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Behavioral Profile of a Heterozygous Mutant Mouse Model for EGF-Like Domain Neuregulin 1

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Human genetic studies have demonstrated that the neuregulin 1 gene (*NRG1*) is involved in the development of schizophrenia. Alternative splicing of *NRG1* results in at least 15 distinct isoforms and all contain an extracellular epidermal growth factor (EGF)-like domain, which is sufficient for Nrg1's biological activity. Here, we characterize a heterozygous mutant model for mouse EGF-like domain neuregulin 1 (*Nrg1*) regarding schizophrenia-related behavioral domains. A comprehensive, multitiered phenotyping strategy was used to investigate locomotion, exploration, anxiety-related behaviors, and sensorimotor gating. *Nrg1* mutant mice exhibited a hyper-locomotive phenotype and an improved ability to habituate to a new environment. Extensive analysis of anxiety-related behaviors revealed a wild type-like phenotype in this domain. However, a moderate impairment in sensorimotor gating was found after pharmacological challenge using psychoactive substances. Our study adds to the increasing behavioral data available from a variety of animal models for *Nrg1* isoforms. We suggest a standardized and comprehensive behavioral phenotyping approach to distinguish between the different models and to clarify their relevance for schizophrenia research. Future behavioral investigations will focus on the negative and cognitive symptoms of schizophrenia.

Keywords: EGF-like domain neuregulin 1, mouse model for schizophrenia, motor activity, exploration, sensorimotor gating

Neuregulin 1 (Nrg1) is part of the growth factor family and is characterized by an epidermal growth factor (EGF)-like domain. The protein activates two ErbB receptor tyrosine kinases (i.e., ErbB3 and ErbB4) whereas a third, ErbB2, does not bind Nrg1 itself but can heterodimerize with either Nrg1-bound ErbB3 or ErbB4. Nrg1 mRNA has been detected in the prefrontal cortex, hippocampus, cerebellum, and substantia nigra—in both humans (Law et al., 2006) and rodents (Kerber, Streif, Schwaiger, Kreutzberg, & Hager, 2003). The human *NRG1* gene is localized on chromosome 8p12-p21, is approximately 1.4 megabases long and less than 0.3% of this span encodes protein (Falls, 2003). Alternative splicing of *NRG1* results in at least 15 distinct isoforms, which are classified by (1) their *N*-terminal sequence (Type I-II have an immunoglobulin-like domain whereas Type III has a cysteine-rich domain), (2) the variant of the EGF-like domain (α or β; the β-type is more potent and predominates in the brain), and (3) the (non-)existence of a transmembrane (TM) region (Type I-II contain a *C*-terminal TM region whereas Type III is characterized by a *C*- and a *N*-terminal TM region) (Walss-Bass et al., 2006). Recently, Steinthorsdottir and colleagues have described transcripts containing additional 5′ exons, which encode for novel isoforms and are designated Type IV-VI (Steinthorsdottir et al., 2004).

In the last decade, genetic, functional, and morphological evidence has accumulated that *NRG1* is implicated in the development of schizophrenia. Stefansson and coworkers discovered an association between *NRG1* and an increased risk for schizophrenia (Stefansson et al., 2002), which was recently confirmed by a meta-analysis (Munafo, Thiselton, Clark, & Flint, 2006). Most of

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the reported *NRG1* polymorphisms associated with schizophrenia are noncoding, suggesting that they have an impact upon expression, transcription, mRNA degradation, or translation of *NRG1* (Harrison & Law, 2006). Within the nervous system, Nrg1 has an impact on key neurodevelopmental processes such as synapse formation, myelination, regulation of expression/activation of receptors (e.g., NMDA and $GABA_A$) and neuronal migration (Corfas, Roy, & Buxbaum, 2004; Harrison & Law, 2006). All these processes are thought to be involved in schizophrenia. Importantly, in the prefrontal cortex Nrg1 stimulation suppresses NMDA receptor activation to a greater extent in schizophrenia patients than in healthy subjects (Hahn et al., 2006) and schizophrenia patients also exhibit increased Nrg1 Type I expression levels as well as decreased Type II/Type I and Type II/type III expression level ratios (Hashimoto et al., 2004).

In the last two decades, laboratories worldwide have developed a multitude of genetic mouse models for a variety of mouse *Nrg1* gene isoforms and their ErbB receptors (developed mostly as heterozygous models when homozygotes are nonviable). The majority of these models have been investigated behaviorally to a varied extent, revealing phenotypes ranging from hyperactivity, impaired habituation to novelty, prepulse and latent inhibition deficits, elevated anxiety and disruption of learning processes to an increased susceptibility to environmental enrichment and Δ^9-tetrahydrocannabinol treatment. Nonetheless, a few models have demonstrated no behavioral abnormalities compared to WT mice (for overview see Table 1).

In this study, we focus on the behavioral profiling of a heterozygous EGF-like domain *Nrg1* mouse model developed by Meyer and Birchmeier (Meyer & Birchmeier, 1995), which has so far been characterized only in regard to basic motor behavior [as measured by bar test and rotarod (Michailov et al., 2004)]. This heterozygous mutant model for the EGF-like domain of *Nrg1* allows the investigation of Nrg1's impact on general behavior and selected schizophrenia-related domains, as all known isoforms of *Nrg1* contain an EGF-like domain. Importantly, the EGF-like domain alone is sufficient for Nrg1's biological activity (i.e., for ErbB receptor-tyrosine kinase binding and activation). Thus, heterozygous EGF-like domain *Nrg1* mutant mice provide a model in which there is a genuine 50% reduction in Nrg1 activity—most other available knockout models for *Nrg1* (except Gerlai, Pisacane, & Erickson, 2000) only provide a 50% depletion of a specific *Nrg1* isoform or Nrg1 type and are therefore limited. In addition to differences in genetic background, such variation in the degree to which Nrg1's biological activity is suppressed might be responsible for the observed variances in behavioral phenotypes of different *Nrg1* mutants (see Table 1).

To evaluate the behavioral impact of a 50% overall reduction in Nrg1 activity, we used a comprehensive, multitiered approach, including tests for basic sensory, neurological and motor functions (so-called "physical exam"; Crawley & Paylor, 1997), for locomotion/exploration, habituation and anxiety as well as for the schizophrenia-related sensorimotor gating (measured in the prepulse inhibition task). Findings are compared specifically with the behavioral phenotype of the only other available EGF-like domain *Nrg1* knockout model (described by Gerlai et al., 2000).

Materials and Method

Animals

The generation of EGF-like domain *Nrg1* mutant mice was described previously (Meyer & Birchmeier, 1995; Michailov et al., 2004). Test animals were heterozygous EGF-like domain $Nrg1^{+/-}$ (*Nrg1* EGF HET) and wild type-like control $Nrg1^{+/+}$ (WT) littermates (backcrossed in 12th generation on C57BL/6 background). We selected test animals of 11 litters from five different breeding pairs (*Nrg1* EGF HET male crossed with WT female). Genotypes were determined by tail tip biopsy and polymerase chain reaction amplification (primers for mutant *Nrg1* EGF HET: NRG4786: 5′-gag atg gtc atg tcc ttg tca cta acc −3′ and Neo2L: 5′- cga att cgc caa tga caa gac gct gg −3′ - primers for WT: NRG4786: 5′- gag atg gtc atg tcc ttg tca cta acc −3′ and NRG4807: 5′- tgc tgc ttt ctt cgc tct tca gaa gc −3′). Age-matched (±7 days), male, adult test animals (WT = 21 vs. *Nrg1* EGF HET = 14) of similar genotype were pair-housed (one cage contained three WT mice) in Macrolon cages without enrichment (except for paper tissues as nesting material) under a 12:12 hour light:dark schedule [light phase: white light (illumination: 80 lx) - dark phase: red light (illumination: <2 lx)]. Microbiological monitoring revealed no infection of the SPF facility holding room; with the exception of the pathogens commonly found in commercial and research facilities, *Pasteurella pneumotropica* and *Helicobacter spp*. All research and animal care procedures were approved by the "Garvan Institute/St Vincent's Hospital Animal Experimentation Ethics Committee" and were in accord with the "Australian Code of Practice for the Care and Use of Animals for Scientific Purposes."

