Overexpression of Neuregulin 1 Type III Confers Hippocampal mRNA Alterations and Schizophrenia-Like Behaviors in Mice

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Neuregulin 1 (NRG1) is a schizophrenia candidate gene whose protein product is involved in neuronal migration, survival, and synaptic plasticity via production of specific isoforms. Importantly, NRG1 type III (NRG1 III) mRNA is increased in humans inheriting a schizophrenia risk haplotype for the NRG1 gene (HapICE), and NRG1 protein levels can be elevated in schizophrenia. The nature by which NRG1 type III overexpression results in schizophrenia-like behavior and brain pathology remains unclear, therefore we constructed a transgenic mouse with Nrg1 III overexpression in forebrain neurons (CamKII kinase+). Here, we demonstrate construct validity for this mouse model, as juvenile and adult Nrg1 III transgenic mice exhibit an overexpression of Nrg1 III mRNA and Nrg1 protein in multiple brain regions. Furthermore, Nrg1 III transgenic mice have face validity as they exhibit schizophrenia-relevant behavioral phenotypes including deficits in social preference, impaired fear-associated memory, and reduced prepulse inhibition. Additionally, microarray assay of hippocampal mRNA uncovered transcriptional alterations downstream of Nrg1 III overexpression, including changes in serotonin receptor 2C and angiotensin-converting enzyme. Transgenic mice did not exhibit other schizophrenia-relevant behaviors including hyperactivity, social withdrawal, or an increased vulnerability to the effects of MK-801 malate. Our results indicate that this novel Nrg1 III mouse is valid for modeling potential pathological mechanisms of some schizophrenia-like behaviors, for determining what other neurobiological changes may be downstream of elevated NRG1 III levels and for preclinically testing therapeutic strategies that may be specifically efficacious in patients with the NRG1 (HapICE) risk genotype.

Key words: schizophrenia/neuregulin 1/type III/transgenic/mouse/behavior

Introduction

Neuregulin 1 (NRG1) has been associated with the risk of developing schizophrenia across multiple ethnic populations,1–3 but not in all genetic studies (ie, Duan et al.,4 for a recent review, see Mostaid and colleagues5). Despite this, continuing to assess the role of abnormal NRG1 signaling in schizophrenia is warranted due to the heterogeneity of NRG1 in schizophrenia6 (which may explain the difficulties in detecting association in genetic studies), the numerous preclinical studies which illustrate the potency by which Nrg1 perturbations induce schizophrenia-like phenotypes, the studies of postmortem brain which implicated altered Nrg1/ErbB4 levels in the disease5 and also a recent meta-analysis of NRG1 in schizophrenia which supports that a genetic association may exist in some populations.7 The NRG1 gene encodes the NRG1 protein, which mediates cell survival, synaptic plasticity, neuronal migration, and myelination.8,9 Over 30 different unique isoforms of NRG1 are categorized into 6 main types (types I–VI) depending on the identity of proteins in the N-terminus.10,11 The type III isoform (NRG1 III) is unique as it contains a cysteine-rich domain in the N-terminus and a transmembrane domain and thus, possesses 2 cleavable transmembrane regions.10,12 NRG1 III is also the most highly expressed NRG1 isoform type in the...
adult human and rodent brain suggesting that it may be the most neurobiologically significant and behaviorally potent NRG1 type.13

Recently, we identified a putative molecular mechanism for increasing genetic risk for schizophrenia whereby inheriting more copies of the NRG1 schizophrenia risk haplotype (named HapICE) increases the cortical expression of NRG1 III mRNA,6 while another study has shown that the ratio of NRG1 II mRNA to NRG1 III is decreased in schizophrenia.14 This compliments earlier studies where elevated levels of brain NRG1 I and IV mRNA,14,15 NRG1 protein16 and increased NRG1 signaling in postmortem tissue from schizophrenia patients have been observed.17 Together, this evidence suggests that a gain of function of NRG1 may serve as a contributing factor to the pathophysiology of this mental disorder although there is debate about which isoform of NRG1 contributes most strongly to schizophrenia.14,18

Genetic disruptions that either model decreased or increased expression of particular Nrg1 isoforms can confer distinct schizophrenia-like behavioral phenotypes. A mouse model with a haploinsufficiency for Nrg1 III has impaired prepulse inhibition (PPI) and cognitive deficits in delayed alternation in the T-maze.19 In gain-of-function Nrg1 mouse models, Nrg1 type I overexpression leads to hyperlocomotion, deficits in learned fear to a context (but not to a cue)20,21 as well as reduced cognitive performance in spatial tasks22 social interaction, impaired social recognition memory, and PPI deficits.23 The behavioral impact of expected neurobiological change resulting from inheriting the NRG1 HapICE, NRG1 III overexpression, has not yet been behaviorally characterized even though an overexpressing Nrg1 III transgenic mouse model was created in 2004.24 This Nrg1 III mouse is not suited for assessing schizophrenia-like behaviors as the neuromuscular junction in these mice is severely compromised and exhibits a tremor.25 Thus, we sought to construct a mouse that could be validated for both overproduction of brain Nrg1 III and for schizophrenia-like behavioral and brain biological phenotypes.

