Neurobehavioral Differences Between Mice Receiving Distinct Neuregulin Variants as Neonates; Impact on Sensitivity to MK-801

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Abstract: Neuregulin-1 (NRG1) is a well-recognized risk gene for schizophrenia and is often implicated in the neurodevelopmental hypothesis of this illness. Alternative splicing and proteolytic processing of the *NRG1* gene produce more than 30 structural variants; however, the neuropathological roles of individual variants remain to be characterized. On the basis of the neurodevelopmental hypothesis of schizophrenia, we administered eNRG1 (0.1~1.0 μg/g), a core epidermal growth factor-like (EGF) domain common for all splicing NRG1 variants, to neonatal mice and compared their behavioral performance with mice challenged with a full mature form of type 1 NRG1 variant. During the neonatal stage, recombinant eNRG1 protein administrated from the periphery passed the blood-brain barrier and activated its receptor (ErbB4) in the brain. In adults, the mice receiving the highest dose exhibited lower locomotor activity and deficits in prepulse inhibition and tone-dependent fear learning, although the hearing reduction of the eNRG1-treated mice may explain these behavioral deficits. Neonatal eNRG1 treatment also significantly potentiated MK-801-driven locomotor activity in an eNRG1 dose-dependent manner. In parallel eNRG1 treatment enhanced MK-801-driven c-Fos induction and decreased immunoreactivity for NMDA receptor subunits in adult brain. In contrast, mice that had been treated with the same molar dose of a full mature form of type 1 NRG1 as neonates did not exhibit hypersensitivity to MK-801. However, both animal models exhibited similar hypersensitivity to methamphetamine. Collectively, our findings suggest that aberrant peripheral NRG1 signals during neurodevelopment alter later behavioral traits and auditory functions in the NRG1 subtype-dependent manner.

Keywords: Behavior, MK-801, Neuregulin-1, NMDA receptor, hearing, schizophrenia.

INTRODUCTION

Genetic polymorphism of neuregulin-1 (*NRG1*) is suggested to associate with vulnerability to psychiatric diseases such as schizophrenia and bipolar disorder [1-3]. The genetic association of *NRG1* with schizophrenia was first demonstrated with the haplotype block called HAPICE that is located in the 5'-end of the gene and consists of three single nucleotide polymorphisms (SNPs) and two microsatellites [1]. This initial finding has been replicated in multiple samples, but not in all populations [1-3]. Alternative splicing or promoter usage of the NRG1 gene produces multiple isoforms of NRG1 precursor proteins (type I-VI) [4-6]. The posttranslational processing of the NRG1 precursors further modifies the protein structures of these isoforms to alter their biological activity [7]. However, the pathological contribution of each NRG1 isoform is not fully understood. Genetic mutant mice that lack the isoform-specific exon(s) or overexpress a specific isoform of NRG1 exhibit distinct behavioral abnormalities relevant to schizophrenia endophenotypes, potentially suggesting the divergence of the isoforms' functions *in vivo* [8-12]. These experiments were often performed by independent researchers and did not allow us to accurately compare the behavioral consequences of the individual isoforms' manipulations. To test the functional difference of the NRG1 isoforms, here, we investigated and compared the behavioral traits of the mice treated with type1 NRG1 (T1-NRG1) and those with its processing product; an epidermal growth factor (EGF) domain peptide (eNRG1). These NRG1 derivatives are potentially induced or recruited in the prenatal and perinatal immune inflammatory processes that are implicated in schizophrenia etiology [13,14].

We have been testing the neurodevelopmental hypothesis of schizophrenia, assessing the neurobehavioral impact of neonatal exposure to T1-NRG1 [15]. In addition to T1-NRG1, here, we challenged mouse neonates with eNRG1 to compare the neurobehavioral effects of the two NRG1 variants, eNRG1 and T1-NRG1. Although there are multiple isoforms of NRG1 precursor proteins (type I-VI), which are generated by alternative splicing or promoter usage of the *NRG1* gene, but all NRG1 isoforms carry this core active domain (i.e., eNRG1) for their receptor binding [5,6]. Moreover, eNRG1 is naturally produced by proteolytic processing of T1-NRG1 with a protease, neuropsin, and suggested to exert a distinct action in

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1875-5666/15 $58.00+.00

hippocampal synaptic plasticity [7]. We assessed basal behavioral traits to evaluate the relevancy of eNRG1-injected mice to an animal model for schizophrenia, comparing the previous results from the mice exposed to T1-NRG1 [15]. As NRG1 signals are known to regulate NMDA receptor expression and function in the brain [16-19]. Thus, we investigated the sensitivity of eNRG1-treated mice to the NMDA receptor antagonist MK-801 as well as the expression of NMDA receptor subunits in these mice [17,18].

MATERIALS AND METHODS

Ethics Statement

All of the animal experiments described were approved by the Animal Use and Care Committee of Niigata University and performed in accordance with the guidelines of NIH (USA).

Animals

C57BL/6NCrj mouse dams of late pregnancy were purchased from Nihon Charles River (Yokohama, Japan) and their pups were used for experiments. Mice were housed with their dam in polypropylene cages (24L $\times$ 17W $\times$ 12H cm) in temperature-controlled colony room maintained under a 12-h light-dark cycle (light on 8:00 h) with free access to food and water. At postnatal day (PND) 25-28, pups were weaned and separated into cage (three or four animals per cage). Both male and female mice were subjected to behavioral tests during PND 56-70 [15]. Behavioral tests were performed during the light-cycle.

Reagents

Recombinant epidermal growth factor domain of human NRG 1β1/heregulin 1β1 was purchased from PeproTech EC (London, UK). This NRG1 peptide is sufficient for activation of the ErbB4 receptor tyrosine kinase. We will refer to it simply as "eNRG1". Alternatively, we used the full mature form of recombinant type-1 NRG1β1 protein (T1-NRG1; MW 25400 Da) [15]. (+)-MK-801 (dizocilpine) was purchased from Tocris Cookson Ltd (Ellisville, MO, USA).

NRG1-Treatment and Psychotomimetic Challenge

eNRG1 or T1-NRG1 was administered subcutaneously (s.c.) to individual litters during postnatal days PND 2-10 at the nape of the neck at a dose of 0.1, 0.3, or 1.0 μg/g body weight (injection volume 15 μl/g). The experimental design and procedures were based on our previous studies of the EGF model for schizophrenia [20]. Control littermates received a phosphate-buffered saline (PBS; vehicle) injection of the same volume. MK-801 (0.3 μg/g; 10 μl/g) and methamphetamine (1.0 μg/g; 10 μl/g) dissolved in saline were administered intraperitoneally (i.p.) to adult mice (PND 56-70) [15,21].

Immunoprecipitation

Protein extracts were prepared from whole brains of eNRG1-treated or vehicle-treated control mice on PND2 [15]. Each brain was homogenized in a RIPA lysis buffer [50 mM Tris-HCl buffer (pH 7.4) with 1% TritonX-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 150 mM NaCl, 5 mM EDTA, 1 mM Na_3VO_4, and 1 mM NaF) plus a protease inhibitor cocktail (Complete Mini; Roche, Mannheim, Germany). After centrifugation, supernatants were harvested and protein content was determined using a Micro BCA kit (Pierce Chemical, Rockland, IL, USA). Each brain lysate (2 mg protein) was then incubated with 2 μg of anti-ErbB4 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight and mixed with Protein G Sepharose beads (20 μl; GE Healthcare Bio-Science AB, Uppsala, Sweden) for 3 h. Sepharose beads were washed with RIPA buffer, and denatured with 100 μl of 2 $\times$ SDS sample buffer (125 mM Tris-HCl pH 6.8, 4% SDS, 10% 2-mercaptoethanol). The specificity of the anti-ErbB4 antibody was ascertained by immuno-blotting of cell lysates containing individual ErbB1-4 proteins (HN, unpublished data). Immunoprecipitates were then analyzed by gel electrophoresis as described below.

Immunoblotting

Brain tissues were homogenized in the sample lysis buffer (62.5 mM Tris-HCl pH 6.8, 2 % SDS, 0.5 % NP-40, 5 mM EDTA) plus the protease inhibitor cocktail (Roche). Protein samples (15-30 μg/lane) were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Membranes were probed with antibodies directed against phosphotyrosine (1:1000, Millipore, Bedford, MA, USA), ErbB4 (1:1000, Santa Cruz Biotechnology). Alter-natively, immunoblots were probed with antibodies directed against excitatory and inhibitory neuronal markers [GluA1 (1:500, Millipore), GluA2/3 (1:1000, Millipore), GluA4 (1:1000, Millipore), GluN1 (1:1000, Millipore), GluN2A (1:500, Millipore), GluN2B (1: 500, Millipore), GluN2C (1:500, Millipore), GluN2D (1:1000, Santa Cruz Biotechnology), GAD65/67 (1:1000, Millipore), parvalbumin (1:3000, Abcam, Cambridge UK), tyrosine hydroxylase (1:1000, Millipore) neuregulin-1 (1:300, RandD Systems, Minneapolis, MN), and β-actin (1:2000, Millipore)]. Immunoreactivity on membranes was detected by peroxidase-conjugated anti-immunoglobulin antibodies followed by chemiluminescence reaction (ECL kit, GE Healthcare) and film exposure. The intensity of an immunoreactive band, whose size matched the authentic molecular weight, was measured by an image processing software, GENETOOLS (Syngene, Cambridge, UK).