Behavioral Phenotyping

For habituation purposes, all test animals were transported to the testing room 1 hour before behavioral testing. Experiments were performed within a time limit of 3 hours per day to avoid the circadian rhythm as a confounding factor on the animals' behavior (Kopp, 2001). Environmental odors were removed from the test apparatus by cleaning the test arena after each trial with 30% ethanol solution. Test order as follows (age[d] included in brackets, for abbreviations see below): GMA I (109) – LD (130) – CM (132) – GMA II (138) – MB (142) – EPM (145) – HB (151) – PPI I (baseline; 158) – PPI II (drug-induced; 170/179/187).

"Physical exam" (PE). General health, sensory abilities, neurological motor reflexes, and motor function/coordination have a significant impact on the animals' behavioral performance (Crawley & Paylor, 1997). Therefore, we used a wide range of basic tasks to control for any gross abnormalities in mutant mice (for details see Karl, Pabst, & von Horsten, 2003), which were performed after completion of all other handling-sensitive paradigms during the light phase of the light cycle.

Comprehensive Behavioral Test Battery

A brief outline of the test paradigms is described below (for more details see Boucher et al., 2007a; Karl, Duffy, Scimone, Harvey, & Schofield, 2007).

General motor activity (GMA). General motor activity was evaluated by placing the mouse into an open field activity test chamber (43.2 cm × 43.2 cm × 30.5 cm; MED Associates Inc.,

Table 1
Overview of Available Genetic Mouse Models for NRG1 and its ErbB Receptors and Their Behavioral Phenotypes

Genetic mouse model for	Described in	Behavioral phenotype of mutant mice
EGF-like domain Nrg1 (heterozygous) Background: C57BL/6	*(Michailov et al., 2004) - developed by (Meyer & Birchmeier, 1995)*	*WT-like performance in bar test and rotarod*
EGF-like domain *Nrg1* (heterozygous) Background: C57BL/6 × 129/SVEV	(Gerlai et al., 2000; Stefansson et al., 2002) - developed by (Erickson et al., 1997)	Hyperactivity in OF but no impaired habituation, WT-like SA, improved rotarod performance, 16% fewer functional NMDA receptors
Transmembrane domain *Nrg1* (heterozygous) Background: C57BL/6	(Boucher et al., 2007a, 2007b; Karl et al., 2007; O'Tuathaigh C et al., 2007; O'Tuathaigh C et al., 2006; Stefansson et al., 2002) - developed by (Stefansson et al., 2002)	Clozapine-reversible hyperactivity in OF, impaired habituation to novelty, reduced anxiety, moderate PPI deficits, WT-like spatial memory and SA, impaired response to social novelty, increased aggression; elevated susceptibility to EE and the neurobehavioural effects of Δ^9-THC - age-dependent and sexually dimorphic phenotype
Immunoglobulin-like domain *Nrg1* (heterozygous) Background: C57BL/6	(Rimer, Barrett, Maldonado, Vock, & Gonzalez-Lima, 2005) - developed by (Kramer et al., 1996)	Increased clozapine suppression of OF and running wheel activity (not at baseline), impaired LI, WT-like T-maze behavior
Nrg1 Type I and *Nrg1* Type III over-expression Background: not described	(Michailov et al., 2004)	Not investigated
ErbB2 (heterozygous) Background: C57BL/6 × 129/SVEV	(Gerlai et al., 2000) - developed by (Erickson et al., 1997)	WT-like performance in rotarod, OF and SA
ErbB2 (heterozygous) Background: C57BL/6 × 129	(Michailov et al., 2004) - developed by (Britsch et al., 1998)	WT-like performance in bar test and rotarod
ErbB3 (heterozygous) Background: C57BL/6 × 129/SVEV	(Gerlai et al., 2000) - developed by (Erickson et al., 1997)	WT-like performance in rotarod, OF and SA
ErbB3 (heterozygous) Background: C57BL/6 × 129 – no information about backcrossing available	(Michailov et al., 2004) - developed by (Riethmacher et al., 1997)	WT-like performance in bar test and rotarod
ErbB4 (heterozygous) Background: C57BL/6	(Stefansson et al., 2002) - developed by (Gassmann et al., 1995)	Hyperactive in OF but no PPI deficit
CNS-specific ErbB4 conditionals (homo- and heterozygous) Background: FVB	(Thuret et al., 2004) - developed by (Long et al., 2003)	WT-like performance in OF, rotarod and footprint patterns
CNS-specific ErbB4 conditionals (homo- and heterozygous) Background: C57BL/6	(Golub, Germann, & Lloyd, 2004)	Homozygotes: High (at weaning) versus low (adult) levels of spontaneous motor activity, reduced grip strength, WT-like rotarod and MWM performance Heterozygotes: delayed motor development (Wahlsen test battery), reduced grip strength, WT-like rotarod performance, males with altered cue use in MWM
Cysteine-rich domain Nrg1 (homozygous – lethal) Background: 129/SV	(Wolpowitz et al., 2000)	Not investigated

WT-like = wild type-like; EE = environmental enrichment; Δ^9-THC = Δ^9-tetrahydrocannabinol; OF = open field; SA = spontaneous alternation; PPI = prepulse inhibition; LI = Latent inhibition; MWM = Morris water maze.
The mutant EGF-like domain *Nrg1* line used for the current study is marked by italics.

Vermont). This paradigm mimics the natural conflict in mice between the tendency to explore a novel environment and to avoid an exposed open area (DeFries, Hegmann, & Weir, 1966) and can be used as an anxiety-related open field paradigm. The arena was divided into a central and a peripheral zone. Animals were tested at the age of (1) 109 days (GMA I: 4 hour before onset of the dark phase) and (2) 138 days (GMA II: 1 hour after onset of the dark phase) for 30 minutes (illumination at floor level for GMA I: 20 lx—for GMA II: <2 lx). The animal's horizontal activity (i.e., overall distance traveled - ODT), ambulatory frequency and episodes, vertical activity, and resting behavior were recorded automatically. The ratio of central to total distance traveled, time spent in the central zone, and the defecation score were taken as measures of anxiety (Denenberg, 1969). We also analyzed habituation to the environment over time (in total ODT per 5-min block and as percentage ODT compared to preceding 5-min block).

Light-Dark (LD). In the LD test the distance traveled, the time spent in a brightly illuminated zone compared to a dark zone, and the occurrence of associated exploratory behaviors can be used to assess anxiety in rodents (Costall, Jones, Kelly, Naylor, & Tomkins, 1989). Four hours after onset of the light phase test animals were placed into the open field activity test chamber, which was equipped with a dark box insert for mice (21.7 cm × 43.2 cm × 30.5 cm; MED Associates Inc. – illumination in light compartment: 20 lx - in dark compartment: <2 lx). At the start of the experiment, mice were placed into the light compartment. The

time spent in, entries into and distance traveled in the differentially illuminated compartments as well as vertical activity, ratio of distance traveled in the light compartment to total distance traveled (distance ratio), and the number of ambulatory episodes were recorded for the following 10 minutes.

Cross maze (CM). This task measures the explorative-like drive of rodents, mimicking the tendency of rodents to alternate their nonreinforced choices of for example, T- or Y-maze arms on successive opportunities (i.e., measure of working memory). We used an X-maze design as described by Stefansson and coworkers (Stefansson et al., 2002) and modified an eight-arm radial arm maze (69.9 cm × 9.8 cm × 20.3 cm; central platform with diameter of 38.1 cm; Pathfinder maze system Model 89000B: Lafayette Instrument, Sagamore Parkway North) for our purposes. Four of the eight arms were blocked so that only four enclosed arms (90 degree angle, illumination on arms: 70 lx) were accessible. The maze was located in a room with different external and internal visual cues. Animals were placed individually 3.5 hours before onset of the dark phase into the center arena of the apparatus. During the following 5 minutes the order of arm visits (defined as all paws being placed inside the arm) was recorded. Parameters investigated were number of repeated arm entries during the first four arm entries, number of arm entries until all arms were visited once, overall number of arm entries, frequency of rearing, and the defecation score. Furthermore, the percentages of triple and quadruple alternations were calculated; a triple/quadruple alternation was defined as the visit of three/four different arms consecutively without entering a previously visited arm.

