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A *NEUREGULIN 1* TRANSMEMBRANE DOMAIN MUTATION CAUSES IMBALANCED GLUTAMATERGIC AND DOPAMINERGIC RECEPTOR EXPRESSION IN MICE

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Abstract—The neuregulin 1 gene has repeatedly been identified as a susceptibility gene for schizophrenia, thus mice with genetic mutations in this gene offer a valuable tool for studying the role of *neuregulin 1* in schizophrenia-related neurotransmission. In this study, slide-based receptor autoradiography was used to quantify glutamatergic *N*-methyl-D-aspartate (NMDA), dopaminergic D2, cannabinoid CB1 and acetylcholine M1/4 receptor levels in the brains of male heterozygous transmembrane domain *neuregulin 1* mutant (*Nrg1*$^{+/-}$) mice at two ages. Mutant mice expressed small but significant increases in NMDA receptor levels in the cingulate cortex (7%, $p = 0.044$), sensory cortex (8%, $p = 0.024$), and motor cortex (8%, $p = 0.047$), effects that were independent of age. In the nucleus accumbens and thalamus *Nrg1*$^{+/-}$ mice exhibited age-dependent alterations in NMDA receptors. *Nrg1*$^{+/-}$ mice showed a statistically significant increase in NMDA receptor levels in the nucleus accumbens of 14-week-old *Nrg1*$^{+/-}$ mice compared to control littermates of the same age (12%, $p = 0.026$), an effect that was not seen in 20-week-old mice. In contrast, NMDA receptor levels in the thalamus, while initially unchanged in 14-week-old mice, were then decreased in the 20-week-old *Nrg1*$^{+/-}$ mice compared to control littermates of the same age (14%, $p = 0.011$). *Nrg1*$^{+/-}$ mutant mice expressed a significant reduction in D2 receptor levels (13–16%) in the striatum compared to controls, independent of age. While there was a borderline significant increase (6%, $p = 0.058$) in cannabinoid CB1 receptor levels in the substantia nigra of *Nrg1*$^{+/-}$ mice compared to controls, CB1 as well as acetylcholine M1/4 receptors showed no change in *Nrg1*$^{+/-}$ mice in any other brain region examined. These data indicate that a *Nrg1* transmembrane mutation produces selective imbalances in glutamatergic and dopaminergic neurotransmission, which are two key systems believed to contribute to schizophrenia pathogenesis. While the effects on these systems are subtle, they may underlie the susceptibility of these mutants to further impacts.

Key words: neuregulin, schizophrenia, NMDA receptor, CB1 receptor, D2 receptor, mouse.

INTRODUCTION

Schizophrenia is a highly heritable psychiatric disorder that affects approximately 50 million people worldwide. Much research indicates a genetic component, with multiple genes identified. Numerous studies replicated in independent populations have demonstrated that variations in the *neuregulin 1* (*NRG1*) gene increase susceptibility for developing schizophrenia (Stefansson et al., 2002, 2003; Williams et al., 2003; Bakker et al., 2004; Corfas et al., 2004; Kim et al., 2006; Walss-Bass et al., 2006) providing strong evidence for an association between the *NRG1* gene and schizophrenia (but also see Sanders et al., 2008). Furthermore, transcript and protein expression of *NRG1* and its receptor v-erb-a erythroblastic leukaemia viral oncogene homologue 4 (ErbB4) are reported to be altered in post-mortem schizophrenia brain (Hashimoto et al., 2004; Law et al., 2006, 2007; Chong et al., 2008; Barakat et al., 2010; Marballi et al., 2012). While *NRG1* is not the only gene underlying schizophrenia susceptibility (Harrison and Weinberger, 2005), and is not implicated in all cases of schizophrenia (Crowley et al., 2008; Moon et al., 2011), it is a promising candidate gene that will provide insights into schizophrenia pathology due to its involvement in major developmental processes and its interactions with key neurotransmitter systems implicated in schizophrenia.

Nrg1 signalling plays a key role in neurodevelopmental processes such as neuronal migration, synapse formation, oligodendrocyte development and axon myelination, as well as synaptic plasticity and neurotransmitter regulation (Corfas et al., 2004; Mei and Xiong, 2008), all of which are believed to be disrupted in schizophrenia (Harrison and Law, 2006). It influences the expression of key neurotransmitters involved in

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Abbreviations: ANOVA, analysis of variance; DISC1, disrupted in schizophrenia 1; EDTA, ethylenediamine tetraacetic acid; EGF, epidermal growth factor; ErbB4, v-erb-a erythroblastic leukaemia viral oncogene homologue 4; GABA, gamma-aminobutyric acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MANOVA, multivariate analysis of variance; NMDA, *N*-methyl-D-aspartate; Nrg1, neuregulin 1; *Nrg1*$^{+/-}$, heterozygous transmembrane domain *neuregulin 1* mutant mouse; WT, wild type-like.

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schizophrenia pathology including glutamate, GABA, dopamine, acetylcholine, and serotonin, and also has a role in neuroendocrine control of puberty (Corfas et al., 2004). Therefore, altering the expression levels or function of *NRG1* could affect the above-mentioned developmental processes and neurotransmission and contribute to the schizophrenia phenotype.