Auditory Brain-Stem Evoked Response

The lowest level, at which an auditory brain-stem evoked response (ABR) pattern is recognized, was

determined in each mouse [15]. Mice were anesthetized with an intramuscular injection of ketamine (75 mg/kg) and xylazine (7.5 mg/kg), and placed in a close acoustic room [15]. A stainless-steel electrode was inserted subcutaneously into the vertex (positive pole), retroauricular region (negative pole), and opposite retroauricular region (background). Acoustic stimuli, consisting of a tone burst (0.1 ms rise and fall no plateau) at a frequency of 10 kHz, were presented to each mouse with a sound stimulator (DPS-725, Diamedical System, Tokyo, Japan) and speaker (PT-R9, Pioneer, Tokyo, Japan) in an open field. For each time point, 500 responses for each mouse were recorded and filtered for band widths of 100–3000 Hz. The Neuropac μ (Nihonkoden, Tokyo, Japan) was used to analyze the ABR response.

c-Fos Immunohistochemistry

Levels of c-Fos expression were assessed by counting the number of c-Fos-immunoreactive cells [22]. Mice were deeply anesthetized with halothane (Takeda Pharmaceutical Co, Osaka, Japan) 3 h after the single injection of MK-801 (0.3 μg/g) and perfused intracardiacally with PBS, followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (pH 7.2). Coronal sections (40 μm) were made by a cryostat and incubated with a blocking solution containing PBS, 5% nonfat milk and 0.25% TritonX-100 for 2 h and incubated with rabbit anti-c-Fos antibody (1:20000; PC-38, Calbiochem, La Jolla, CA, USA) in the blocking solution 48 h at 4°C, followed by biotinylated anti-rabbit immunoglobulin antibody (1:200; BA-1000, Vector Laboratories). Sections were washed and processed with the avidin-biotinylated horseradish peroxidase complex (Vector ABC kit Vector Laboratories, Burlingame, CA, USA). Immunoreactivity was visualized with the chromogen 3, 3'- diaminobenzidine. To quantify the number of c-Fos positive cells in the brain, we used an all-in-one microscope (BZ-9000, Keyence, Osaka, Japan) and a BZ-Analyzer (Keyence) to scan the sections, and quantified the cell numbers from the digital images using NIH Image (ver. 1.61) cell-counting system [23].

Behavioral Testing

Acoustic startle response and prepulse inhibition (PPI) were measured as fully described previously [10,15]. Test paradigm of context- and tone-dependent fear learning, locomotor measurement, resident-intruder assessment [24] and further procedural details of individual behavioral tests, see Supplementary Information. Three independent cohorts of mice were made for each NRG1 subtypes and each doses and assigned to the following three types of experiments; basal behavioral tests (locomotor assessment, sound startle test, and fear conditioning in the less stressful order), the MK-801 experiments, and the resident-intruder test (see below).

Statistical Analysis

Data are presented as mean ± SEM. Computation was carried out using the SPSS 11.0 for Windows (Tokyo, Japan). Behavioral or immunohistochemical data were first subjected to the Shapiro-Wilk test (for the fitting to Gesian distribution) and Levene's test (for the equality of standard deviations) and then either one-way analysis of variance (ANOVA) or Student's t-test. ANOVA adopts NRG1-treatment as a between-subject factor, NRG1-treatment as a between-subject factor and time as a within-subject factor, or eNRG1/T1-NRG1 treatment and drug injection as between-subject factors. ABR data were subjected to two-way ANOVA with eNRG1-treatment as a between-subject factor and frequency of auditory stimulus as a within-subject factor. Other data stem from experimental groups were subjected to Student's t-test. Some of the data in the frequency of c-fos, startle responses and PPI scores had showed biased distributions or difference in deviations among groups, suggesting the risk of type 1 errors in ANOVA (Supplemental Table **S1**). Subsequently, a Tukey HSD test was applied to the data carrying equal deviations as a *post hoc* test. Alternatively, a Scheffe's test was applied to the data having non-equal deviations to validate the initial ANOVA assessment. When there was significant interaction between eNRG1-treatment and drug, data were analyzed separately to avoid the interaction for the final analysis. A p value < 0.05 was regarded as statistically significant.

RESULTS

eNRG1 Penetration into the Brain

Since the blood-brain barrier of neonatal mice is immature, peripherally administered cytokines can reach the brain [15,25]. To confirm that eNRG1 in circulation penetrates the blood-brain barrier and activates intracerebral NRG1 receptors, we subcutaneously administered eNRG1 (1.0 μg/g) to mouse pups (PND2) and examined eNRG1 immunoreactivity and the phosphorylation of ErbB4 in the cerebrum. Immunoblotting revealed that there was eNRG1 immunoreactivity in the cerebrum of the eNRG1-injeced mice but not in that of saline-injected controls (Fig. **1A**). Peripheral injection of eNRG1 also significantly increased phosphorylation levels of ErbB4 [$F(4,11) = 11.4$, $p < 0.01$]. The increase persisted between 1-6 h after the injection with a peak of ErbB4 phosphorylation at 3 h (Fig. **1B**, **C**). Thus, a peripheral injection of eNRG1 to mouse pups can produce hyper-signaling of NRG1/ErbB4 in the neonatal mouse brain.

Exploratory Activity in Novel Environment

We administered various concentrations (vehicle alone, 0.1, 0.3, and 1.0 μg/g) of eNRG1 repeatedly to mouse neonates. We monitored several parameters of

mouse development; tooth eruption, eyelid opening, and body weight gain (Table **1**). NRG1, which belongs to the EGF polypeptide family, promotes tooth eruption and eyelid opening [15,26]. A one-way ANOVA revealed that repeated eNRG1 injections significantly stimulated eyelid opening [dose: $F(3,56) = 90.31$, $p < 0.001$] and tooth eruption [dose: $F(3,56) = 35.54$, $p < 0.001$] in a dose-dependent manner. These results verified the physiological activity of injected eNRG1 *in vivo*. In addition, injections with higher doses of eNRG1 (i.e. 0.3 μg/g and 1.0 μg/g), produced a 10-20% decrease in body weight gain that persisted until adulthood (Table **1**).

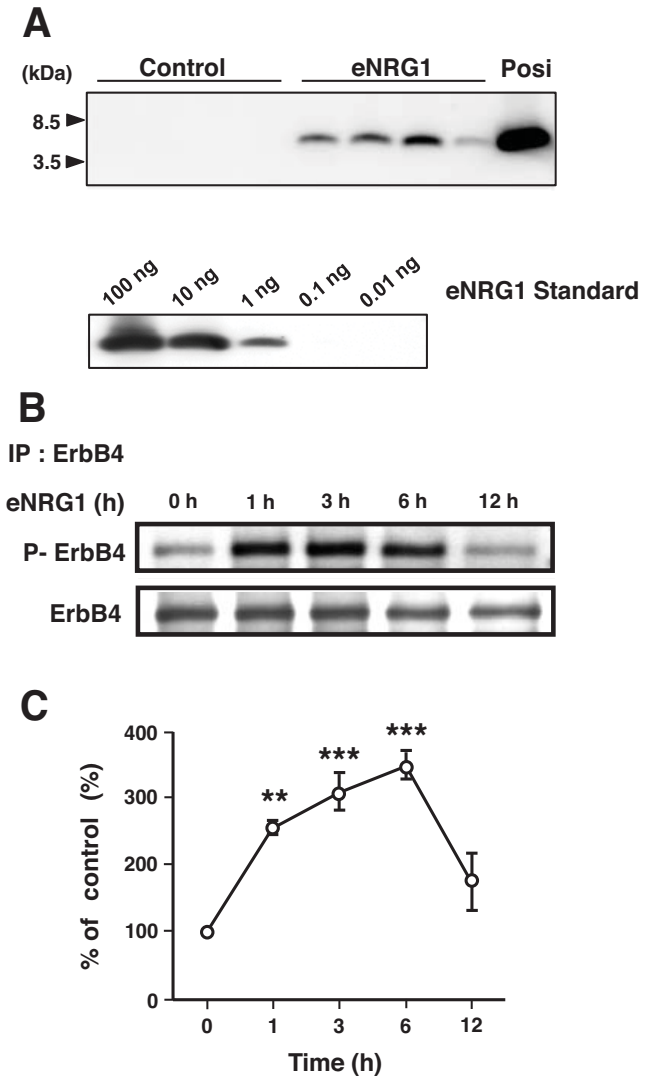


Fig. (1). ErbB4 activation and eNRG1 immunoreactivity in the brain following peripheral eNRG1 injection. (**A**) Neonatal mice (PND2) were subcutaneously challenged with eNRG1 (1.0 μg/g) and intracardiacally perfused with PBS 3 h after eNRG1 injection. Brain lysates (200 μg/lane) were subjected to SDS polyacrylamide electrophoresis with the authentic eNRG1 protein (1 ng; Posi). The presence of eNRG1 protein in the brain was examined by immunoblotting ($n = 4$ mice per group). (**B**) Whole brain lysates were prepared 0, 1, 3, 6, and 12 h after subcutaneous injection of eNRG1 (1.0 μg/g) to neonatal mice (PND2). ErbB4 phosphorylation levels were examined using immunoblotting for phosphotyrosine combined with ErbB4 immunoprecipitation. (**C**) Relative levels of ErbB4 phosphorylation in the brains of eNRG1-injected mice are presented (% of 0 time; mean ± SEM, $n = 3$ per each time points). *$p < 0.05$ compared with 0 time by Tukey HSD.