Marble burying (MB). The marble burying test was performed in the open field activity test chambers (MED Associates Inc.). Glass marbles ($n = 12$) were spaced evenly on a 2 cm deep layer of sawdust in each chamber. Animals were placed individually into the test chambers and could explore the area without any disturbance for the following 30 minutes (3.5 hours before onset of the dark phase). After that time the number of marbles completely buried or partially buried (covered with sawdust more than one half of the marble size but not completely) was recorded as a measure of general activity level (Gyertyan, 1995).

Elevated plus maze (EPM). The EPM represents the natural conflict between the tendency of mice to explore a novel environment and the tendency to avoid a brightly lit, elevated (71.5 cm), open area (Montgomery, 1958). Open arms (50 cm × 6.0 cm—ledges 0.3 cm high) of the gray plus maze were highly illuminated (70 lx) whereas enclosed arms (50 cm × 6.0 cm × 10 cm) were only dimly illuminated (10 lx). Three hours before onset of the dark phase mice were placed individually onto the center field (6.0 cm × 6.0 cm) of the EPM facing an enclosed arm and were allowed to explore the maze for 5 minutes. Behavior was measured online. Frequency of stretch-attend postures, time spent on open arms as well as the percentage of open arm entries were recorded as measures of anxiety (Pellow, Chopin, File, & Briley, 1985). An individual entry was recorded when the animal entered the arm with at least half of its body length. The number of total arm entries was recorded as a measure of general motor activity. Frequencies of head dipping over the edges and rearing in the junction area between arms were analyzed as parameters for exploration.

Hole board (HB). The hole board test provides independent measures of locomotor activity and directed exploration (van Gaalen & Steckler, 2000). Mice were placed into the open field activity test chamber, which was equipped with a hole board floor insert for mice (16 holes—diameter 1.6 cm; MED Associates Inc.). Animals were tested 1 hour after onset of the dark phase (illumination: <2 lx). The system provided automated measures of the distance traveled, ambulatory frequency, head dipping frequency (into novel and already investigated holes), and working memory ratio (number of head dippings into novel holes divided by total number of head dippings) in a 7 minute test session.

Prepulse Inhibition (PPI). PPI is the operational measure of sensorimotor gating, in which a weak prestimulus (prepulse) attenuates the startle response (Wang, Short, Ledent, Lawrence, & van den Buuse, 2003). PPI was tested in two startle chambers (SR-Lab: San Diego Instruments, San Diego, CA; animal enclosure: length = 12.7 cm; diameter = 3.8 cm; actual length between animal enclosure inserts = 7.6 cm). The protocol used was adapted from methods developed by Geyer and Swerdlow (Geyer & Swerdlow, 1998). Briefly, after a 5 minute acclimation period with a 70 dB background noise the test session began (2 hours after onset of the light phase). Each session consisted of 76 trials in a pseudorandomized order: 10x 90 dB and 18x 120 dB startle response trials, 2x prepulse alone trials (prepulse intensities of 74/78/82/86 dB), 8x PPI response trials (prepulse followed 80 ms later by a 120 dB startle stimulus), and 8x background noise only trials. We chose a variable intertrial interval of 15 seconds on average (range 10–20 seconds), prepulse duration of 20 ms and startle duration of 40 ms. Startle response was measured as the average mean amplitude. Percentage of PPI (%PPI) was calculated as [(startle response 120 dB – PPI response) × 100/startle response 120 dB]. We analyzed PPI performance (1) at baseline (PPI I) and (2) 15 minutes after ip treatment with either saline, MK-801 [noncompetitive glutamate receptor (NMDA) antagonist; 0.5 mg/kg body weight; Sigma] or d-amphetamine [dopamine (DA) agonist; 5.0 mg/kg body weight; Sigma] (PPI II). Mice were treated with saline, MK-801 and d-amphetamine in pseudorandom order (intertrial interval of at least 7 days to guarantee drug washout), allowing within-animal comparisons.

Statistical Analysis

One-way ANOVA was applied to assess the effects of the factor "genotype" on the different behavioral domains. For the analysis of the drug-induced prepulse inhibition performance two-way ANOVAs were used for the factors "genotype" and "drug treatment." In the GMA, habituation over time (overall distance traveled for 5-min blocks during a 30-min session and ODT as percentage of prior 5-min block) was investigated using a one-way ANOVA with repeated measures. Tests were followed by post hoc comparison (i.e., Fisher-PLSD test), if appropriate. Differences were regarded as statistically significant if $p < .05$ (nonsignificant = *ns*). The number of animals (n) was WT = 21 versus *Nrg1* EGF HET = 14. The "Results" section presents degrees of freedom, F- and p values of the ANOVAs, while figures and tables show the p values of the corresponding post hoc effects using asterisks ($^{*}p < .05$, $^{**}p < .01$ and $^{***}p < .001$). All data are presented as means ± *SEM*.

Results

The parameters investigated in the "physical exam" revealed that all animals were in a state of good health and physical

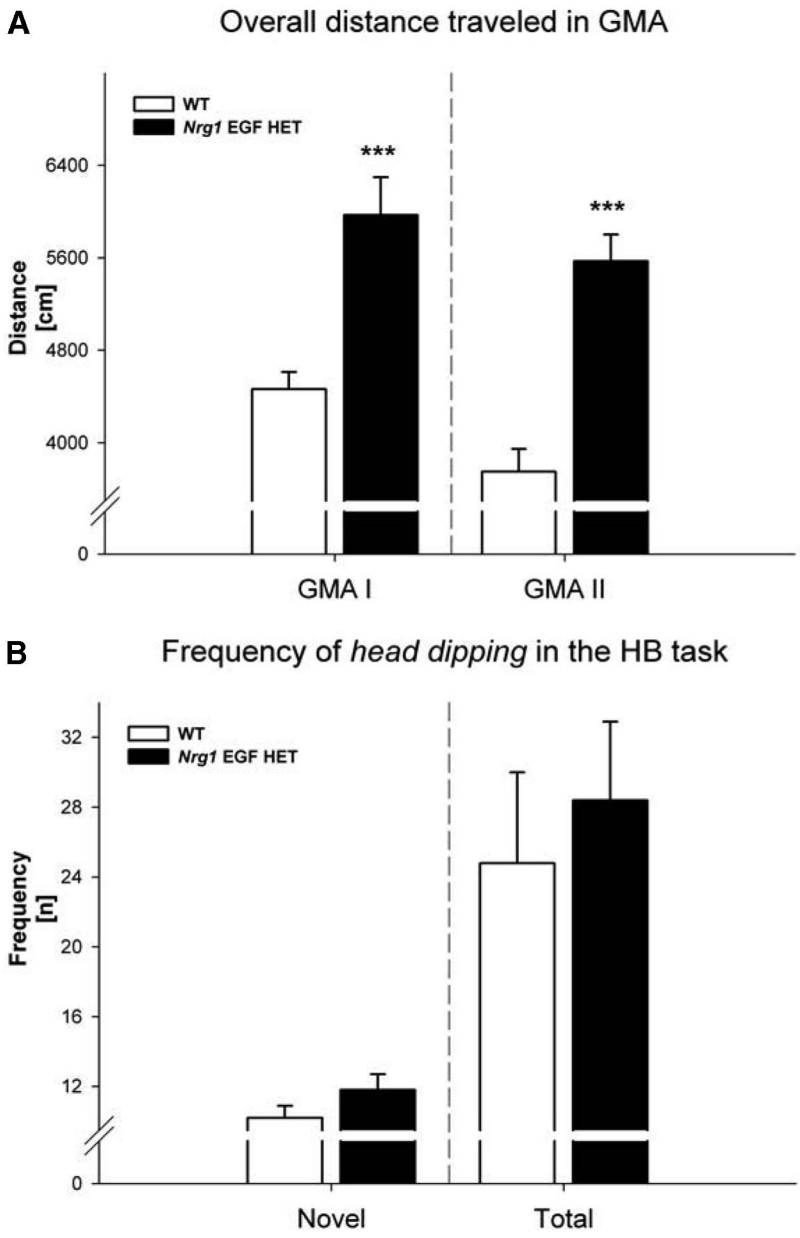

Figure 1. Motor activity and exploration: (A) Overall distance traveled (ODT) [cm] in GMA I and II and (B) frequency of head dippings [n] into novel holes (novel) and overall (total) in the HB task. Significant post hoc effects of *Nrg1* EGF HETs versus WT mice are indicated by asterisks ($^{***}p < .001$). Data are presented as means + *SEM*.

condition and exhibited normal neurological reflexes and sensory abilities. Furthermore, there were no differences in motor function and coordination between WT and *Nrg1* EGF HET mice (data not shown).