Materials and Methods

Generation of Transgenic Animals

Briefly, a transgenic mouse with CamKII promoter driven Neuregulin 1 type III was generated on a C57BL/6NTac background (for details, see supplementary methods).

Test Animals

For our behavioral studies, male heterozygous Neuregulin 1 type III overexpressing mice (Nrg1 III tg) (n = 10) and male wild type (WT) (n = 10) control littermates aged 28 (±2) weeks were used. For mRNA assays, male Nrg1 III tg mice and WT aged 28 days and 39.5 (±2.5) weeks were used, while mice aged 39.5 (±2.5 weeks) were used for protein western blotting. 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 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (ACEC#14/49A) (refer to supplementary methods).

Molecular Confirmation of Nrg1 Type III mRNA

Overexpression and Transcriptional Changes of 4 Genes from Hippocampal Microarray

Total RNA from the olfactory bulb, prefrontal cortex, dorsal striatum, hippocampus, and cerebellum of Nrg1 III tg and WT mice was assayed via standardized quantitative real-time polymerase chain reaction procedure with TaqMan probes to Nrg1 III, and to targets identified by microarray (RIKEN cDNA 181005G02, potassium inwardly rectifying channel member 13, and transthyretin). mRNA expression was normalized to the geometric mean of reference genes (see supplementary materials for full details). Total protein was isolated from the prefrontal cortex and Nrg1 was quantified using western blotting with an antibody specific to the C-terminus of the Nrg1 protein (1:300, sc-348, Santa Cruz Biotechnology, refer to supplementary materials for details).

Microarray

Hippocampal RNA of WT (n = 10) and Nrg1 III tg (n = 8) mice (aged 39.5 [±2.5] weeks) were assessed using the Affymetrix GeneChip Mouse Gene 2.0 ST Array, performed by The Ramaciotti Centre for Genomics as per the manufacturer’s instructions (for further details, see supplementary materials).

Behavioral Testing

Behavioral tests were performed in the following order: elevated plus maze (EPM; anxiety and exploration), social interaction (sociability), Y-maze (spatial memory), social preference task (social preference and recognition memory), prepulse inhibition (sensorimotor gating), fear conditioning (fear associated memory), and 15 min baseline open field (exploration and locomotion) with an additional 90-min period in open field after MK-801 hydrogen malate (0.25 mg/kg, [injection volume of 10 ml/kg body weight] subcutaneous injection: Sigma–Aldrich) challenge (behavioral sensitivity to N-methyl-D-aspartate [NMDA] receptor antagonism). Finally, mice underwent a comprehensive physical exam, which screened for sensory, motor function, and neurological behavioral deficits. The behavioral tasks and protocols used are described in our previous publications26–32 (for details of all tests, see supplementary material).

Statistical Analysis

For statistical procedures, please refer to the supplementary materials.
Results

*Nrg1* Type III mRNA and Protein Levels

We found a >5 times increase of normalized *Nrg1* III mRNA in the olfactory bulb [$t(17) = 14.0, P < .0001$], prefrontal cortex [$t(17) = 34.3, P < .0001$], dorsal striatum [$t(17) = 6.8, P < .0001$], hippocampus [$t(17) = 13.0, P < .0001$] and a ~30% increase in the cerebellum of heterozygous *Nrg1* III overexpressing (*Nrg1* III tg) mice compared with WT mice [$t(16) = 3.0, P < .01$] (figure 1A) confirming that insertion of multiple copies of the transgene lead to robust forebrain *Nrg1* type III overexpression. Supporting this, relative levels of full-length and cleaved Nrg1 protein from the prefrontal cortex across

![Graph A: Normalized relative levels of Nrg1 type III mRNA expression in the olfactory bulb, prefrontal cortex, dorsal striatum, hippocampus, and cerebellum.](image)

![Graph B: Representative immunoreactive bands for Nrg1 protein with interpolated approximate band sizes.](image)