At the young adult stage (PND54-70), we assessed the locomotor activity of eNRG1-treated mice in a novel environment. A one-way ANOVA yielded a significant difference among eNRG1 doses [$F(3,36) = 4.6$, $p < 0.01$]. *Post hoc* comparisons revealed that the mice treated with the highest dose (1.0 μg/g) of eNRG1 displayed significantly fewer horizontal movements compared with the other three groups ($p < 0.05$) (Fig. **2A**), whereas stereotypic behaviors of these mice were similar among the groups [dose: $F(3,36) = 0.7$, $p = 0.54$] (Fig. **2B**). There were no significant differences in the above indices between males and females [gender × dose: $F(3,32) = 1.23$, $p = 0.31$ for horizontal movements; gender × dose: $F(3,32) = 0.27$, $p = 0.85$ for stereotypic movements], although stereotypic movements fundamentally differed between males and females [gender: $F(3,32) = 1.23$, $p < 0.01$].

Effects of Neonatal eNRG1-Treatment on Other Behavioral Traits

Using the mice treated with 1.0 and 0.3 μg/g doses of eNRG1, we further analyzed other behaviors of these animals in adulthood (PND56-70) such as fear learning ability, sensorimotor gating and social behavioral traits. The mice treated with the 1.0 μg/g dose of eNRG1 as neonates exhibited a marked reduction of PPI [eNRG1: $F(1,49) = 61.3$, $p < 0.001$, repeated ANOVA for 4 prepulses] (Table **2**). There were no significant differences in eNRG1 effects between males and females [gender × eNRG1: $F(1,47) = 2.26$, $p = 0.14$]. These mice also showed a reduction in tone-dependent learning scores but did not alter context-dependent learning scores [$F(1,39) = 0.004$, $p = 0.95$ for context-dependent learning; $F(1,39) = 23.8$, $p < 0.001$ for tone-dependent learning, both repeated ANOVA for 5 bins]. There was no significant difference in shock sensitivity [$F(1,39) = 0.005$, $p = 0.95$] (Table **2**). There were no significant differences between males and females in eNRG1 effects on the above indices, either [gender × eNRG1; $F(1,37) = 1.20$, $p = 0.28$ for conditioning: gender × eNRG1; $F(1,37) = 0.41$, $p = 0.53$ for context-dependent learning: gender × eNRG1: $F(1,37) = 1.87$, $p = 0.18$ for tone-dependent learning]. Similar behavioral alterations were observed when we administered the lower dose of eNRG1 (0.3 μ/g) to mouse pups (Supplemental Fig. **S1**). This dose (0.3 μ/g) was approximately 40 pmol per gram body weight and equivalent to the molar dose of T1-NRG1 that had been given to mouse pups in our previous study [15].

To investigate the impact of neonatal eNRG1-treatment on social behaviors in adulthood, we used a resident-intruder behavioral assay. In this assay, a group-housed male mouse (intruder) was placed in another home cage where a resident male mouse had

Table 1. Summary of physical development.

Dose	Vehicle		eNRG1	
Tooth Eruption (PND)				
0.1 μg/g	11.6 ± 0.2		10.4 ± 0.2***	
0.3 μg/g	11.6 ± 0.2		10.2 ± 0.2***	
1.0 μg/g	10.9 ± 0.2		9.4 ± 0.2***	
Eyelid Opening (PND)				
0.1 μg/g	13.9 ± 0.1		11.5 ± 0.2***	
0.3 μg/g	13.7 ± 0.2		10.8 ± 0.1***	
1.0 μg/g	13.1 ± 0.2		10.5 ± 0.2***	
Body Growth; P11				
0.1 μg/g	5.72 ± 0.13		5.52 ± 0.07	
0.3 μg/g	6.18 ± 0.20		5.62 ± 0.14*	
1.0 μg/g	6.05 ± 0.17		5.08 ± 0.22**	
Body Growth; P56	**Male**	**Female**	**Male**	**Female**
0.1 μg/g	24.2 ± 0.6	20.2 ± 0.3	23.8 ± 0.2	19.3 ± 0.3
0.3 μg/g	24.2 ± 0.6	20.3 ± 0.4	21.4 ± 0.3**	18.4 ± 0.5*
1.0 μg/g	19.4 ± 0.3	16.6 ± 0.3	17.6 ± 0.4**	14.3 ± 0.6**

eNRG1 or vehicle was administered (s.c.) daily to neonatal mice during PND2-10. During and after treatment, we monitored eyelid opening, tooth eruption and body weight of all mice. Data are expressed as mean ± SEM (n = 10 per group). P-values, compared between eNRG1-treated and vehicle-treated groups by unpaired two-tailed t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

been housed alone until the test day (Table **2**). eNRG1-treated mice exhibited decreased sniffing duration compared with vehicle-treated controls ($p < 0.05$, unpaired t-test), whereas no significant difference between groups was found with sniffing counts or fighting counts (Table **2**). The lower frequency of aggressive behaviors of the present mice can be ascribed to the mouse strain used in the present study (i.e., C57BL/6N) [27,28].

Effects of Neonatal eNRG1-Treatment on MK-801-Driven-Locomotor Activity

Sensitivity to psychostimulants, such as NMDA receptor antagonists, have often been evaluated in animal models to assess their relevancy to a schizophrenia model. To test whether neonatal eNRG1-treatment (0.1, 0.3, and 1.0 μg/g) alters acute responsiveness to an NMDA receptor antagonist, MK-801, in adulthood, mice were first acclimated to a novel environment to minimize the substantial difference of exploratory activity and/or motivation between groups. After one hour acclimation, mice were challenged with a 0.3 μg/g dose of MK-801 (Fig. **3**). The given dose of MK-801 was the minimum dose that can induce hyperlocomotion in mice [29]. MK-801 markedly increased the locomotor activity of mice for approximately 2 h in both the eNRG1-treated and control groups [drug: $F(1,36) = 29.7$, $p < 0.001$]. However, the magnitude of MK-801-induced locomotor activity was significantly higher in the eNRG1-treated group than in the control group [eNRG1-treatment: $F(1,36) = 5.2$, $p < 0.05$; eNRG1 × drug: $F(1,36) = 6.0$, $p < 0.05$] (Fig. **3A**). The sensitivity appeared to depend on the dose of eNRG1 [eNRG1 dose: $F(1,36) = 3.2$, $p < 0.05$] (Fig. **3C**). Thus, these results indicate that neonatal exposure to eNRG1 enhances behavioral sensitivity to MK-801 in a dose-dependent manner. However, there was no significant difference in MK-801-triggered stereotypic scores between eNRG1-treated and vehicle-treated control mice at any dose of eNRG1 [eNRG1 dose: $F(3,36) = 1.2$, $p = 0.34$] (Fig. **3B**, **D**).

Effects of Neonatal eNRG1-Treatment on c-Fos-Expression in the Brain

The NMDA antagonist MK-801 increases both dopamine and glutamate efflux primarily in the forebrain regions [30,31] and induces the gene expression of c-Fos through MK-801-evoked glutamatergic neurotransmission and subsequent MAP kinase/AP-1 activation [22]. Employing c-Fos expression as a marker of neuronal activity, we examined the effects of neonatal eNRG1 treatment on MK-801-induced c-Fos expression and attempted to confirm the above observation; the higher sensitivity of eNRG1-treated mice to MK-801. We counted the number of c-Fos positive cells in the medial prefrontal cortex (mPFC), nucleus accumbens (NAc), piriform cortex, and

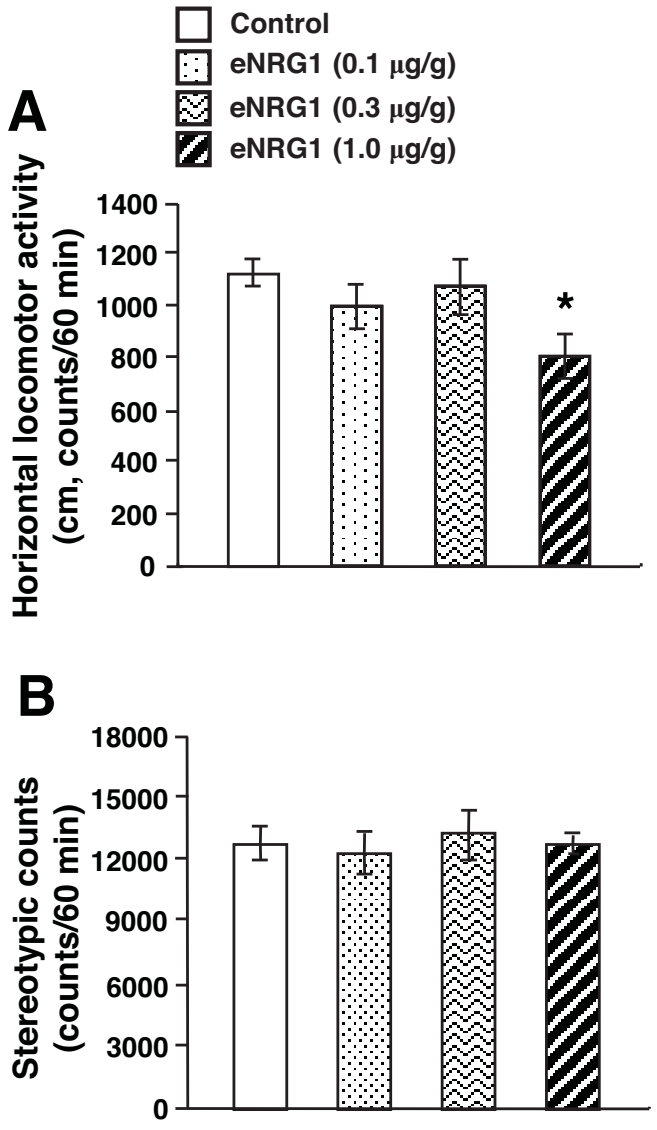


Fig. (2). Baseline locomotor activity and stereotypic behavior in a novel environment. (**A**) Total locomotor activity and (**B**) stereotypic behavior counts of eNRG1 (0.1, 0.3 and 1.0 μg/g)-treated and vehicle-treated control mice for 60 min (n = 10 mice per group). Data is expressed as mean ± SEM. *p < 0.05 compared with control mice by Tukey HSD.