General Motor Activity (GMA)

As expected, *Nrg1* EGF HET mice displayed a pronounced hyper-locomotive phenotype in both versions of the general motor activity task. Testing the mice in the GMA I under high illumination levels revealed higher levels of arousal/activity than in the GMA II with low illumination; the overall distance traveled was significantly increased in mutant mice [GMA I: $F(1/33) = 17.6$; $p < .001$ - GMA II: $F(1/33) = 29.0$; $p < .001$; Figure 1A]. Importantly, elevated locomotion was also evident for *Nrg1* mutants in the peripheral part of the test arena, which is less anxiety-provoking [GMA I: $F(1/33) = 13.7$; $p < .001$ - WT = 3078.3 ± 112.0 vs. *Nrg1* EGF HET = 3982.6 ± 248.6 – GMA II: $F(1/33) = 11.6$; $p < .001$ - WT = 2520.4 ± 140.4 vs. *Nrg1* EGF HET = 3507.6 ± 199.6]. Furthermore, the overall time spent resting was significantly decreased in these mice [GMA I: $F(1/33) = 14.9$; $p <$

.001 - WT = 1015.2 ± 16.3 vs. *Nrg1* EGF HET = 905.7 ± 24.7 – GMA II: $F(1/33) = 26.9$; $p < .001$ - WT = 1127.6 ± 20.9 vs. *Nrg1* EGF HET = 973.9 ± 18.2]. Further data supporting this prominent phenotype were ambulatory frequency and episodes in both GMA tasks (data not shown).

Nrg1 EGF HET mice habituated their locomotive response to a novel environment more readily than WT mice, as measured by overall distance traveled (OTD) in the GMA II over time (in 5-min blocks). Repeated measures ANOVA revealed a significant effect of "genotype" [$F(1/165) = 35.9$; $p < .001$], of "habituation over time" [$F(1/165) = 29.4$; $p < .001$] and a significant interaction between both factors [$F(1/165) = 3.0$; $p = .01$] (Figure 2A). Analyzing the percentage change in OTD compared to the preceding 5-min block confirmed the significant interaction between factors "genotype" and "habituation over time" [$F(1/132) = 3.7$; $p = .007$; Figure 2B].

Comparing the exploration-related parameter vertical activity in WT and *Nrg1* EGF HET mice revealed no differences, indicating that the hyperactive phenotype of *Nrg1* EGF HETs was specific to motor activity but not exploration in GMA I [$F(1/33) = 2.0$; *ns* - WT = 331.5 ± 17.2 vs. *Nrg1* EGF HET = 369.6 ± 19.5] as well as GMA II [$F(1/33) = 0.4$; *ns* - WT = 252.8 ± 18.6 vs. *Nrg1* EGF HET = 270.1 ± 20.9].

Anxiety-related parameters in the GMA tests revealed that the distance mutant mice traveled in the more aversive central zone of both GMA paradigms was significantly increased [GMA I: $F(1/33) = 17.6$; $p = .0002$ – GMA II: $F(1/33) = 29.0$ $p < .001$; Figure 3A]. However, *Nrg1* EGF deficient mice displayed wild type-like levels of anxiety for the locomotion-independent ratio of distance traveled in the center of the GMA I [$F(1/33) = 1.5$; *ns*; Figure 3B] and only a subtle trend for a less anxious phenotype in the GMA II for the same parameter ($F(1/33) = 3.2$; $p = .08$; Figure 3B].

Light-Dark (LD)

Light-Dark testing revealed a significantly increased hyperactive phenotype in the Nrg1 mutants [overall distance traveled: $F(1/33) = 8.0$; $p = .008$ - WT = 1866.8 ± 75.6 vs. *Nrg1* EGF HET = 2335.1 ± 168.6], which is consistent with the results of the GMA testing. This hyperactivity was confirmed by the LD parameter ambulatory frequency ($F(1/33) = 7.5$; $p < .01$ - WT = 1390.7 ± 66.4 vs. *Nrg1* EGF HET = 1770.2 ± 138.2). The vertical activity of both *Nrg1* mutants and WT mice did not differ confirming the findings of both GMA paradigms [$F(1/33) = 2.8$; *ns* – WT = 69.4 ± 4.6 vs. *Nrg1* EGF HET = 85.4 ± 9.6]. The anxiety-related parameter ratio of traveled distance in the light compartment of the paradigm did not reveal any genotype differences [LD: $F(1/33) = .07$; *ns*].

Cross Maze (CM)

Mutant mice displayed an increased rearing frequency in the cross maze task. However, this elevation was only significant for the central area of the CM [rearing in center: $F(1/33) = 14.1$; $p < .001$ – WT = 1.5 ± 0.3 vs. *Nrg1* EGF HET = 3.4 ± 0.5] whereas no differences were found for the total rearing frequency (including rearing within the four arms of the X-maze) [$F(1/33) = 0.02$; *ns* – WT = 35.5 ± 2.3 vs. *Nrg1* EGF HET = 35.0 ± 3.1]. No differences were found between the two genotypes regarding quadruple alternations [$F(1/33) = 2.3$; *ns* – WT = 1.9 ± 0.2 vs. *Nrg1* EGF HET = 2.4 ± 0.3].

Marble Burying (MB)

No differences were detected in regard to general behavioral activity (i.e., marble burying frequency) between the two groups of mice. The number of marbles completely buried by *Nrg1* EGF HET and WT mice was similar [$F(1/33) = 0.5$; *ns* – WT = 3.8 ± 0.5 vs. *Nrg1* EGF HET = 3.2 ± 0.5].

Elevated Plus Maze (EPM)

This commonly used paradigm did not reveal any gross abnormalities for anxiety-related parameters in *Nrg1* mutant mice. The main parameter for anxiety of this task, the open arm entry ratio was similar for mutant and wild type-like mice [$F(1/33) = 0.2$; *ns*; Figure 3B].

There were no differences in the explorative-like phenotype of mutant and WT mice in the EPM [frequency of rearing: $F(1/33) = 0.06$; *ns* – WT = 21.9 ± 1.8 vs. *Nrg1* EGF HET = 21.1 ± 2.6] supporting the findings from other tasks such as the HB and LD test. Frequency of stretch attend postures [$F(1/33) = 1.1$; *ns* – WT = 2.6 ± 0.3 vs. *Nrg1* EGF HET = 2.0 ± 0.5] and time spent on open arms [$F(1/33) = 0.03$; *ns* – WT = 35.6 ± 4.8 vs. *Nrg1* EGF HET = 36.9 ± 6.6] confirmed the wild type-like phenotype of *Nrg1* mutants for this task.

Hole Board (HB)

Nrg1 mutants exhibited a significantly increased locomotor activity in the HB task [overall distance traveled: $F(1/33) = 34.9$; $p < .0001$. – WT = 1379.7 ± 59.1 vs. *Nrg1* EGF HET = 1952.3 ± 79.0]. However, contrary to locomotor activity, heterozygous EGF-like domain *Nrg1* deficient mice did not exhibit altered levels of exploration. The hole board task failed to reveal differences in the frequency of head dips (HD) into novel holes and in the total number of HDs [novel holes: $F(1/33) = 2.0$; *ns* – total number of HDs: $F(1/33) = 0.2$; *ns*; Figure 1B]. Furthermore, a similar working memory ratio was detected for both genotypes in the HB task [$F(1/33) = 1.5$; *ns* – WT = 0.55 ± 0.04 vs. *Nrg1* EGF HET = 0.48 ± 0.04].

