type II, is expressed in the neurocircuitry involved in regulating the HPA axis response to stressors, including the hypothalamic paraventricular nucleus, which integrates and controls the neuroendocrine response to stress (Taylor et al., 2011a). Type II NRG1 is an important isoform to investigate as it is encoded by exons in the 5′ region of the *Nrg1* gene that contains many of the risk haplotype associations with schizophrenia and bipolar disorder (Stefansson et al., 2002, 2003; Harrison and Law, 2006; Prata et al., 2009; Greenwood et al., 2011, 2012). Using a rat model of disrupted Type II NRG1, i.e. the hypomorphic $Nrg1^{Tn}$ rats, we found that male $Nrg1^{Tn}$ rats have significantly higher basal corticosterone (CORT) levels, while female $Nrg1^{Tn}$ rats show enhanced suppression of CORT secretion after recovery from acute restraint stress (Taylor et al., 2011a,b). In addition, sex-specific changes in glucocorticoid (GR) and mineralocorticoid (MR) receptor expression were found, leading to a disrupted MR/GR balance in the hippocampus of males and amygdala of females (Taylor et al., 2011a,b). These findings implicate Type II NRG1 in stress regulation. Behaviorally, we found that male $Nrg1^{Tn}$ rats may be more sensitive to changes in their environment, while female rats are less impacted by mildly stressful aspects in their environment (Taylor et al., 2011a,b).

Based on our previous findings that Type II NRG1 plays a sex-specific role in stress regulation and behavioral responses to the environment in adult rats, we sought to test the hypothesis that chronic stress during adolescence interacts with disruption of Type II NRG1 to produce sex-specific behavioral changes in adult anxiety-like behavior, fear conditioning and basal HPA axis activity. In non-stressed animals, we predicted that male $Nrg1^{Tn}$ rats would show increased anxiety-like behavior, enhanced acquisition and impaired extinction of conditioned fear and increased basal CORT, while female $Nrg1^{Tn}$ rats would exhibit decreased anxiety, reduced acquisition and enhanced extinction of fear conditioning and wild type-like basal CORT. The interaction between disrupted Type II NRG1 and adolescent chronic variable stress was predicted to potentiate these genotype-specific alterations in the behavioral measures.

EXPERIMENTAL PROCEDURES

Animals

Male and random cycling female Fischer 344 wild-type (WT) and homozygous $Nrg1^{Tn}$ 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 and has been previously described (Lu et al., 2007; Taylor et al., 2011a,b). The $Nrg1^{Tn}$ rats have reduced brain expression of both Type II NRG1 mRNA and protein (Taylor et al., 2011a). All animals were housed in same-sex cages of 2–3 rats in a temperature- and light-controlled (lights on 06.00–20.00 h) facility. Water and chow (Harlan Teklad, Frederick, MD, USA) were available *ad libitum*. All procedures conform to the guidelines for animal research established by the National Institutes of Health, and were