striatum. MK-801 challenge significantly increased the number of c-Fos-positive cells in these regions in both control and eNRG1-treated mice [drug: $F(1,12)$ = 384.1, p < 0.001 for mPFC; $F(1,12)$ = 139, p < 0.001 for NAc; $F(1,12)$ = 69.6, p < 0.001 for piriform cortex]. In the mPFC and NAc, however, we found significant statistical interactions between MK-801 challenge and eNRG treatment [eNRG1 × drug: $F(1,12)$ = 8.5, p < 0.05 for mPFC, $F(1,12)$ = 12.4, p < 0.01 for NAc]. The interactions indicate that MK-801-triggered increases in the number of c-Fos-positive cells were greater in eNRG1-treated mice than in control mice (Fig. **4A-H**). In contrast, the number of c-Fos positive cells in the piriform cortex was indistinguishable in control and eNRG1-treated mice following MK-801 challenges [eNRG1-treatment: $F(1,12)$ = 1.4, p = 0.27, eNRG1-treatment × drug F(1,12) = 0.6, p = 0.45] (Fig. **4I**). As compared between control and eNRG1-treated mice in the saline challenged-groups, however, there was a significant difference in the basal frequency of c-Fos-positive cells in the mPFC (p < 0.001). In the striatum, we found no detectable increase in c-Fos expression following MK-801 challenge [drug: $F(1,12)$ = 0.1, p = 0.79] (Fig. **4I**).

Table 2. Summary of behavioral traits.

	Vehicle	eNRG1
Prepulse inhibition (% inhibition)		
73dB	20.5 ± 3.2	-2.5 ± 3.9***
76dB	38.5 ± 3.5	4.7 ± 4.8***
79dB	47.2 ± 3.3	3.7 ± 5.3***
82dB	57.6 ± 3.2	8.7 ± 4.9***
Learning (% freezing)		
Shock sensitivity	61.3 ± 3.4	60.0 ± 4.7
Context-dependent	66.5 ± 1.5	65.7 ± 2.1
Tone-dependent	66.0 ± 1.3	41.5 ± 3.2***
Resident-intruder test		
Sniffing Duration (sec)	178.3 ± 8.9	145.8 ± 8.1*
Sniffing Counts (times)	9.7 ± 0.8	8.1 ± 0.7
Aggression (frequency)	2.28 ± 0.14	3.61 ± 0.23

Neonatal mice were treated with eNRG1 (1.0 μg/g) or vehicle (control) as described in the Materials and Methods. In adulthood, PPI levels (%) were measured with 73, 76, 79 and 82 dB prepulse stimuli (n = 25-26 mice per group) and a main pulse of 120 dB. eNRG1-treated and vehicle-treated mice were also subjected to context-tone-foot shock pairs to evaluate their fear learning. Mean freezing rates (%) were measured for 150 sec (30 sec/ bin) during conditioning or during the same context exposure and during tone exposure in the test paradigm (n = 20-21 mice per group). In the resident-intruder assay, we measured the total time duration of sniffing behaviors and frequency of sniffing and fighting behaviors of the resident males over a 10-min period (n = 18 mice per group). Data are expressed as mean ± SEM. *p < 0.05, *** p < 0.001 by Tukey HSD or Student's t-test.

NMDA Receptor-Like Immunoreactivity in eNRG1-Treated Mice

Alterations in brain expression of NMDA receptors are often implicated in the behavioral sensitivity to the NMDA receptor blockers MK-801, phencyclidine, and ketamine [24,32]. We aimed to elucidate the molecular phenotypes underlying the hypersensitivity of eNRG1 (1.0 μg/g eNRG1)-treated mice to MK-801. Using Western blot techniques, we estimated protein levels for NMDA receptor subunits (GluN1, GluN2A-2D) and AMPA receptor subunits (GluA1-A4) in the frontal cortex, hippocampus, and striatum plus NAc (Fig. **5A**). In addition to these receptors, we also examined molecular markers for GABAergic and dopaminergic neurons. Among the receptors examined, there were significant decreases in hippocampal levels of GluN2C-like immunoreactivity and frontal cortex levels of GluN2D-like immunoreactivity in eNRG1-treated mice compared with those of control mice (Fig. **5B**). In addition, there was a decrease in GAD67-like

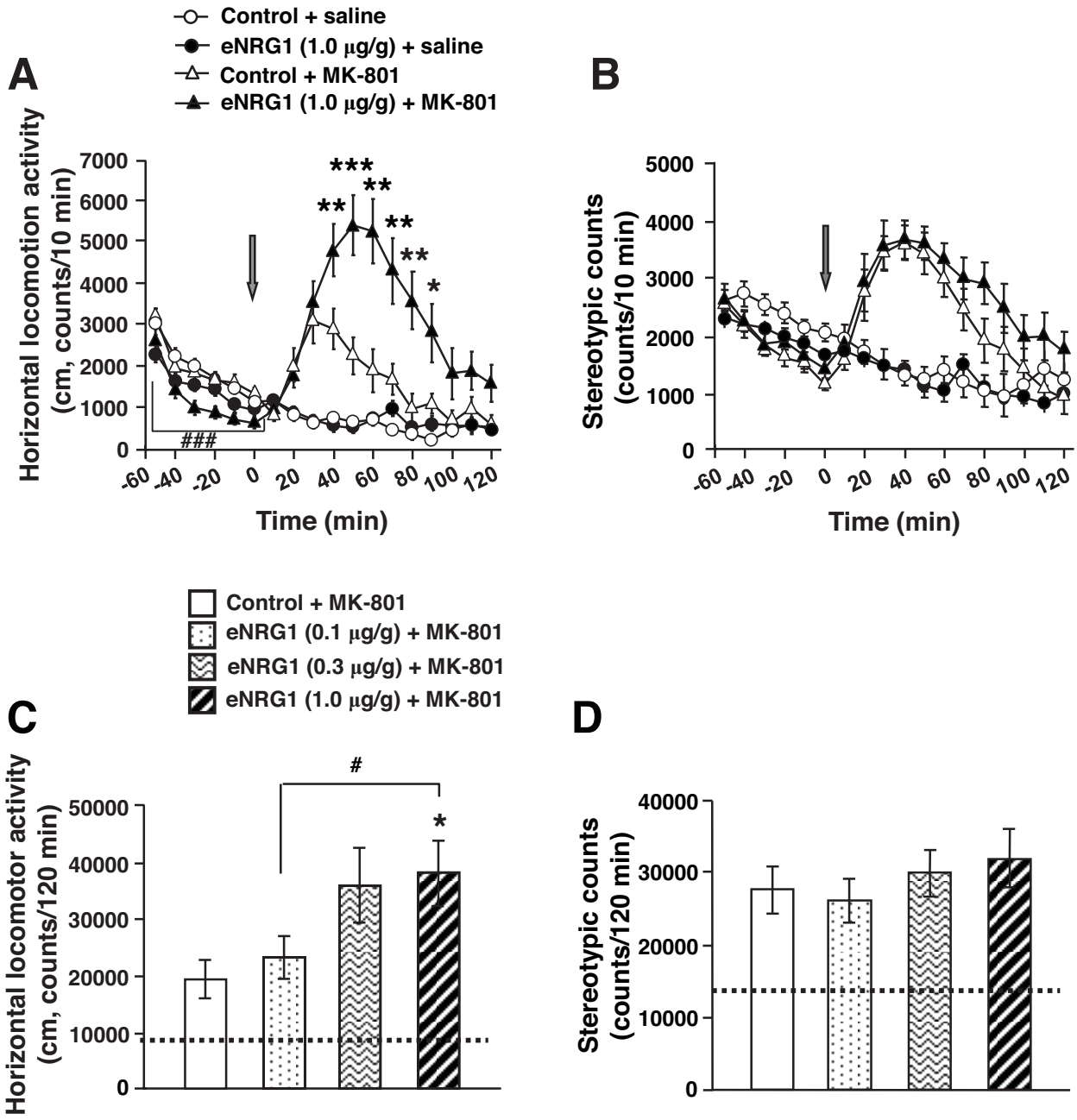


Fig. (3). Hyper-locomotor activity and stereotypic behavior induced by MK-801. (**A**, **C**) Horizontal locomotor activity and (**B**, **D**) stereotypic behavior of eNRG1 (0.1, 0.3 and 1.0 μg/g)-treated and vehicle-treated control mice were monitored before and after MK-801 (0.3 μg/g, i.p.) or saline challenge every 10 min. Prior to MK-801 challenge, mice were acclimated to a test chamber to minimize their exploratory movement. Total locomotor activity and stereotypic counts of eNRG1-treated and vehicle-treated control mice were calculated and presented (n = 10 mice per group). Data is expressed as mean ± SEM. Arrows mark the timing of MK-801 challenge. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared with vehicle-treated control mice challenged by MK-801 and $^{\#}p < 0.05$ compared between marked groups by Tukey HSD. Note; There was a significant main effect of eNRG1 treatment on total locomotor activity during the acclimination period of the four groups of mice; $F(1, 36) = 19.3$, ###$p < 0.001$ for eNRG1, $F(1,36) = 0.46$, $p = 0.503$ for MK-801, $F(1,36) = 1.46$, $p = 0.234$ for eNRG1 x MK-801.