Prepulse Inhibition (PPI)/Sensorimotor Gating

Based on the prepulse inhibition protocol used, EGF-like domain *Nrg1* mutant and WT mice showed similar baseline %PPI for the various individual prepulse intensities [74 dB: $F(1/33) = 0.02$ – 78 dB: $F(1/33) = 0.5$ – 82 dB: $F(1/33) = 1.3$ – 86 dB: $F(1/33) = 2.5$; all *ns*; Figure 4A]. Treatment with MK-801 and d-amphetamine revealed a significant effect of the factor "genotype" on %PPI averaged over different prepulses [two-way ANOVA: $F(1/77) = 4.4$; $p = .04$]. One-way ANOVA (split by the factor "drug treatment") detected a significantly impaired %PPI for mutant mice after MK-801 treatment [$F(1/23) = 6.0$; $p = .02$] but not d-amphetamine treatment (Figure 4B).

Discussion

The animal model for reduced human EGF-like domain *NRG1* exhibits a variety of behavioral abnormalities compared to their

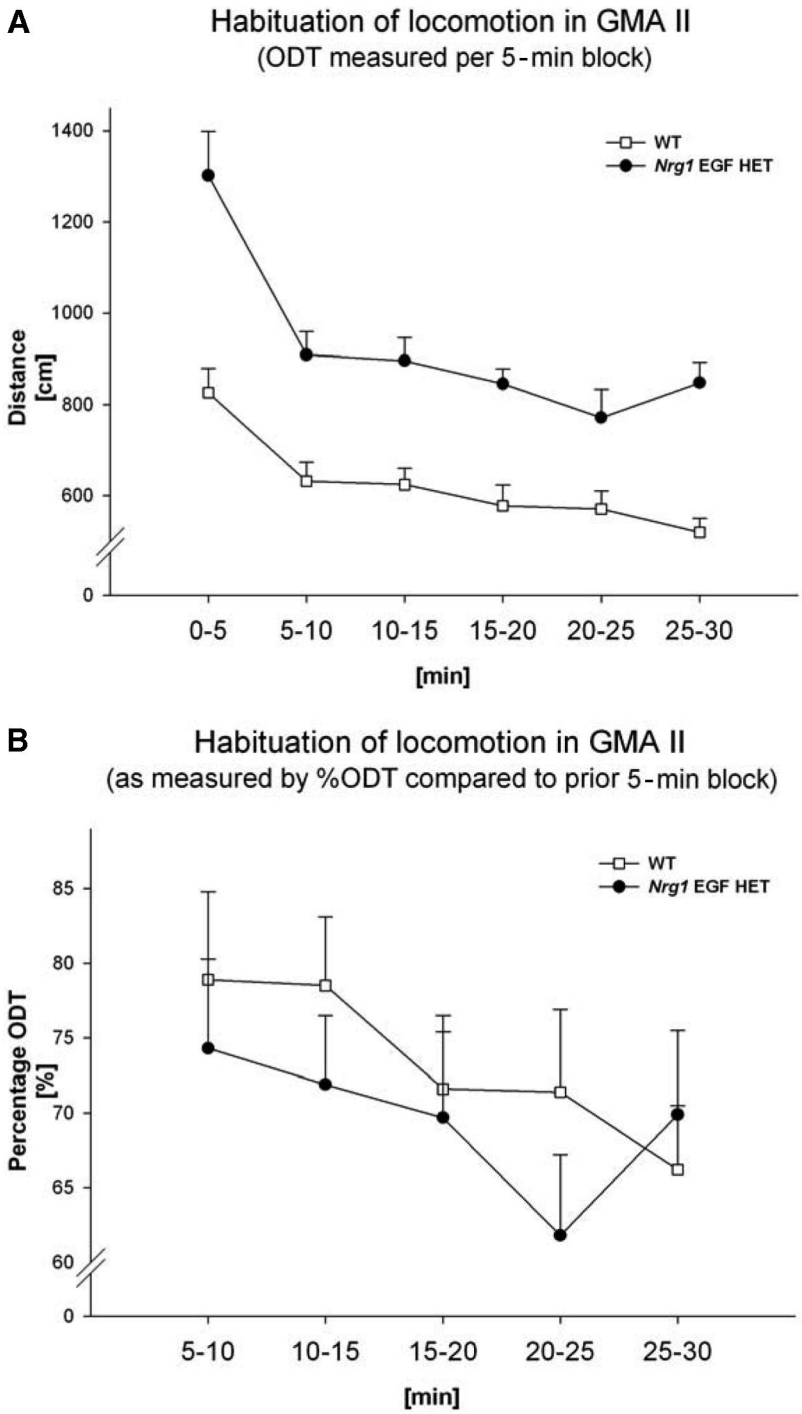

Figure 2. Habituation of locomotion over time in GMA II: Overall distance traveled (ODT – in cm) for (A) 5-min blocks during a 30-min trial (summed ODT for 0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 minutes) and (B) as a percentage ODT compared to the preceding 5 minutes of testing [%]. Repeated measures ANOVA revealed significant effects of "genotype," "habituation over time" and a significant "genotype × habituation over time" interaction for (A) and a significant "genotype × habituation over time" interaction for (B). All data are presented as means + *SEM*.

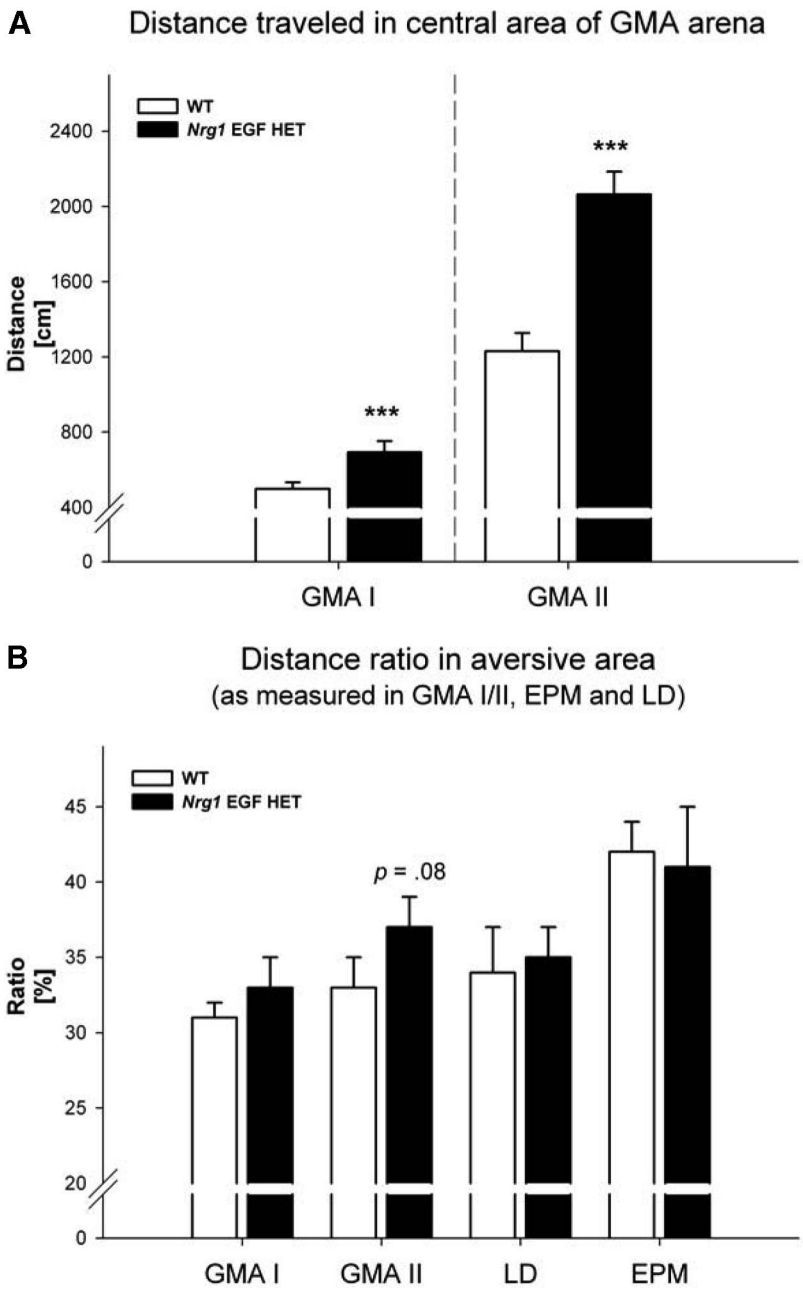

Figure 3. Anxiety-related parameters in various paradigms: (A) Distance traveled in the central zone of the GMA [cm] and (B) ratio of traveled distance (i.e., GMA I/II and LD) or open arm entries (i.e., EPM) to total traveled distance/total arm entries [%]. Significant post hoc effects of *Nrg1* EGF HETs versus WT mice are indicated by asterisks ($^{***}p < .001$). Data are presented as means + *SEM*.