![Graph C: Normalized levels of Nrg1 protein expression in the prefrontal cortex.](image)

![Graph D: Normalized Nrg1 type III mRNA levels in the prefrontal cortex and hippocampus of postnatal day 28–29 and adult (40–42 weeks old) Nrg1 type III transgenic and WT mice.](image)

![Graph E: Volcano plot of hippocampal microarray with 4 representative significantly changed genes across genotype identified.](image)

![Graph F: qPCR mRNA expression of the 3 representative genes.](image)

Fig. 1. *Nrg1* type III mRNA and protein levels, hippocampal microarray, and validating qPCR in WT and *Nrg1* III transgenic mice. (A) Normalized relative levels of *Nrg1* type III mRNA expression in the olfactory bulb, prefrontal cortex, dorsal striatum, hippocampus, and cerebellum, (B) representative immunoreactive bands for Nrg1 protein with interpolated approximate band sizes, (C) normalized levels of Nrg1 protein levels in the prefrontal cortex, (D) normalized *Nrg1* type III mRNA levels in the prefrontal cortex and hippocampus of postnatal day 28–29 and adult (40–42 weeks old) *Nrg1* type III transgenic and WT mice, (E) volcano plot of hippocampal microarray with 4 representative significantly changed genes across genotype identified and (F) qPCR mRNA expression of the 3 representative genes. Data for control (WT) and *Nrg1* type III transgenic (*Nrg1* III tg) mice are shown as mean + SEM. Significant genotype effects vs WT are shown as “*” (*P < .05, **P < .01, and ***P < .001). Trend genotype effects vs WT is shown as “T” (T P = .053). Significant effect between postnatal day 28–29 and adult (40–42 weeks old) in *Nrg1* III tg mice is shown as “++” (++P < .01).
several band sizes were elevated in transgenic mice compared with WT mice (126 kDa) \[t(14) = 2.4, P < .02\], (82 kDa) \[t(13) = 2.6, P < .02\], (77 kDa) \[t(13) = 1.7, P = .06\], (64 kDa) \[t(13) = 3.6, P < .002\], (54 kDa) \[t(13) = 1.6, P = .07\], (44 kDa) \[t(14) = 2.4, P < .02\], (40 kDa) \[t(12) = 3.1, P < .004\] (figures 1B and 1C).

To assess whether the transgene expression of Nrg1 type III mRNA varies with age, Nrg1 type III mRNA expression levels in Nrg1 III tg and WT mice were measured at 2 time points: juvenile (postnatal day 28 [P28]) and adult (39.5 \[±2.5\] weeks) and in 2 brain regions (prefrontal cortex and hippocampus) (figure 1E). We found a significant “genotype” by “age” interaction in the prefrontal cortex \[F(1, 25) = 11.8, P = .002\] whereby Nrg1 type III mRNA in Nrg1 III tg adult mice was significantly elevated compared with Nrg1 IIItg P28 mice \(P < .001\). This effect was not seen in WT mice. For the hippocampus, we did not detect an “age” by “genotype” interaction effect for Nrg1 type III mRNA levels. For both ages and for both regions, Nrg1 type III mRNA levels were significantly increased in Nrg1 IIItg mice compared with the respective age group of WT mice \(P < .0001\).

**Hippocampal Microarray mRNA Assay Results and Confirmation via quantitative polymerase chain reaction**

A total of 187 differentially expressed transcripts were detected in Nrg1 IIItg mice compared with WT (figure 1D and supplementary materials). Of the 187 transcripts, we selected 3 (2 of those most differentially expressed and 1 at moderate level of significance) and assessed the mRNA expression of these transcripts via qPCR in order to verify the results and robustness of the microarray analysis: RIKEN cDNA 181005G020 (upregulated compared with WT) \[t(15) = 16.9, P < .0001\], Potassium inwardly rectifying channel, subfamily J member 13 (downregulated compared with WT) \[t(16) = 2.7, P = .02\] and Transhyretin (trend downregulated compared with WT) \[t(17) = 2.1, P = .053\] (figure 1F). After validating the microarray, we performed a PubMed literature search on the 187 differentially expressed transcripts and found several downregulated genes that have been implicated in schizophrenia, including 5-hydroxytryptamine (serotonin) receptor 2C,\(^{23}\) angiotensin converting enzyme,\(^{34}\) sulfatase 1,\(^{35}\) and transhyretin.\(^{36}\) Additionally, we screened for immunologically associated transcripts as Nrg1 has been shown to regulate the immune response in the brain\(^{37}\) and abnormal neuroinflammatory processes have been linked to schizophrenia.\(^{38,39}\) In total, we found several altered immune-related transcripts including Serpinb1b, Mcf7d2, Fgl2, Cldn9, Gnas, Prlfr, Gulp1, Cldk6, Spint2, Lbp, Ltc4s, Mir9-1, Sulfl, Igi27, KI, Ddb2, Trpm3, Bcld2a1a, C1qtnf5, Cldn2, F5, Lepr, Psmnb5-ps, Ucp2, Slek39a4, and 1500015Q10Rik (Ecrg4). Finally, several members of the insulin-like growth factor (Igf) pathway including Igf2, Igf binding protein 2 (Igfbp2), and Igfbp7 appear to be downregulated in Nrg1 IIItg mice. These transcripts may be of interest as Igf signaling, like Nrg1 signaling, mediates neurogenesis and cell survival.\(^{40}\)