immunoreactivity in eNRG1-treated mice. As we performed multiple comparisons here, we attempted to compensate the statistics with a Bonferroni's method. However, the differences in GluN2C-like and GluN2D-like immunoreactivites still remained to be significant. There were no significant differences in other receptors and other GABAergic/dopaminergic markers between groups after compensation.

Distinct Sensitivity of eNRG1-Treated and T1-NRG1-Treated Mice to MK-801 But Not to Methamphetamine

Recently, we reported that neonatal exposure to full length T1-NRG1 results in behavioral impairments and hypersensitivity to methamphetamine in mice [15]. To compare the *in vivo* effects of eNRG1 and T1-NRG1,

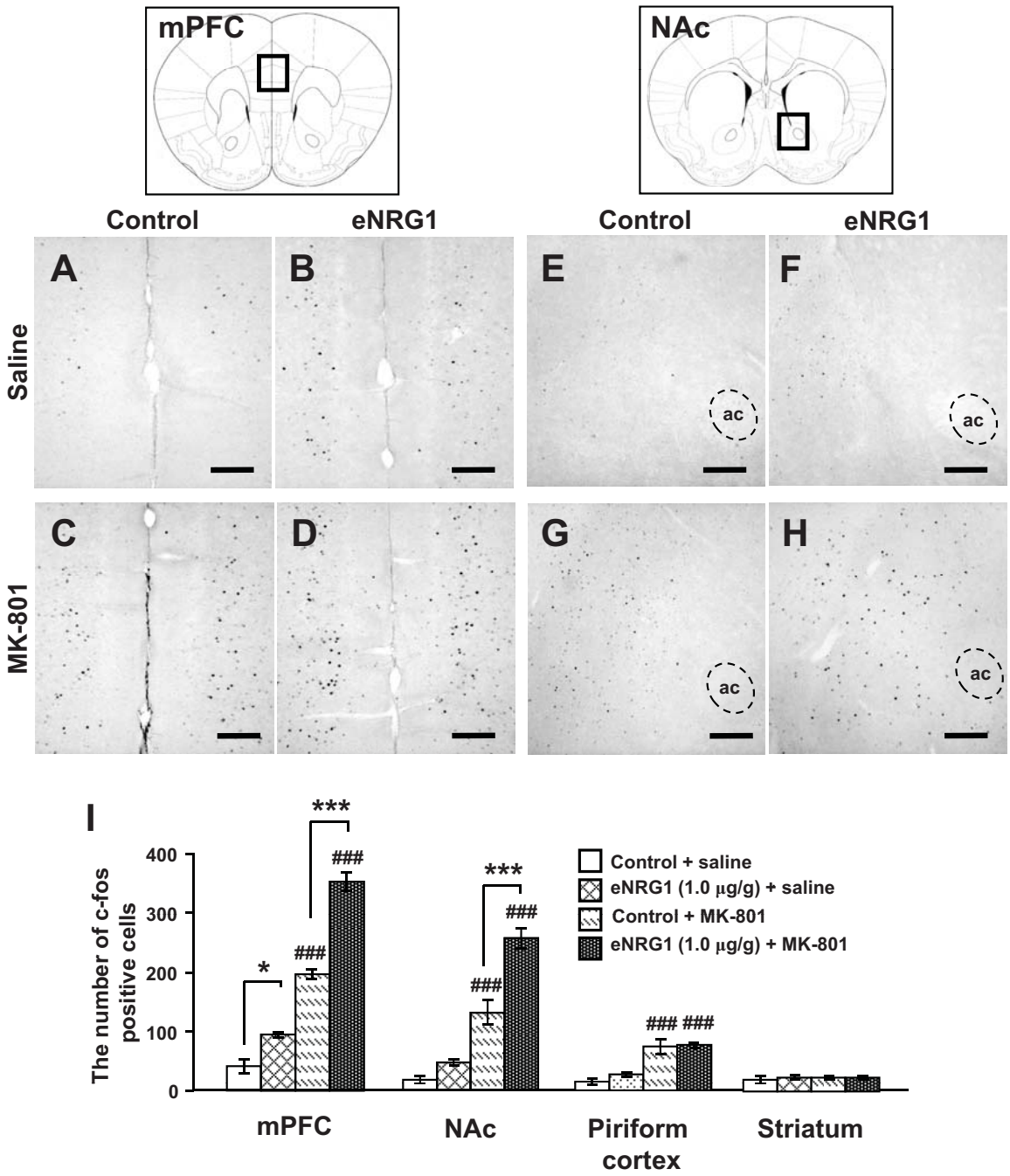
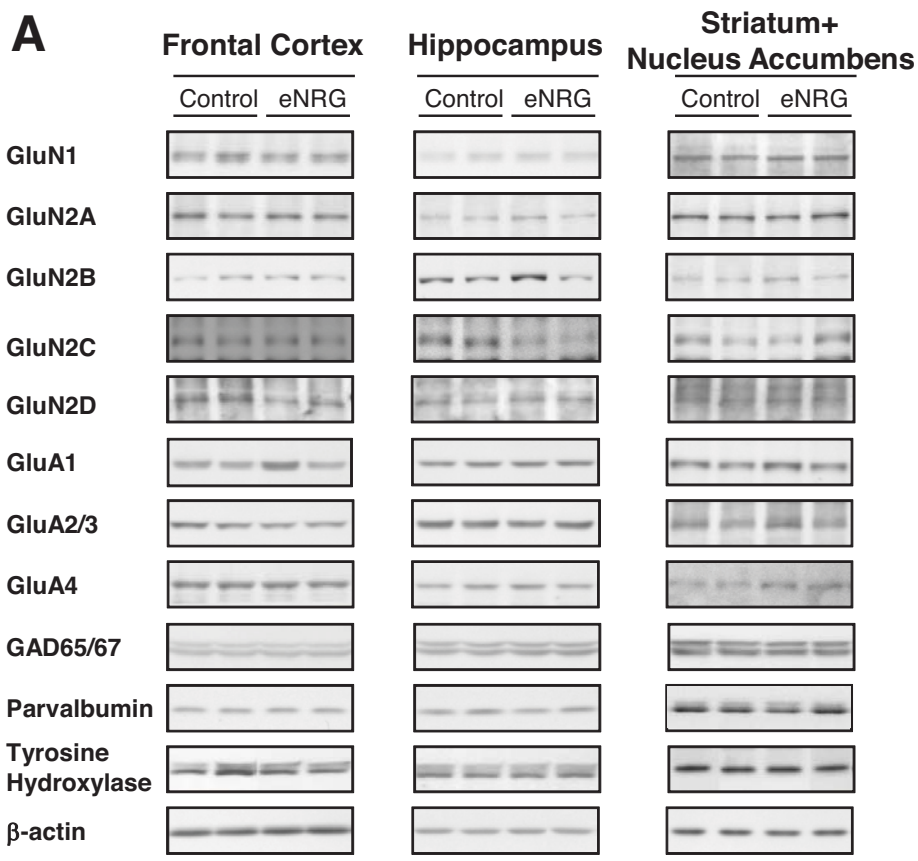


Fig. (4). Neonatal exposure to eNRG1 enhances c-Fos expression following MK-801-challenge. In adulthood, vehicle-treated mice (**A**, **C**, **E**, **G**) and eNRG1-treated mice (**B**, **D**, **F**, **H**) were subjected to c-Fos immunohistochemistry 2 h after saline (**A**, **B**, **E**, **F**) or MK-801 (**C**, **D**, **G**, **H**) challenge. Representative pictures of mPFC (prelimbic cortex; **A - D**) and NAc (**E - H**) are shown. Scale bar, 100 μm. (**I**) The number of c-Fos-positive cells in the microscopic field (725 × 965 μm) was counted bilaterally using five to seven sections of mPFC (+1.70 ~ +1.98 mm from Bregma), NAc, and striatum (+1.18 ~ +1.54 mm from Bregma), piriform cortex (+1.18 ~ +1.98 mm from Bregma), averaged for each mouse, and subjected to statistical analysis (n = 4 mice per group). Values are expressed as mean ± SEM. *p < 0.05, ***p < 0.001 compared between marked groups. $^{###}p$ < 0.001 compared with saline-challenged groups by Scheffe's test.

we measured the sensitivity of T1-NRG1-treated and eNRG1-treated mice to the NMDA receptor antagonist and methamphetamine (Figs. **6**, **7**). We prepared both types of mouse models by daily administering the same molar dose of T1-NRG1 (1.0 μg/g body weight) and eNRG1 (0.3 μg/g body weight) to mouse pups, allowed them to reach 2 months of age, and challenged with MK-801 or saline. This dose was approximately 40 pmol per gram body weight and equivalent to the molar dose of T1-NRG1 that had been given to mouse pups in our previous study [15].