WT-like littermates, demonstrating the important influence of Nrg1 on a variety of behavioral domains. *Nrg1* EGF HET mice exhibited a hyper-locomotive phenotype in several different behavioral paradigms. Importantly, this increase in motor activity was highly specific, as neither exploratory behaviors nor general activity levels were altered in mutant mice. Furthermore, heterozygous EGF-like domain *Nrg1* deficient mice habituated more readily to a new environment compared to WT-like control mice. Extensive analysis of anxiety-related behaviors revealed no pronounced changes in anxiety levels for *Nrg1* EGF HET mice. Interestingly, a 50% inhibition of Nrg1s biological activity resulted in a moderate overall impairment in sensorimotor gating performance in mice, although this effect was only evident after pharmacological challenge with the psychoactive substance MK-801.

Our multitiered behavioral test battery revealed a pronounced increase in locomotion over a variety of behavioral tasks in

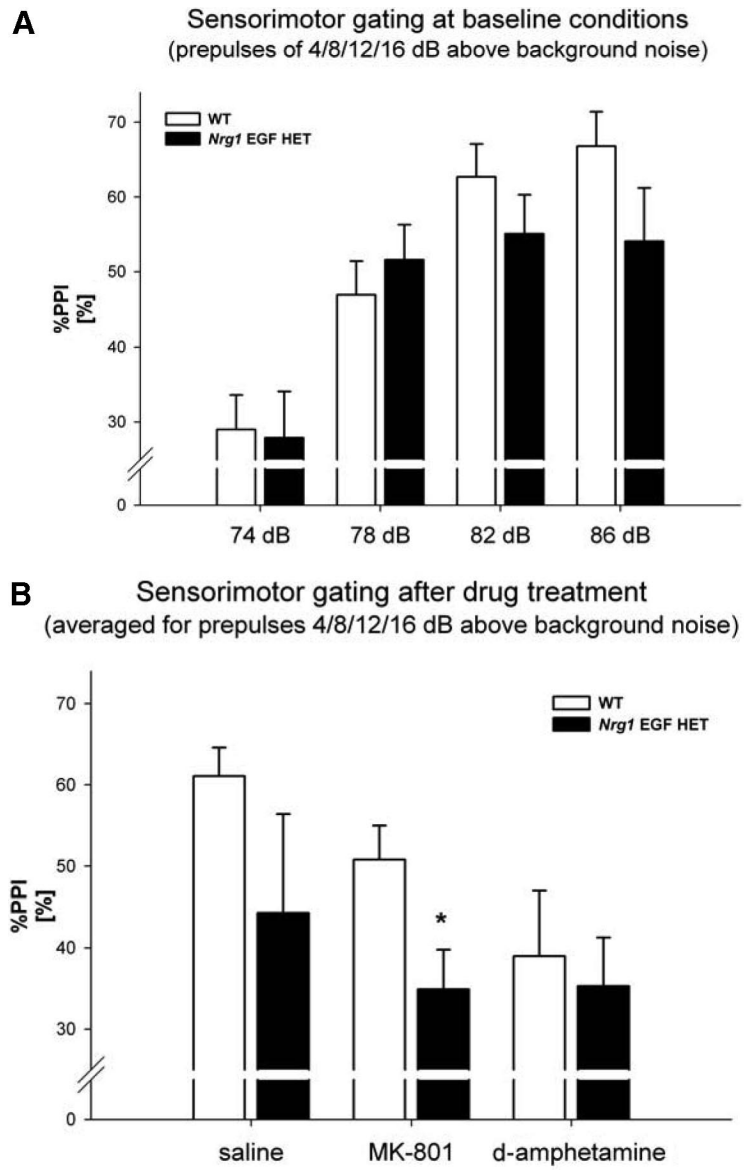

Figure 4. Sensorimotor gating: (A) At baseline and (B) after drug treatment [ip treatment with either saline, MK-801 (0.5 mg/kg BW) or d-amphetamine (5 mg/kg BW)] 15 minutes before testing. %PPI was calculated as: (startle response 120 dB – PPI response) × 100/startle response 120 dB]. Significant post hoc effects of *Nrg1* EGF HETs versus WT mice are indicated by asterisks ($^{*}p < .05$). Data are presented as means + *SEM* (A) for the individual prepulse intensities and (B) averaged over the different prepulse intensities.

heterozygous EGF-like domain *Nrg1* deficient mice. The hyperlocomotive phenotype appears to be independent of the stress level induced by experimental testing, as it was apparent both in a highly illuminated (i.e., stress-inducing Hascoet, Bourin, & Dhonnchadha, 2001) and a dimly illuminated version of the GMA, as well as the less aversive peripheral area of both GMA tests. This finding was confirmed by a variety of other parameters, including a decrease in time spent resting in mutant mice.

Importantly, locomotion-related hyperactivity is one of the nonspecific features believed to be relevant for animal models of schizophrenia (Gainetdinov, Mohn, & Caron, 2001), as pharmacological models for schizophrenia-like symptoms, including dopaminergic hyper-stimulation (i.e., amphetamine treatment) and glutamatergic hypo-function (i.e., MK-801 or ketamine) result in increased motor activity (Javitt & Coyle, 2004; Wong & Van Tol, 2003). Interestingly, Nrg1 regulates aspects of dopaminergic neurotransmission (Yurek, Zhang, Fletcher-Turner, & Seroogy, 2004) and NMDA receptor function (Gu, Jiang, Fu, Ip, & Yan, 2005; Ozaki, Sasner, Yano, Lu, & Buonanno, 1997); an inhibition of these functions in the striatum and nucleus accumbens might have caused the locomotive response observed in our model (Adriani et al., 1998). Central administration of Nrg1 itself was reported to inhibit wheel-running behavior in hamsters (Snodgrass-Belt, Gilbert, & Davis, 2005). Conversely, an animal model for reduced

Nrg1 activity is expected to show increased motor activity. The mutated EGF-like domain *Nrg1* specifically affects locomotion, as mutant mice showed neither an increase in general activity measures [i.e., digging behavior in marble burying paradigm (Gyertyan, 1995)] nor in the majority of explorative-like domains investigated (i.e., number of head dips in the hole board test, vertical activity in general motor activity and Light-Dark test and rearing frequency in elevated plus maze). The effect of Nrg1 suppression on mouse locomotion is consistent with the findings for another available model for EGF-like *NRG1* (Gerlai et al., 2000; Table 1).

As described previously in mice carrying a transmembrane domain *Nrg1* mutation (Karl et al., 2007) and extending the findings of Gerlai and coworkers (2000), *Nrg1* inhibition led to improved locomotive habituation to a novel environment. Hippocampal nuclei are involved in habituation-like processes (Clark, Hines, Hamilton, & Whishaw, 2005; Lee, Hunsaker, & Kesner, 2005), suggesting that morphological alterations in the dentate gyrus-CA3 network might have occurred in our animal model.

A comprehensive analysis of anxiety-related behaviors in *Nrg1* EGF HET mice revealed no major changes in trait anxiety. Mutant mice showed increased locomotion in aversive areas of GMA and LD but this effect was confounded by the hyper-locomotive phenotype evident in these animals, as it disappeared when locomotion ratios were analyzed. Furthermore, animals did not spend more time in anxiety-provoking areas and the frequency of anxiety-related behaviors such as stretch attend postures was not altered compared to WT-like mice. Surprisingly, only one other animal model for human transmembrane domain *NRG1* has been investigated in regard to its anxiety-like phenotype. These animals were reported to have a task-specific anxiolytic-like phenotype (Boucher et al., 2007a; Karl et al., 2007). The EGF-like domain *Nrg1* mutants investigated by Gerlai and colleagues (2000) were tested for open field behavior but no anxiety-related parameters have been described (see Table 1).