Nrg1 mutant mouse models provide a valuable tool to directly assess the gene's role in schizophrenia-related neurotransmission. The *Nrg1* gene gives rise to at least 30 different isoforms, which are classified as type I–VI depending on the characteristics of their epidermal growth factor (EGF)-like domain, N-terminal sequence, and transmembrane domain (Mei and Xiong, 2008). While various polymorphisms in the *NRG1* gene have been reported in schizophrenia, the identification of a missense mutation on exon 11, which codes for the transmembrane domain of the gene, is one of the few variations that have been found in a coding region of the gene (Walss-Bass et al., 2006). Mice mutated through a targeted deletion of the transmembrane domain of the *Nrg1* gene demonstrate hypophosphorylation of the NR2B subunit of the glutamate *N*-methyl-D-aspartate (NMDA) receptor (Bjarnadottir et al., 2007) and increases in cortical serotonergic 2A receptors and transporters (Dean et al., 2008). No changes have been found in acetylcholine muscarinic, glutamate NMDA or dopamine D2 receptor levels when examination is limited to the rostral cortex and striatum in animals older than 5 months (Dean et al., 2008; Van den Buuse et al., 2009). In addition, a recent study reports no changes in $GABA_A$ or cannabinoid CB1 receptors in these mutants at 6 months of age (Long et al., 2012). The lack of change in systems that are clearly implicated in schizophrenia does question the relevance of this model to schizophrenia. However, in support of its validity as a schizophrenia mouse model, *Nrg1* mutant mice exhibit schizophrenia-like behaviors, including a hyperlocomotive phenotype (Stefansson et al., 2002; Karl et al., 2007; Van den Buuse et al., 2009), disrupted social functioning (O'Tuathaigh et al., 2007), task-specific cognitive deficits (Duffy et al., 2010), and increased susceptibility to environmental impacts such as the administration of cannabis, methamphetamine and enriched housing conditions (Karl et al., 2007; Boucher et al., 2007a,b; Spencer et al., 2012). In addition, *Nrg1* mutants are shown to have sex-specific behavioral phenotypes (Duffy et al., 2010; Long et al., 2010; Chesworth et al., 2012). Furthermore, consistent with the neurodevelopmental theory of schizophrenia, aspects of the behavioral phenotype within this animal model are age-dependent, as one study reports that the hyperlocomotive phenotype of *Nrg1* mutants is not present at 14 weeks of age, only becoming apparent when these mice reach 20 weeks of age (Karl et al., 2007). This phenomenon suggests age-dependent differential modulations of neurotransmission systems may occur in this animal model.

In this study we examined key receptors in the glutamate (NMDA), dopamine (D2), cannabinoid (CB1) and acetylcholine (M1) neurotransmission systems in various regions of the brain of male mice with a mutation in the transmembrane domain of *Nrg1* at 14 and 20 weeks of age. All these receptor targets are implicated in schizophrenia symptoms and are altered in schizophrenia post-mortem brain (Knable et al., 1994; Newell et al., 2005, 2006, 2007a). As this paper will show, this *Nrg1* mutation alters schizophrenia-related neurotransmission, especially glutamatergic NMDA and dopaminergic D2, but this effect can be dependent on age and brain region. Cannabinoid CB1 and cholinergic M1/4 receptors remained largely unaltered in the *Nrg1* mutant brains.

EXPERIMENTAL PROCEDURES

Animals

Frozen brains from male adult (14 ± 1 week and 20 ± 1 week) heterozygous neuregulin 1 transmembrane domain mice (*Nrg1*$^{+/-}$: targeted allele: *Nrg1*^{tm2Zhou}; backcrossed for over 10 generations to C57BL/6JArc background) and wild type-like (WT) littermates were obtained from the colony maintained at the Garvan Institute of Medical Research under standard pair-housing conditions as previously described (Karl et al., 2007). These mice were previously tested in open field and light/dark tests, with aspects of the schizophrenia-like behavioral phenotype only becoming evident in the 20 ± 1-week age group (Karl et al., 2007). All research and animal care procedures were approved by the Garvan Institute/St. Vincent's Hospital Animal Experimentation Ethics Committee and were in agreement with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Every effort was made to minimize the number of animals used.

Receptor autoradiography

Brain sections from six mice per group were cut at 14 μm on a cryotome at Bregma levels +2.34 (containing prefrontal cortex), +0.98 (containing cingulate cortex, rostral caudate-putamen, nucleus accumbens, motor cortex), −0.22 (containing intermediate caudate-putamen, globus pallidus), −0.94 (containing caudal caudate-putamen), −1.82 (containing hippocampus, thalamus, sensory cortex), and −3.08 (containing auditory cortex, visual cortex, retrosplenial cortex, substantia nigra) (Fig. 1). Brain sections were thaw mounted onto polysine coated slides and stored at −20 °C until the day of the receptor binding assay, when sections were thawed to room temperature before the experiments began. The binding of [^{3}H]MK-801 to NMDA (Newell et al., 2005), [^{3}H]raclopride to D2 (du Bois et al., 2008), [^{3}H]CP,55940 to CB1 (Newell et al., 2006), and [^{3}H]pirenzepine to M1/4 receptors (Newell et al., 2007b) were as we have described previously. Furthermore, concentrations of radioligands used are reported to be higher than the previously reported Kd's for these radioligands (Dean et al., 2001; Scarr et al., 2003; Uchida et al., 2007), indicating that the

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