A



B

	Frontal Cortex (% of control)	Hippocampus (% of control)	Striatum + Nucleus Accumbens (% of control)
GluN1 #	93± 4	105 ± 15	99 ± 5
GluN2A	88 ± 11	111 ± 12	99 ± 8
GluN2B #	100 ± 6	110 ± 17	98 ± 13
GluN2C	103 ± 17	50 ± 7**	79 ± 11
GluN2D #	58 ± 7**	124 ± 16	96 ± 10
GluA1 #	121 ± 13	96 ± 7	104 ± 6
GluA2/3	107 ± 3	80 ± 11	105 ± 14
GluA4 #	99 ± 2	92 ± 7	109 ± 12
GAD65	92 ± 3	116 ± 3	94 ± 3
GAD67	84 ± 3*	99 ± 2	90 ± 8
Parvalbumin	104 ± 8	96 ± 6	96 ± 7
TH	92 ± 3	80 ± 5	90 ± 7
β-actin (n=5-6)	101 ± 6	98 ± 10	97 ± 9
(n=9-10) #	104 ± 3	99 ± 5	98 ± 4

Fig. (5). Influences of neonatal eNRG1 treatment on the immunoreactivity of glutamate receptors, GABAergic and dopaminergic markers. Protein extracts were prepared from the frontal cortex, hippocampus, and striatum puls NAc of vehicle-treated and eNRG1 (1.0 μg/g)-treated mice (*n* = 4-5 mice per group) at the adult stage (PND70-80) and subjected to immunoblotting with the antibodies directed against NMDA receptors (GluN1, GluN2A, GluN2B, GluN2C, GluN2D), AMPA receptors (GluA1, GluA2/3 and GluA4), GABAergic markers (glutamic acid decarboxylase; GAD65/67 and parvalbumin), a dopaminergic marker (TH; tyrosine hydroxylase), and a loading control (β-actin). (**A**) Typical signals of two samples in each brain region are displayed. (**B**) The intensity of the immunoreactivity appearing at the authentic size(s) was measured by densitometoric analysis and subjected to an unpaired two-tailed *t*-test. **p* < 0.05, ***p* < 0.01 without Bonferroni's compensation. #) The number of samples was increased to 9 or 10 when the values of SEM from 4-5 mice had been larger than 20%; GluN1, GluN2B, GluA1, GluA4, and β-actin. Relative levels of the markers in eNRG1-treated mice are presented (% of control; mean ± SEM; *n* = 4-5 or *n* = 9-10 for #).

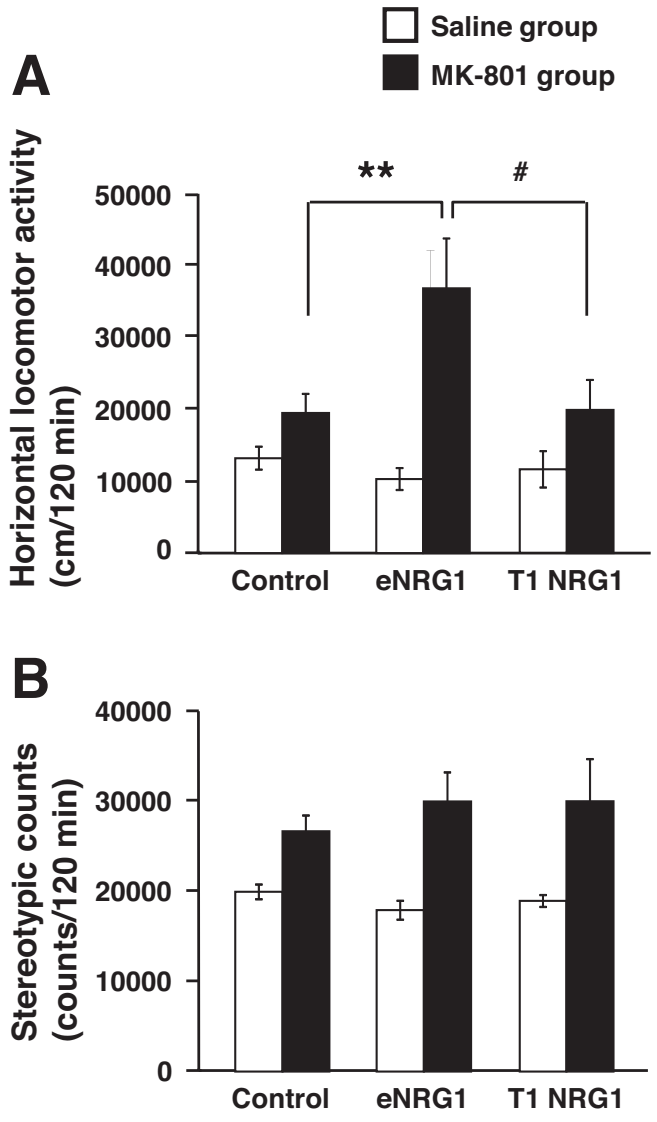


Fig. (6). Difference of eNRG1-treated and T1-NRG1-treated mice in MK-801-driven locomotor activity and stereotypic behaviors. eNRG1 (0.3 μg/g)-treated, T1-NRG1 (1.0 μg/g)-treated and vehicle-treated mice (control) as neonates were challenged with MK-801 (0.3 μg/g; black boxes) or saline (white boxes) and their horizontal locomotor activity (**A**) and stereotypic behavior (**B**) were compared. Total horizontal activity and stereotypic score of mice were measured for the 120 min period after challenge and presented with the scales of cm/120 min and counts/120 min, respectively (n = 10 - 23 mice per group). Data is expressed as mean ± SEM. $^{**}p < 0.01$, compared between control and eNRG1-treated mice challenged with MK-801 and $^{\#}p < 0.05$ compared between eNRG1-treated mice challenged with MK-801 and T1-NRG1-treated mice challenged with MK-801 by Tukey HSD.

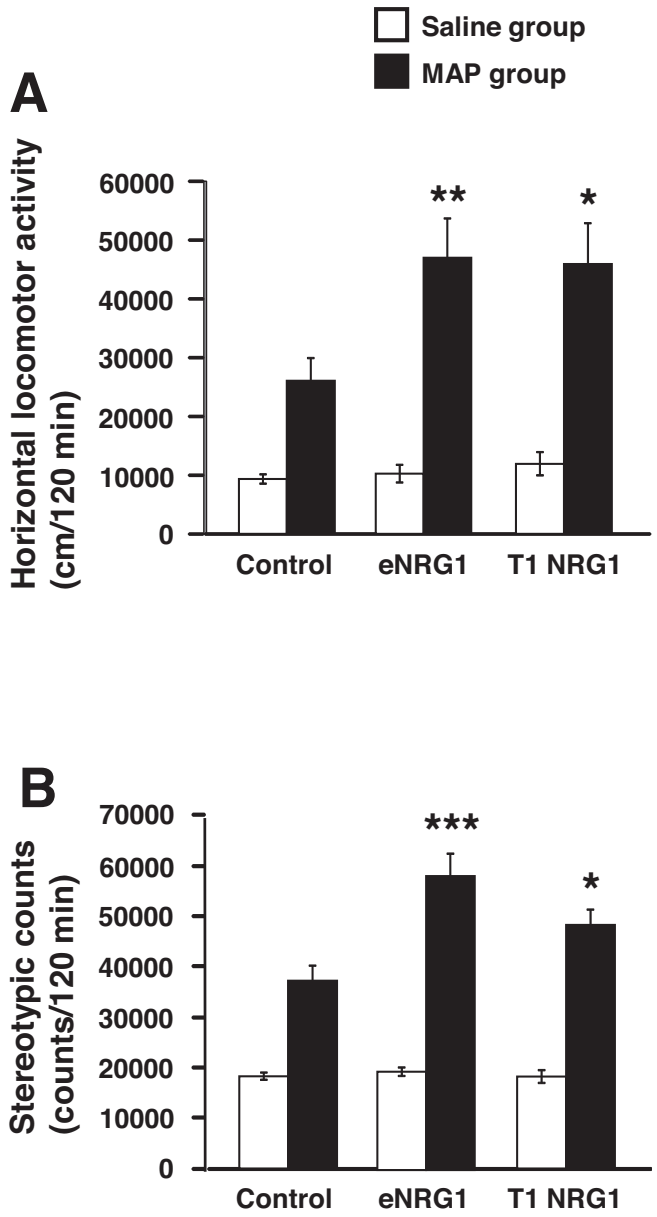


Fig. (7). Similar enhancement of eNRG1-treated and T1-NRG1-treated mice in methamphetamine-driven locomotor activity and stereotypic behaviors. eNRG1 (0.3 μg/g)-treated, T1-NRG1 (1.0 μg/g)-treated and vehicle-treated mice (control) as neonates were challenged with methamphetamine (MAP; 1.0 μg/g black boxes) or saline (white boxes) and their horizontal locomotor activity (**A**) and stereotypic behavior (**B**) were compared. Data is expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ between control and eNRG1-treated or T1-NRG1 treated mice challenged with MAP by Tukey HSD.