One of the main schizophrenia-related behaviors examined in comparative neurobiology is sensorimotor gating as measured by prepulse inhibition (PPI). PPI is a phenomenon whereby a prestimulus reduces the magnitude of the response to a startle-inducing stimulus (Geyer & Swerdlow, 1998). Schizophrenia patients show a reduction in PPI which can be rescued by atypical antipsychotic medication (Ellenbroek & Cools, 1990). Importantly, motor activity differences do not influence the acoustic startle response (Leng, Yee, Feldon, & Ferger, 2004), meaning that the hyper-locomotion of our *Nrg1* mutants would not have had an impact on their PPI performance. *Nrg1* EGF HET mice revealed no differential PPI performance at baseline. However, impairment in overall sensorimotor gating was evident in the mutant mice after pharmacological challenge with MK-801, a noncompetitive NMDA receptor antagonist. Glutamatergic mechanisms are involved in PPI (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001) suggesting that alterations especially in the expression of NMDA receptors might be responsible for the observed moderate changes in PPI behavior of *Nrg1* mutant mice after MK-801 treatment—such an alteration was reported for the other mouse line available for EGF-like domain *Nrg1* (Stefansson et al., 2002). Importantly, a recent study confirms an involvement of Nrg1 in the modulation of NMDA receptor phosphorylation (Bjarnadottir et al., 2007). Thus, changes to NMDA receptor expression in the mutants would explain the observed alterations in locomotive behavior as well as the impaired sensorimotor gating. Treatment with another psychotomimetic drug (i.e., d-amphetamine) did not produce impairments in the prepulse inhibition performance of *Nrg1* EFG HET mice suggesting a specific effect of Nrg1 on the glutamatergic but not the dopaminergic system. Interestingly, the sensorimotor gating performance of only two other mouse models for human *NRG1* isoforms and its receptors have been described (Boucher et al., 2007a; Stefansson et al., 2002) and neither exhibited baseline PPI impairments (see Table 1).

Although PPI is commonly used in schizophrenia-related animal research and is one of the few paradigms which can be studied in both humans and animals in a similar fashion, it has to be stressed that (1) PPI deficits are not specific to only schizophrenia but are also seen for example, in Huntingon's disease and Tourette Syndrome (Geyer et al., 2001); (2) PPI performance should be analyzed both at baseline and after pharmacological intervention using psychoactive compounds (i.e., MK-801, ketamine or amphetamine); and (3) the substantial range in PPI protocols used in the literature complicates comparisons between different studies investigating animal models for schizophrenia.

In conclusion, we report here that a mutation in mouse EGF-like domain *Nrg1* results in pronounced effects on motor activity and habituation to an environment and subtle changes in sensorimotor gating. Further research is needed to address the biochemical mechanisms underlying this behavioral phenotype, focusing initially on the glutamatergic system. Our study adds to the increasing amount of behavioral data available on a variety of animal models for different isoforms/types of *NRG1* and suggests a standardized and comprehensive behavioral phenotyping approach is required to distinguish between the different available mouse models. Future behavioral investigations should focus not only on the positive symptoms of schizophrenia (as measured by psychomotor agitation and sensitivity to psychotomimetic drugs; Powell & Miyakawa, 2006) but also on the negative (e.g., social withdrawal and adhedonia) and cognitive (further tests for working memory and executive functions) symptoms of this mental disorder. The behavioral phenotyping of female *Nrg1* mutants to identify sex-specific effects of this mutation as well as a comprehensive pharmacological profiling of this mouse line using typical and atypical antipsychotics would be necessary to further investigate this animal model. Importantly, such a strategy should also include environmental risk factors for schizophrenia (e.g., Boucher et al., 2007a; Karl et al., 2007) to validate the current and other animal models for *NRG1* in regard to their potential for schizophrenia research.

References

Adriani, W., Felici, A., Sargolini, F., Roullet, P., Usiello, A., Oliverio, A., et al. (1998). N-methyl-D-aspartate and dopamine receptor involvement in the modulation of locomotor activity and memory processes. *Experimental Brain Research, 123,* 52–59.

Bjarnadottir, M. B., Misner, D. L., Haverfield-Gross, S., Bruun, S., Helgason, V. G., Stefansson, H., et al. (2007). Neuregulin (NRG1) signaling through Fyn modulates NMDA receptor phosphorylation: Differential synaptic function in NRG1$^{+/-}$ knock-outs compared with wild-type mice. *Journal of Neuroscience, 27,* 4519–4529.

Boucher, A. A., Arnold, J. C., Duffy, L., Schofield, P. R., Micheau, J., & Karl, T. (2007a). Heterozygous neuregulin 1 mice are more sensitive to

the behavioural effects of Delta9-tetrahydrocannabinol. *Psychopharmacology (Berlin), 192,* 325–336.

Boucher, A. A., Hunt, G. E., Karl, T., Micheau, J., McGregor, I. S., & Arnold, J. C. (2007b). Heterozygous neuregulin 1 mice display greater baseline and Δ9-tetrahydrocannbinol-induced c-Fos expression. *Neuroscience, 149,* 861–870.

Britsch, S., Li, L., Kirchhoff, S., Theuring, F., Brinkmann, V., Birchmeier, C., & Riethmacher, D. (1998). The ErbB2 and ErbB3 receptors and their ligand, neuregulin-1, are essential for development of the sympathetic nervous system. *Genes & Development, 12,* 1825–1836.

Clark, B. J., Hines, D. J., Hamilton, D. A., & Whishaw, I. Q. (2005). Movements of exploration intact in rats with hippocampal lesions. *Behavioural Brain Research, 163,* 91–99.

Corfas, G., Roy, K., & Buxbaum, J. D. (2004). Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. *Nature Neuroscience, 7,* 575–580.

Costall, B., Jones, B. J., Kelly, M. E., Naylor, R. J., & Tomkins, D. M. (1989). Exploration of mice in a black and white test box: Validation as a model of anxiety. *Pharmacology, Biochemistry, and Behavior, 32,* 777–785.

Crawley, J. N., & Paylor, R. (1997). A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. *Hormones and Behavior, 31,* 197–211.

DeFries, J. C., Hegmann, J. P., & Weir, M. W. (1966). Open-field behavior in mice: Evidence for a major gene effect mediated by the visual system. *Science, 154,* 1577–1579.

Denenberg, V. H. (1969). Open-field behavior in the rat: What does it mean? *Annals of the New York Academy of Sciences, 159,* 852–859.

Ellenbroek, B. A., & Cools, A. R. (1990). Animal models with construct validity for schizophrenia. *Behavioural Pharmacology, 1,* 469–490.

Erickson, S. L., O'Shea, K. S., Ghaboosi, N., Loverro, L., Frantz, G., Bauer, M., et al. (1997). ErbB3 is required for normal cerebellar and cardiac development: A comparison with ErbB2-and heregulin-deficient mice. *Development, 124,* 4999–5011.

Falls, D. L. (2003). Neuregulins: Functions, forms, and signaling strategies. *Experimental Cell Research, 284,* 14–30.

Gainetdinov, R. R., Mohn, A. R., & Caron, M. G. (2001). Genetic animal models: Focus on schizophrenia. *Trends in Neurosciences, 24,* 527–533.

Gassmann, M., Casagranda, F., Orioli, D., Simon, H., Lai, C., Klein, R., et al. (1995). Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature, 378,* 390–394.

Gerlai, R., Pisacane, P., & Erickson, S. (2000). Heregulin, but not ErbB2 or ErbB3, heterozygous mutant mice exhibit hyperactivity in multiple behavioral tasks. *Behavioural Brain Research, 109,* 219–227.

Geyer, M. A., Krebs-Thomson, K., Braff, D. L., & Swerdlow, N. R. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: A decade in review. *Psychopharmacology (Berl), 156,* 117–154.

Geyer, M. A., & Swerdlow, N. R. (1998). Measurement of startle response, prepulse inhibition, and habituation. In J. N. Crawley & P. Skolnick (Eds.), *Current protocols in neuroscience* (pp. 8.7.1–8.7.15). New York: Wiley.

Golub, M. S., Germann, S. L., & Lloyd, K. C. (2004). Behavioral characteristics of a nervous system-specific erbB4 knock-out mouse. *Behavioural Brain Research, 153,* 159–170.