**Physical Exam and General EPM Behavior**

Nrg1 IIItg mice did not display deficits in motor functions, neurological behaviors or in any of the sensory abilities measured in this test compared with WT mice (all \(P > .05\); table 1). Using one-way ANOVA, no differences between Nrg1 IIItg and WT mice were observed for measures of time spent in open arms, percentage of open arm entries, overall distance travelled in the arms of the maze nor for duration or frequency of grooming, head-dipping, rearing, and stretch-attend posture (all \(P > .05\); table 1).

**Social Behaviors**

No differences were detected in the social interaction test between Nrg1 IIItg and WT mice for duration and frequency of social behaviors or for overall active social interaction time (all \(P > .05\); table 1). When analyzing the sociability trial of the social preference test, we found that both WT and Nrg1 IIItg mice spent more time in the chamber containing a mouse than the empty chamber \("chamber" \(F(1, 18) = 9.3, P = .007\); no “chamber” by “genotype” interaction] (figure 2A) indicating normal preference for social stimuli, supporting our findings in the social interaction test. However, in the social recognition/preference memory test, we found a significant “chamber” by “genotype” interaction effect \(F(1, 18) = 4.7, P = .04\) where WT mice spent a significantly higher percentage of time in the chamber containing the novel mouse as compared with the familiar mouse \(P = .03\) whereas the Nrg1 IIItg mice did not demonstrate this preference for the novel mouse \(P = .5\). In agreement with this, WT mice spent a significantly higher percentage of time in the chamber containing the novel mouse than by chance \(58.97\%, t(18) = 2.2, P = .04\), whilst the time spent by Nrg1 IIItg mice in the chamber containing the novel mouse was lower and was not significantly different than chance \(47.20\%, t(18) = 8, P = .44\).

**Cognitive Behaviors**

For the Y-maze, we did not detect a “genotype” effect on percentage distance travelled in the novel arm, which was above chance \(33\%\) for both genotypes \([t\text{-test: }\text{WT: }42.3\%, t(18) = 5.0, P < .0001; \text{Nrg1 IIItg: }41.4\%, t(18) = 4.2, P < .0001\]; and both genotypes performed similarly for percentage novel arm entries and for time spent in novel arm: table 1). For fear-associated learning, Nrg1 IIItg mice had WT levels of baseline freezing in the first 2 min of the conditioning trial \(F(1, 17) = 0.4, P = .5\) and in the first 2 min of the cue trial, prior to cue presentation \(F(1, 17) = 1.2, P = .3\). In contrast, Nrg1 IIItg mice displayed reduced freezing levels compared with WT mice.
Transgenic Mouse Model for Nrg1 Type III

during the first 2 min of the context trial \(F(1, 17) = 6.4, P = .02\) (figure 3A). Additionally, Nrg1 III tg mice exhibited an overall reduction in total time spent freezing to the context compared with WT mice \(F(1, 17) = 4.7, P < .05\) indicating a deficit in contextual fear conditioning (figure 3B). In the cue test, there was no difference in total freezing duration between genotypes during cue presentation \(F(1, 15) = 1.8, P = .2\). There was a significant “genotype” by “time” interaction across 1 min blocks during cue presentation \(F(4, 15) = 3.4, P = .02\); however, we did not find any significant differences across genotype at individual time-points by post hoc analysis (figure 3C).

Furthermore, freezing duration prior to cue presentation (ie, first 2 min) and during cue presentation (ie, third to seventh min) was not different between Nrg1 III tg mice and WT.