An initial 2-way ANOVA on horizontal locomotor activity, using the subject factors of NRG1 isoform and MK-801, revealed a main effect of MK-801 [$F(1,74) = 23.57$, $p < 0.001$] and a significant interaction between NRG1 isoform and MK-801 [$F(2,74) = 5.17$, $p < 0.01$]. We then stratified on MK-801 treatment (MK-801-challenged and saline-challenged groups; Fig. **6A**). In the saline-challenged control groups, there was no difference in basal locomotor activity among T1-NRG1-treated, eNRG1-treated and control mice [NRG1 isoform : $F(2,37) = 0.70$, $p = 0.50$]. A one-way ANOVA for the MK-801-challenged group revealed a significant effect of isoform [$F(2,37) = 4.80$, $p < 0.05$]. *Post-hoc* analyses suggest that MK-801-driven horizontal locomotor activity of T1-NRG1-treated mice was indistinguishable from that of control mice receiving

MK-801 whereas eNRG1-treated mice displayed a larger locomotor response to MK-801 than control mice. A two-way ANOVA for stereotypic movement revealed no significant effect of NRG1 isoform or no interaction between NRG1 isoform and MK-801 [NRG1 isoform: $F(2,74) = 0.15$, $p = 0.86$; NRG1 isoform × MK-801: $F(2,74) = 0.99$, $p = 0.37$] (Fig. **6B**). The results from the horizontal activity test revealed a significant difference in behavioral sensitivity to the NMDA receptor antagonist between T1-NRG1-treated and eNRG1-treated mice.

We also compared the magnitude of methamphetamine effects on locomotor activity. An initial 2-way ANOVA on horizontal movement, with between subject factors of NRG1 isoform and methamphetamine, revealed a main effect of methamphetamine [$F(1,92) = 60.2$, $p < 0.001$] and an interaction between NRG1 isoform and methamphetamine [$F(2,92) = 5.19$, $p < 0.01$]. We then stratified on methamphetamine treatment (methamphetamine-challenged and saline-challenged groups; Fig. **7A**). In the saline-challenged groups, there were no significant differences among eNRG1-treated, T1-NRG1-treated, and control groups [$F(2,46) = 0.60$, $p = 0.56$]. A one-way ANOVA for the methamphetamine-challenged groups revealed a significant effect of NRG1 isoform [$F(2,46) = 5.68$, $p < 0.01$]. *Post-hoc* analyses detected a significant difference between eNRG1-treated and control groups and between T1-NRG1-treated and control groups, but not between eNRG1-treated and T1-NRG1-treated groups. These results suggest that neonatal treatments with eNRG1 and T1-NRG1 similarly enhance methamphetamine-induced horizontal locomotion. We analyzed the effects of NRG1 isoform on stereotypic movements in a similar manner (Fig. **7B**). There was a main effect of methamphetamine [$F(1,92) = 101$, $p < 0.001$] and an interaction between NRG1 isoform and methamphetamine [$F(2,92) = 4.74$, $p < 0.05$]. Thus, we stratified on methamphetamine treatment. In the saline-challenged groups, there were no significant differences among groups [$F(2,46) = 0.0064$, $p = 0.99$]. In the methamphetamine-challenged groups, however, there was a significant effect of NRG1 isoform [$F(2,46) = 5.19$, $p < 0.01$]. *Post-hoc* tests detected a significant difference between eNRG1-treated and control groups and between T1-NRG1-treated and control groups, but not between eNRG1-treated and T1-NRG1-treated groups. These analyses suggest that neonatal treatments with eNRG1 and T1-NRG1 similarly enhance methamphetamine-triggered stereotypic movements.

Auditory Perception of eNRG1-Treated Mice

NRG1 signaling is involved in the survival of cochlear sensory neurons; thus, we tested sound startle responses of the mice treated with eNRG1 (0.3 and 1.0 μg/g) to increasing noises and also measured the auditory stimulus thresholds in different tone frequencies. In the sound startle response test, the lower dose of eNRG1-treated mice exhibited a marked increase in startle responses to 110-dB and 120-dB tones, compared with control mice [eNRG1 × pulse: $F(6,114) = 30.6$, $p < 0.001$] (Fig. **8A**). Conversely, the eNRG1-treated mice exhibited marked increases in the auditory stimulus thresholds at all stimulus frequencies at the ABR test [eNRG1 × stimulus frequencies: $F(5,90) = 11.2$, $p < 0.001$] (Fig. **8B**). The mice treated with the higher dose of eNRG1 (1.0 μg/g) similarly exhibited the increases in startle responses and the auditory stimulus thresholds [eNRG1 × pulse: $F(6,114) = 21.4$, $p < 0.001$ for startle responses; eNRG1 × stimulus frequencies: $F(5,90) = 13.4$, $p < 0.001$ for auditory stimulus thresholds] (Fig. **8A**, **B**). The results from the ABR test suggest deterioration in hearing of eNRG1-trearted mice. We speculate that, in the sound startle response test, the saturated levels of tone stimuli appear to produce higher startle responses in burst-noise-naïve eNRG1-treated mice with deterioration in hearing.

DISCUSSION

In our preceding study, mice pups were treated with T1-NRG1 and their behavioral consequences were analyzed with respect to the relevancy to an animal model for schizophrenia [15]. The T1-NRG1-treated animals exhibit various behavioral abnormalities in PPI and social traits but their gross learning performance and hearing ability are normal [15]. In addition to the isoform of T1-NRG1, here mouse pups were challenged with eNRG1, the core domain peptide that is produced by the neuropsin-dependent processing of T1-NRG1 [7]. In agreement with the preceding results from T1-NRG1-treated mice, transient exposure of eNRG1 during the neonatal stage similarly altered adult behavioral traits in PPI and resident-intruder test. Even though the mice were treated with the same molar dose of NRG1, eNRG1-treated mice exhibited additional behavioral deficits in tone-dependent fear learning, sound-startle responses and sensitivity to the NMDA receptor antagonist MK-801.

We also found that eNRG1-treated mice have a hearing disability. This finding is consistent with the previous report that ErbB4 signals regulate the development of hair follicles in the inner ear and that the manipulation of NRG1 signals indeed impair hearing in mice [33,34]. In this context, we can suggest that the deficits in prepulse inhibition, sound-startle responses, and tone-dependent learning of eNRG1-treated mice are, in part, due to the deterioration in hearing.

The decrease of 1.0 μg/g eNRG1-treated mice in exploratory movement is also noteworthy. In contrast to the basal locomotor reduction, these mice rather exhibited the higher locomotor responses following MK-801 challenge. Although the lower basal movement might reflect the elevation of their anxiety levels, we do not rule out the possibility that these behavioral features of eNRG1-treated mice might be indirectly influenced by their deterioration in hearing as well.

In the present study, we additionally prepared the animal treated with the full soluble type 1 isoform of T1-NRG1 at the same molar dose of eNRG1 and