Gu, Z., Jiang, Q., Fu, A. K., Ip, N. Y., & Yan, Z. (2005). Regulation of NMDA receptors by neuregulin signaling in prefrontal cortex. *Journal of Neuroscience, 25,* 4974–4984.

Gyertyan, I. (1995). Analysis of the marble burying response: Marbles serve to measure digging rather than evoke burying. *Behavioural Pharmacology, 6,* 24–31.

Hahn, C. G., Wang, H. Y., Cho, D. S., Talbot, K., Gur, R. E., Berrettini, W. H., et al. (2006). Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. *Nature Medicine, 12,* 824–828.

Harrison, P. J., & Law, A. J. (2006). Neuregulin 1 and Schizophrenia: Genetics, gene expression, and neurobiology. *Biological Psychiatry, 60,* 132–140.

Hascoet, M., Bourin, M., & Dhonnchadha, B. A. (2001). The mouse light-dark paradigm: A review. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 25,* 141–166.

Hashimoto, R., Straub, R. E., Weickert, C. S., Hyde, T. M., Kleinman, J. E., & Weinberger, D. R. (2004). Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. *Molecular Psychiatry, 9,* 299–307.

Javitt, D. C., & Coyle, J. T. (2004). Decoding schizophrenia. *Scientific American, 290,* 48–55.

Karl, T., Duffy, L., Scimone, A., Harvey, R. P., & Schofield, P. R. (2007). Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: Implications for understanding schizophrenia. *Genes, Brain, and Behavior, 6,* 677–687.

Karl, T., Pabst, R., & von Horsten, S. (2003). Behavioral phenotyping of mice in pharmacological and toxicological research. *Experimental and Toxicologic Patholology, 55,* 69–83.

Kerber, G., Streif, R., Schwaiger, F. W., Kreutzberg, G. W., & Hager, G. (2003). Neuregulin-1 isoforms are differentially expressed in the intact and regenerating adult rat nervous system. *Journal of Molecular Neuroscience, 21,* 149–165.

Kopp, C. (2001). Locomotor activity rhythm in inbred strains of mice: Implications for behavioural studies. *Behavioural Brain Research, 125,* 93–96.

Kramer, R., Bucay, N., Kane, D. J., Martin, L. E., Tarpley, J. E., & Theill, L. E. (1996). Neuregulins with an Ig-like domain are essential for mouse myocardial and neuronal development. *Proceedings of the National Academy of Sciences of the United States of America, 93,* 4833–4838.

Law, A. J., Lipska, B. K., Weickert, C. S., Hyde, T. M., Straub, R. E., Hashimoto, R., et al. (2006). Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5′ SNPs associated with the disease. *Proceedings of the National Academy of Sciences of the United States of America, 103,* 6747–6752.

Lee, I., Hunsaker, M. R., & Kesner, R. P. (2005). The role of hippocampal subregions in detecting spatial novelty. *Behavioral Neuroscience, 119,* 145–153.

Leng, A., Yee, B. K., Feldon, J., & Ferger, B. (2004). Acoustic startle response, prepulse inhibition, and spontaneous locomotor activity in MPTP-treated mice. *Behavioural Brain Research, 154,* 449–456.

Long, W., Wagner, K. U., Lloyd, K. C., Binart, N., Shillingford, J. M., Hennighausen, L., et al. (2003). Impaired differentiation and lactational failure of Erbb4-deficient mammary glands identify ERBB4 as an obligate mediator of STAT5. *Development, 130,* 5257–5268.

Meyer, D., & Birchmeier, C. (1995). Multiple essential functions of neuregulin in development. *Nature, 378,* 386–390.

Michailov, G. V., Sereda, M. W., Brinkmann, B. G., Fischer, T. M., Haug, B., Birchmeier, C., et al. (2004). Axonal neuregulin-1 regulates myelin sheath thickness. *Science, 304,* 700–703.

Montgomery, K. C. (1958). The relation between fear induced by novel stimulation and exploratory behavior. *Journal of Comparative and Physiological Psychology, 48,* 254–260.

Munafo, M. R., Thiselton, D. L., Clark, T. G., & Flint, J. (2006). Association of the NRG1 gene and schizophrenia: A meta-analysis. *Molecular Psychiatry, 11,* 539–546.

O'Tuathaigh, C., M., Babovic, D., O'Sullivan, G., J., Clifford, J. J., Tighe, O., Croke, D. T., et al. (2007). Phenotypic characterization of spatial cognition and social behavior in mice with 'knockout' of the schizophrenia risk gene neuregulin 1. *Neuroscience, 147,* 18–27.

O'Tuathaigh, C., M., O'Sullivan, G., J., Kinsella, A., Harvey, R. P., Tighe, O., Croke, D. T., et al. (2006). Sexually dimorphic changes in the

exploratory and habituation profiles of heterozygous neuregulin-1 knockout mice. *Neuroreport, 17,* 79–83.

Ozaki, M., Sasner, M., Yano, R., Lu, H. S., & Buonanno, A. (1997). Neuregulin-beta induces expression of an NMDA-receptor subunit. *Nature, 390,* 691–694.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods, 14,* 149–167.

Powell, C. M., & Miyakawa, T. (2006). Schizophrenia-relevant behavioral testing in rodent models: A uniquely human disorder? *Biological Psychiatry, 59,* 1198–1207.

Riethmacher, D., Sonnenberg-Riethmacher, E., Brinkmann, V., Yamaai, T., Lewin, G. R., & Birchmeier, C. (1997). Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. *Nature, 389,* 725–730.

Rimer, M., Barrett, D. W., Maldonado, M. A., Vock, V. M., & Gonzalez-Lima, F. (2005). Neuregulin-1 immunoglobulin-like domain mutant mice: Clozapine sensitivity and impaired latent inhibition. *Neuroreport, 16,* 271–275.

Snodgrass-Belt, P., Gilbert, J. L., & Davis, F. C. (2005). Central administration of transforming growth factor-alpha and neuregulin-1 suppress active behaviors and cause weight loss in hamsters. *Brain Research, 1038,* 171–182.

Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., et al. (2002). Neuregulin 1 and susceptibility to schizophrenia. *American Journal of Human Genetics, 71,* 877–892.

Steinthorsdottir, V., Stefansson, H., Ghosh, S., Birgisdottir, B., Bjornsdottir, S., Fasquel, A. C., et al. (2004). Multiple novel transcription initiation sites for NRG1. *Gene, 342,* 97–105.

Thuret, S., Alavian, K. N., Gassmann, M., Lloyd, C. K., Smits, S. M., Smidt, M. P., et al. (2004). The neuregulin receptor, ErbB4, is not required for normal development and adult maintenance of the substantia nigra pars compacta. *Journal of Neurochemistry, 91,* 1302–1311.

van Gaalen, M. M., & Steckler, T. (2000). Behavioural analysis of four mouse strains in an anxiety test battery. *Behavioural Brain Research, 115,* 95–106.

Walss-Bass, C., Liu, W., Lew, D. F., Villegas, R., Montero, P., Dassori, A., et al. (2006). A Novel Missense Mutation in the Transmembrane Domain of Neuregulin 1 is Associated with Schizophrenia. *Biological Psychiatry, 60,* 548–553.

Wang, J. H., Short, J., Ledent, C., Lawrence, A. J., & van den Buuse, M. (2003). Reduced startle habituation and prepulse inhibition in mice lacking the adenosine A2A receptor. *Behavioural Brain Research, 143,* 201–207.

Wolpowitz, D., Mason, T. B., Dietrich, P., Mendelsohn, M., Talmage, D. A., & Role, L. W. (2000). Cysteine-rich domain isoforms of the neuregulin-1 gene are required for maintenance of peripheral synapses. *Neuron, 25,* 79–91.

Wong, A. H., & Van Tol, H. H. (2003). Schizophrenia: From phenomenology to neurobiology. *Neuroscience and Biobehavioral Reviews, 27,* 269–306.

Yurek, D. M., Zhang, L., Fletcher-Turner, A., & Seroogy, K. B. (2004). Supranigral injection of neuregulin1-beta induces striatal dopamine overflow. *Brain Research, 1028,* 116–119.

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