Prepulse Inhibition

For PPI testing, we found that all mice exhibited an increasing startle response to increasing startle stimuli \(F(1, 18) = 40.6, P < .001\); “startle” by “genotype”:

\[\text{Table 1. Results for Behavioral Measures from the Elevated Plus Maze (EPM), Social Interaction Test (SI), Y-Maze (YM), Open Field (OF), Prepulse Inhibition (PPI), Accelerod, and Beam Walking Task}\]

<table>
<thead>
<tr>
<th>Behavioral Test</th>
<th>Parameter</th>
<th>WT</th>
<th>Nrg1 III tg</th>
<th>F (df)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPM</td>
<td>Time in open arms [s]</td>
<td>9.4 ± 6.9</td>
<td>20.8 ± 6.2</td>
<td>1.5 (1, 18)</td>
<td>P = .2</td>
</tr>
<tr>
<td></td>
<td>Time in open arms [%]</td>
<td>4.2 ± 3.2</td>
<td>9.4 ± 2.9</td>
<td>1.5 (1, 18)</td>
<td>P = .2</td>
</tr>
<tr>
<td></td>
<td>Overall distance travelled [m] (meters)</td>
<td>10.5 ± 0.8</td>
<td>10.4 ± 0.9</td>
<td>0.002 (1, 18)</td>
<td>P = 1</td>
</tr>
<tr>
<td></td>
<td>Stretch-attend posture [n]</td>
<td>27.3 ± 2.3</td>
<td>23.7 ± 2.2</td>
<td>1.3 (1, 19)</td>
<td>P = .3</td>
</tr>
<tr>
<td></td>
<td>Stretch-attend posture [s] (seconds)</td>
<td>34.6 ± 3.3</td>
<td>28.9 ± 3.0</td>
<td>1.6 (1, 18)</td>
<td>P = .2</td>
</tr>
<tr>
<td></td>
<td>Head-dipping [n]</td>
<td>17.2 ± 2.4</td>
<td>14.3 ± 2.4</td>
<td>0.8 (1, 18)</td>
<td>P = .4</td>
</tr>
<tr>
<td></td>
<td>Head-dipping [s]</td>
<td>12.3 ± 1.7</td>
<td>10.3 ± 1.6</td>
<td>0.7 (1, 18)</td>
<td>P = .4</td>
</tr>
<tr>
<td></td>
<td>Rearing [n]</td>
<td>13.5 ± 2.5</td>
<td>14.8 ± 2.4</td>
<td>0.1 (1, 18)</td>
<td>P = .7</td>
</tr>
<tr>
<td></td>
<td>Rearing [s]</td>
<td>9.3 ± 1.7</td>
<td>10.9 ± 1.7</td>
<td>0.4 (1, 18)</td>
<td>P = .5</td>
</tr>
<tr>
<td></td>
<td>Grooming [n]</td>
<td>8.2 ± 1.3</td>
<td>7.2 ± 1.2</td>
<td>0.3 (1, 18)</td>
<td>P = .6</td>
</tr>
<tr>
<td></td>
<td>Grooming [s]</td>
<td>17.2 ± 2.9</td>
<td>12.0 ± 2.8</td>
<td>1.7 (1, 18)</td>
<td>P = .2</td>
</tr>
<tr>
<td>SI</td>
<td>Total social interaction time [s]</td>
<td>83.7 ± 10.3</td>
<td>87.3 ± 10.8</td>
<td>0.1 (1, 19)</td>
<td>P = .8</td>
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<tr>
<td></td>
<td>Nosing/sniffing [n]</td>
<td>55.6 ± 3.8</td>
<td>57.5 ± 4.0</td>
<td>0.1 (1, 19)</td>
<td>P = .7</td>
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<tr>
<td></td>
<td>Nosing/sniffing [s]</td>
<td>54.7 ± 7.4</td>
<td>58.7 ± 7.8</td>
<td>0.1 (1, 19)</td>
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<td></td>
<td>Anogenital sniffing [n]</td>
<td>19.6 ± 2.7</td>
<td>19.3 ± 2.8</td>
<td>0.01 (1, 19)</td>
<td>P = .9</td>
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<td>Anogenital sniffing [s]</td>
<td>17.5 ± 2.5</td>
<td>18.4 ± 2.6</td>
<td>0.1 (1, 19)</td>
<td>P = .8</td>
</tr>
<tr>
<td></td>
<td>Crawling over [n]</td>
<td>9.1 ± 1.4</td>
<td>8.2 ± 1.4</td>
<td>0.2 (1, 18)</td>
<td>P = .6</td>
</tr>
<tr>
<td></td>
<td>Crawling over [s]</td>
<td>8.1 ± 1.8</td>
<td>6.2 ± 1.9</td>
<td>0.5 (1, 19)</td>
<td>P = .5</td>
</tr>
<tr>
<td></td>
<td>Following [n]</td>
<td>4.3 ± 1.1</td>
<td>5.2 ± 1.2</td>
<td>0.4 (1, 18)</td>
<td>P = .6</td>
</tr>
<tr>
<td></td>
<td>Following [s]</td>
<td>3.4 ± 0.8</td>
<td>3.0 ± 0.9</td>
<td>0.1 (1, 18)</td>
<td>P = .7</td>
</tr>
<tr>
<td>YM</td>
<td>Time in novel arm [%]</td>
<td>32.1 ± 7.7</td>
<td>35.3 ± 6.2</td>
<td>1.0 (1, 18)</td>
<td>P = .3</td>
</tr>
<tr>
<td></td>
<td>Distance in novel arm [%]</td>
<td>42.3 ± 5.0</td>
<td>41.4 ± 5.2</td>
<td>0.1 (1, 18)</td>
<td>P = .7</td>
</tr>
<tr>
<td></td>
<td>Time in entries in novel arm [%]</td>
<td>37.3 ± 5.2</td>
<td>39.7 ± 4.8</td>
<td>1.2 (1, 18)</td>
<td>P = .3</td>
</tr>
<tr>
<td>OF</td>
<td>Distance travelled at baseline [cm]</td>
<td>1678.9 ± 503.1</td>
<td>1758.0 ± 258.9</td>
<td>0.2 (1, 19)</td>
<td>P = .7</td>
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<tr>
<td>PPI</td>
<td>Overall average PPI [%]</td>
<td>50.0 ± 4.4</td>
<td>32.6 ± 4.4</td>
<td>5.3 (1, 18)</td>
<td>P = .03</td>
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<tr>
<td>Accelerod</td>
<td>Latency to fall [s]</td>
<td>158.7 ± 48.3</td>
<td>199.4 ± 60.4</td>
<td>2.7 (1, 16)</td>
<td>P = .1</td>
</tr>
<tr>
<td>Beam walking task</td>
<td>Latency to reach platform [s]</td>
<td>40.2 ± 25.1</td>
<td>26.4 ± 17.9</td>
<td>1.6 (1, 16)</td>
<td>P = .2</td>
</tr>
</tbody>
</table>