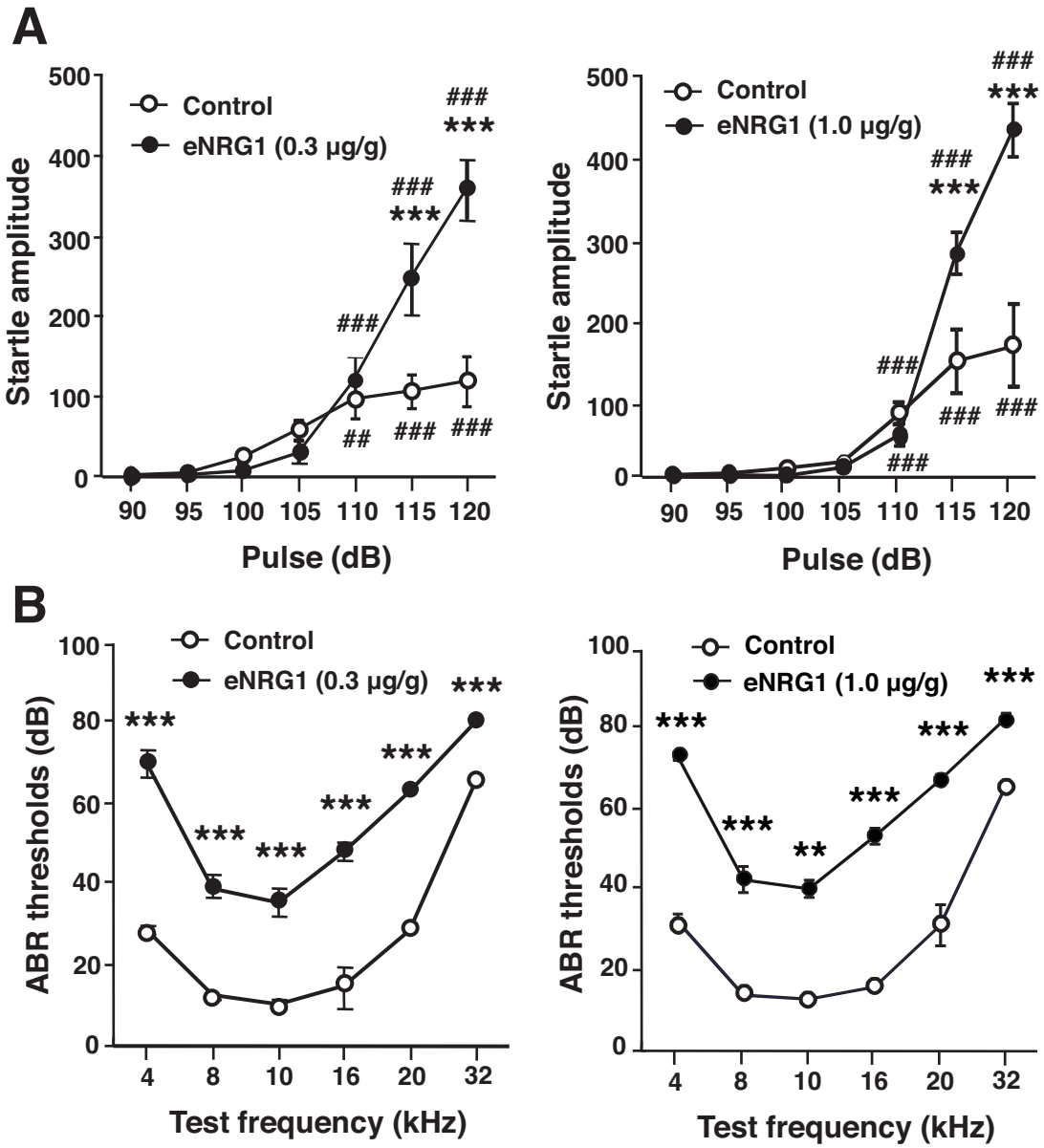


Fig. (8). Auditory responses of eNRG1-treated mice as adults. (**A**) Relative amplitudes of startle responses of eNRG1 (left; 0.3 µg/g and right; 1.0 µg/g)-treated and vehicle-treated mice (control) were monitored with 90 – 120 dB tones (n = 10 mice per group). Data is expressed as mean ± SEM. *** $p < 0.001$ compared between groups. ## $p < 0.01$ and ### $p < 0.001$ compared with startle levels at 90 dB by Tukey HSD. (**B**) ABR thresholds of eNRG1 (left; 0.3 µg/g and right; 1.0 µg/g)-treated and vehicle-treated mice were determined with specific auditory stimuli (4, 8, 10, 16, 20, and 32 kHz) by varying the sound pressure levels (n = 9-11 mice per group). Data is expressed as mean ± SEM. ** $p < 0.01$ and *** $p < 0.001$ compared between groups by Scheffe's test.

compared the sensitivities of eNRG1-treated and T1-NRG1-treated mice to two psychotomimetics, MK-801 and methamphetamine. The MK-801-driven locomotor enhancement was larger in mice treated with eNRG1 as neonates, compared with T1-NRG1-treated mice, even though the given molar concentration of T1-NRG1 was nearly equal to the dose of eNRG1. The hypersensitivity of the eNRG1-treated mice to MK-801 was also apparent in the magnitude of c-Fos induction in the brain. Following MK-801 challenge, eNRG1-treated mice contained a higher number of c-Fos-positive cells in the mPFC as well as in the NAc than control mice receiving MK-801. Both brain regions are implicated as target regions of MK-801 [35]. In contrast to the responses to MK-801, there were no significant difference in methamphetamine-triggered locomotor enhancement between T1-NRG1-treated and eNRG1-treated mice. The behavioral difference of T1-NRG1-treated and eNRG1-treated mice might reflect qualitative differences in *in vivo* activities of eNRG1 and T1-NRG1. Alternatively, the quantitative difference in the efficacy of blood-brain barrier permeability or in metabolic rates in the brain might underly the behavioral difference between these NRG1 variants. However, the magnitude and peak time of ErbB4 phosphoryration appeared to be similar between

eNRG1 and T1-NRG1 (i.e. both ~3-fold increases three hours after injection of their 1.0 μg/g dose) [15].

Several reports indicate the activity difference among NRG1 splicing variants. Major splicing variants of NRG1 (type 1; NDF, type 2; GGF, and type 3; SMDF) exhibit distinct interactions to individual ErbB1-4 receptor subtypes, presumably leading to distinct biological effects in the brain [35,36]. NRG1 isoforms exhibit qualitative differences due to their specific structures such as an immunoglobulin-like domain. This domain, which is found in T1-NRG1 but not eNRG1, carries a heparin binding activity and regulates tissue distribution and receptor interactions of the ligand [7,37,38].

eNRG1 is produced from T1-NRG1 as well as potentially from the type2 isoform of NRG1 through the neuropsin-dependent proteolytic processing [7]. The protease neuropsin recognizes and cleaves the immunoglobulin-like domain of the NRG1 precursors and liberate their carboxyl terminal domain; eNRG1. Tamura *et al*. [7] demonstrate the biological implication of the neuropsin-dependent NRG1 processing in hippocampal synapse plasticity. As the molar dose of eNRG1 was adjusted to that of T1-NRG1 in some of the present experiments, their dose difference cannot illustrate the behavioral differences between T1-NRG1-treated and eNRG1-treated mice. Nonetheless, the present results demonstrate that the biological actions of individual NRG1 variants significantly differ *in vivo* and result in distinct behavioral consequences. Corroborating our findings, mice deficient in type 3 NRG1 exhibit distinct behavioral deficits from type 1 NRG1-knockout mice [8,9,11]. However, it remains to be explored whether the present behavioral differences of T1-NRG1-treated and eNRG1-treated mice are ascribed to the unique activity of the immunoglobulin-like domain or that of the neuropsin-cleaving product eNRG1 [7,38].

To elucidate the molecular underpinnings of the given hypersensitivity to the NMDA receptor antagonist, we assessed the neurochemical influences of eNRG1 in adulthood, focusing on NMDA-type and AMPA-type glutamate receptors. There were reductions in NMDA receptor-like immunoreactivities (i.e. GluN2C and GluN2D) in the frontal cortex and hippocampus of eNRG1-treated mice, which can be involved in the hypersensitivity to MK-801. The specificity of the anti-GluN2D antibody, but not that for the anti-GluN2C antibody, has been verified with the use of knockout mice [39,40] Although the molecular nature of the present GluN2C-like immunoreactivity required further validation, the above arguments are in agreement with the observation that NMDA receptor-hypomorphic mutant mice show hypersensitivity to an NMDA receptor blocker [24,41]. Recently, Belforte *et al.* (2010) also provide supportive evidence that the psychomimetric activity of MK-801 is ascribed to a decrease in the NMDA receptor sensitivity of parvalbumin-positive GABA neurons during postnatal development [42]. As parvalbumin-positive GABA neurons are enriched with ErbB4 receptors for NRG1, the expression of NMDA receptors in this cell population might be affected by excess eNRG1 in the present model [43-46]. However, we do not rule out the possibility that the eNRG1-driven hypersensitivity to MK-801 may involve glutamatergic, dopaminergic, or cholinergic brain circuits as suggested previously [47].

NRG1 is one of the most promising candidate genes for schizophrenia. Most of the single nucleotide polymorphisms (SNPs) associating with schizophrenia are located in the genomic promoter region and introns of the 5'-side of the *NRG1* gene [4,48,49]. Although the functional effects of these polymorphisms on gene transcription or alternative splicing of NRG1 isoforms are largely speculative, postmortem studies indicate that schizophrenic alterations of NRG1 mRNA levels are isoform-specific [3,50,51]. In agreement, the individual SNPs in human *NRG1* are differentially implicated in psychotic symptoms, spatial working memory capacity, and white matter density and integrity [52,53]. In addition to schizophrenia, the genetic and neuropathologic association with NRG1 is implicated in several other psychiatric disease major depression, and bipolar disorder as well although several controversies still remain [54,55]. The differential expression of NRG1 processing enzymes is reported in schizophrenia and bipolar disorder [56]. Thus differential processing of NRG1 protein may contribute to the disease specificity as well.

The present results indicate that the composition of NRG1 isoforms might influence behavioral or cognitive traits in humans as well. Future studies should investigate the biological or pathological actions of individual NRG1 splicing isoforms underlying the SNP-dependent cognitive and anatomical traits in normal subjects and/or patients with schizophrenia.

ABBREVIATIONS

ABR	=	Auditory brain-stem evoked response
ANOVA	=	Analysis of variance
EGF	=	Epidermal growth factor
eNRG1	=	An epidermal growth factor domain peptide of neuregulin-1
mPFC	=	Medial prefrontal cortex
NAc	=	Nucleus accumbens
NRG1	=	Neuregulin-1
PBS	=	Phosphate-buffered saline
PND	=	Postnatal day
PPI	=	Prepulse inhibition
SNP	=	Single nucleotide polymorphisms
T1-NRG1	=	Type1 neuregulin-1

CONFLICT OF INTEREST

The authors confirm that this article content has not conflicts of interest.

ACKNOWLEDGEMENTS

The authors are grateful to Minoru Tanaka and Michisuke Yokoyama for their assistance with mouse breeding. This work was supported by MEXT KAKENHI (No.24116010), JSPS KAKENHI (No. 22300107), and a grant for Promotion of Niigata University Research Projects.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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Received: November 11, 2014 Revised: December 20, 2014 Accepted: January 17, 2015