Fig. 2. Social preference testing. (A) Sociability (ie, preference for spending time in social chamber/empty chamber) (%) and (B) social recognition memory (ie, preference for spending time with a novel mouse/familiar mouse) (%), where significant genotype effects vs WT results are shown as “*” (*P < .04), while “#” above line indicates “chamber” by “genotype” interaction (*P < .04). Data for control (WT) and Nrg1 type III transgenic (Nrg1 III tg) mice are shown as mean ± SEM.
$F(2, 18) = 0.8, P = .4$] and increased PPI with increasing prepulse intensities [$F(1, 18) = 75.4, P < .001$] (figure 3D). A PPI deficit was detected as the %PPI of Nrg1 III $tg$ mice was significantly reduced at prepulse intensities of 74 [$F(1, 18) = 5, P = .04$] and 86 dB [$F(1, 18) = 7.6, P = .01$], but not at 82 dB [$F(1, 18) = 1.7, P = .2$] (figure 3D). In line with this finding, Nrg1 III $tg$ mice exhibited significantly lower PPI averaged across prepulse intensities compared with WT mice [$F(1, 18) = 5.3, P = .03$] (table 1). We also assessed whether the level of Nrg1 type III mRNA overexpression negatively correlated with PPI performance by correlating Nrg1 type III mRNA levels in the prefrontal cortex of behaviorally tested Nrg1 III $tg$ and WT mice with their %PPI. We found that increasing

Fig. 3. Fear-associated memory, sensorimotor gating (ie, prepulse inhibition) and correlation analysis: Nrg1 type III mRNA levels with percentage prepulse inhibition. (A) Time spent freezing (s) in the first 2 min of conditioning trial, context trial and cue trial, (B) total freezing time (s) during context test, (C) time spent freezing (s) during cue trial, shown in 1-min blocks, (D) percent prepulse inhibition (%) at 3 different prepulse intensities, and (E) inverse correlation of prefrontal cortex Nrg1 type III expression with percentage prepulse inhibition performance ($r = $ Pearson's correlation co-efficient). Significant genotype effects vs WT results are shown as “*” and “**” ($*p < .05$, **$p < .001$), while “#” above line indicates “time” by “genotype” interaction ($*p < .05$). Data for control (WT) and Nrg1 type III transgenic (Nrg1 III $tg$) mice are shown as mean + SEM.

Nrg1 type III mRNA levels correlated with impaired PPI performance \( r = -.53, P = .03 \) (figure 3E).

**Baseline and MK-801-Induced Open Field Behavior**

We detected a significant effect of “time” for distance travelled during the first 15 min of the open field test indicating intact habituation \( F(14, 16) = 3.3, P = .007 \); however, we did not detect a significant “time” by “genotype” interaction effect (figure 4). Furthermore, overall baseline locomotion during these 15 min was almost identical between genotypes \( F(1, 19) = .2, P > .7 \) (table 1). Comparing baseline locomotion to the first 15 min following drug treatment showed a significant effect of “time” for total distance travelled \( F(1, 16) = 29.2, P < .001 \), while no “time” by “genotype” interaction was found \( F(1, 16) = .1, P = .7 \). Locomotion post drug treatment across 5-min blocks was increased as a result of the MK-801 treatment [RM ANOVA for “drug”:

\[ F(1, 17) = 37.6, P < .001 \] ; however, no “genotype” or “drug” by “genotype” interaction effect was detected (figure 4B).

**Discussion**

Here, we created a transgenic mouse that mimics the overexpression of Nrg1 type III in the brain that is linked to increased genetic risk of developing schizophrenia. We find that this mouse can serve as a model of aspects of schizophrenia-like behavior (ie, endophenotypes) as deficits in social recognition memory, contextual fear conditioning, and sensorimotor gating were detected. However, more general social behaviors, baseline locomotion and exploration, locomotor response to MK-801, and anxiety did not appear to be altered. Additionally, we have uncovered schizophrenia-related transcriptional changes in the hippocampus of Nrg1 III tg mice.

Nrg1 III tg mice did not possess sensory, neurological reflex, or motor deficits in the domains measured; nor do they have a generalized anxiety phenotype. This suggests that effects of Nrg1 III overexpression on schizophrenia-like behavior is a consequence of impaired higher order processing of information, and not of an underlying physical impairment or stress response-related differences.

Assessing social interaction in our mice was of interest as schizophrenia patients have difficulty in forming and maintaining meaningful social relationships.41 We found that Nrg1 III tg mice preferred the presence of another mouse over an empty chamber. However, Nrg1 III tg mice failed to exhibit a normal preference toward a novel mouse, suggesting that an overexpression of Nrg1 type III might contribute toward an aversion or lack of preference toward social novelty. Similarly, Nrg1 transmembrane domain heterozygous mice have normal levels of sociability in social preference task but have reduced social preference for the novel mouse. 42 Two separate mouse lines with CamKIIα promoter driven overproduction of Nrg1 type I exhibit both social withdrawal and a loss of preference for the novel mouse. 21,43 These results, in tandem with ours, highlight the importance of isoform specific levels of Nrg1 in relation to each other in mediating social deficits that may resemble the negative symptoms of schizophrenia.

Cognitive symptoms in schizophrenia patients correlate with structural and functional abnormalities in brain regions including the hippocampus (HPC).44 Fear conditioning in rodents requires highly integrated brain circuitry which includes the HPC.45 We found impoverished fear-associated memory to context in the Nrg1 III tg mouse, a result which occurs across numerous other Nrg1 perturbations. 35,43,46,47 We found alterations in the hippocampal transcriptome of Nrg1 III tg mice that may provide clues in uncovering the mechanisms behind this behavioral phenotype. One downregulated transcript...
relating to the serotonin pathway, 5-hydroxytryptamine (serotonin) receptor 2C, has been shown to be reduced in schizophrenia patients and has also been shown to modulate conditioned fear to context in rodents. Due to our findings, we intend to undertake further investigations into the signaling pathway of a few of these candidate genes and assess whether Nrg1 type III upregulation may disrupt these pathways in the hippocampus and cause deficits in fear conditioning in Nrg1 III tg mice.

In addition to altered 5-hydroxytryptamine (serotonin) receptor 2C, our microarray revealed other altered mRNA transcripts in the hippocampus of Nrg1 III tg mice which bear relevance to schizophrenia including changes in angiotensin converting enzyme, sulfatase 1, and transhyretin. Interestingly, the DiGeorge critical region gene 8 has been shown to be reduced in schizophrenia and has been implicated in the signaling pathway of a few of these candidate genes. Our findings suggest that fear response to the conditioned cue over an extended period of exposure may be altered and future work on the extinction of a learned fear might be warranted. At present, only one study has assessed the role of Nrg1 on extinction fear memory, wherein hypomorphic Nrg1 II rats froze less during extinction trials to a discrete cue 96 h after conditioning. Similarly to our finding, both full-length and N-terminal fragment overexpression of Nrg1 does not appear to elicit a deficit in learned fear to cue, suggesting that an upregulation of Nrg1 may not play a role in disrupting basic learned fear to a discrete cue.

Our results suggest that short-term spatial memory is intact with forebrain Nrg1 type III overexpression as Nrg1 III tg mice exhibited WT levels of exploration of the novel arm on the Y-maze. Other Nrg1 mouse models, however, exhibit impairments in short-term working spatial memory in a variety of cognitive tasks. The heterozygous Nrg1 type III knockout mouse has deficits in 60 s delayed spontaneous alternation while an Nrg1 type I overexpressing mouse has decreased performance in several spatial memory tasks, which is rescued when transgene expression is shut-off in adulthood. Together, these results suggest that short-term spatial memory deficits in Nrg1 mutant mice might be isoform dependent and/or vulnerable to changes in the specific concentration of these Nrg1 isoforms relative to one another.

Our Nrg1 III tg mouse failed to have the expected baseline or drug-induced increases in locomotion. Baseline ambulatory distance travelled in an open field arena did not appear to be impacted by Nrg1 type III overexpression. This is in alignment with the heterozygous Nrg1 type III knockout mouse that also does not exhibit hyperlocomotion. Furthermore, several other Nrg1 I overexpressing mice only show a locomotor phenotype under certain conditions (eg, dependent on age or drug treatment). We did not observe a genotype difference in mice exposed to MK-801, suggesting that Nrg1 type III overexpression does not alter the response to NMDA receptor perturbation known to induce hyper-locomotion. Our study possesses limitations, including the use of only male mice. It is important to interrogate the molecular and behavioral phenotypes in both male and female
mice in order to assess how abnormal Nrg1 signaling may be contributing to the schizophrenia-like symptoms in both males and females. Thus, using the effect sizes from this study, we intend to follow-up with comprehensive characterization of female Nrg1 III tg mice. Another limitation is that we only used one transgenic line and thus cannot rule out the impact of transgene insertion on the phenotypes observed in the Nrg1 III tg mouse. Additionally, the overexpression of forebrain Nrg1 type III mRNA in Nrg1 III tg mice was >5 times higher than WT mice while schizophrenia patients carrying NRG1 HapICE alleles only have a ~30% elevation in Nrg1 type III mRNA compared with non NRG1 HapICE allele carriers. However, it is worth mentioning that several Nrg1 type I transgenic mouse models also overexpress Nrg1 by a magnitude of >4 times. Another limitation of the study is that we did not assess whether antipsychotics are capable of reversing the schizophrenia-like behavioral deficits exhibited by Nrg1 III tg mice (ie, test predictive validity of model). Future studies may consider including an antipsychotic treatment in this transgenic mouse model.

In sum, the results of this study indicate that forebrain driven overexpression of Nrg1 III in a novel mouse model causes schizophrenia-like deficits across several behavioral domains and supports the candidacy of this transgenic mouse model for future studies. Given that a subset of schizophrenia patients carry copies of the NRG1 HapICE gene, which appears to upregulate NRG1 III in their forebrain, evidence from the current study gives weight toward the role of forebrain NRG1 III overexpression contributing toward some of the clinical symptoms of the disease, at least in a subset of individuals. Additionally, these findings also highlight the use of this Nrg1 III overexpressing model to assess the biological consequences of the upregulation of this isoform type. Future research will need to be conducted in order to extend our understanding of forebrain driven Nrg1 type III overexpression on schizophrenia related phenotypes. For example, it will be relevant to assess if NRG1 type III overexpression (eg, in PFC) is linked to increased ErbB4 activity. In line with our previous work, we will also consider sex effects and gene–environment interactions in our new model.

Supplementary Material

Supplementary data are available at Schizophrenia Bulletin online.

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Contributions

T.K. and C.S.W. with J.C.O. designed the study and wrote the protocol. C.L.M. and M.M. constructed and generated the mouse. J.C.O. performed the experiments. J.C.O. wrote the first draft. J.C.O., T.K., D.S., and M.K. were responsible for the data analysis. J.C.O., T.K., C.L.M., M.M., D.S., M.K., and C.S.W. reviewed and revised the manuscript draft. Each of the authors has reviewed the manuscript and has approved the final manuscript.

References


