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Research Article

RESEARCH ARTICLE

Restoration of *Sp4* in Forebrain GABAergic Neurons Rescues Hypersensitivity to Ketamine in *Sp4* Hypomorphic Mice

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Abstract

Background: Ketamine produces schizophrenia-like behavioral phenotypes in healthy people. Prolonged ketamine effects and exacerbation of symptoms after the administration of ketamine have been observed in patients with schizophrenia. More recently, ketamine has been used as a potent antidepressant to treat patients with major depression. The genes and neurons that regulate behavioral responses to ketamine, however, remain poorly understood. Sp4 is a transcription factor for which gene expression is restricted to neuronal cells in the brain. Our previous studies demonstrated that *Sp4* hypomorphic mice display several behavioral phenotypes relevant to psychiatric disorders, consistent with human *SP4* gene associations with schizophrenia, bipolar disorder, and major depression. Among those behavioral phenotypes, hypersensitivity to ketamine-induced hyperlocomotion has been observed in *Sp4* hypomorphic mice.
Methods: In the present study, we used the *Cre-LoxP* system to restore *Sp4* gene expression, specifically in either forebrain excitatory or GABAergic inhibitory neurons in *Sp4* hypomorphic mice. Mouse behavioral phenotypes related to psychiatric disorders were examined in these distinct rescue mice.
Results: Restoration of *Sp4* in forebrain excitatory neurons did not rescue deficient sensorimotor gating nor ketamine-induced hyperlocomotion. Restoration of *Sp4* in forebrain GABAergic neurons, however, rescued ketamine-induced hyperlocomotion, but did not rescue deficient sensorimotor gating.
Conclusions: Our studies suggest that the *Sp4* gene in forebrain GABAergic neurons regulates ketamine-induced hyperlocomotion.

Keywords: GABAergic, genetic rescue, ketamine, locomotor activity, Sp4

Introduction

Sp4 is a transcription factor that recognizes GC-rich sequences in the "CpG islands" around the promoters of many genes (Heisler et al., 2005). In contrast to the *Sp1* gene, *Sp4* gene expression is restricted to neuronal cells in the brain (Supp et al., 1996; Zhou et al., 2005). Our previous studies found that *Sp4* hypomorphic mice displayed several behavioral phenotypes

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relevant to psychiatric disorders, including deficits in prepulse inhibition and hypersensitivity to ketamine (Zhou et al., 2005, 2007, 2010). In humans, the *SP4* gene was reported to be sporadically deleted in patients with schizophrenia (Tam et al., 2010; Zhou et al., 2010), and the SP4 protein is reduced in the post-mortem brains of bipolar patients (Pinacho et al., 2011). Additionally, human genetic studies reported the association of the *SP4* gene with bipolar disorder, schizophrenia, and major depression (Shyn et al., 2009; Zhou et al., 2009; Shi et al., 2010; Greenwood et al., 2011). These studies suggest the *Sp4* hypomorphic mice as a promising mouse genetic model for human psychiatric disorders.

Administration of ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptors antagonist, induces behaviors that resemble several aspects of psychiatric disorders in healthy people (Krystal et al., 1994; Xu et al., 2015). Prolonged ketamine effects and exacerbation of symptoms were reported in schizophrenia patients after administration of ketamine (Lahti et al., 1995; Malhotra et al., 1997; Holcomb et al., 2005). On the other hand, ketamine was recently found to act as a potent antidepressant that provides rapid relief to patients who are resistant to treatment with classical antidepressants (Zarate et al., 2006; Price et al., 2009; aan het Rot et al., 2010; Diazgranados et al., 2010; Li et al., 2010). In rodents, ketamine also disrupts prepulse inhibition of startle (PPI), a form of sensorimotor gating that is deficient in patients with either schizophrenia or bipolar disorder, but not in patients with depression (Braff et al., 2001; Geyer, 2006). It is unclear however, what genes and neurons are the key regulators of such responses to ketamine.

Sp4 hypomorphic mice have robust behavioral phenotypes such as deficient PPI and hypersensitivity to ketamine-induced hyperlocomotion (Ji et al., 2013). By taking advantage of a genetic rescue strategy used in the generation of *Sp4* hypomorphic mice (Zhou et al., 2005), we restored *Sp4* expression in forebrain excitatory and GABAergic inhibitory neurons, respectively. Deficient PPI and ketamine-induced hyperlocomotion were examined in the two different neuron-specific *Sp4* rescue mouse lines.

Materials and Methods

Mouse Strains and Breeding

Following the recommendation of the Banbury Conference on Genetic Background in Mice (Banbury, 1997) to use the F1 genetic background, we generated *Sp4* hypomorphic mice on an F1 129S/Black Swiss genetic background for behavioral studies, as previously described (Ji et al., 2013). The *Emx1-Cre* mouse line (stock number: 005628) and *Dlx6a-Cre* mouse line (stock number: 008199) were purchased from Jackson laboratory. Both lines were backcrossed with Black Swiss mice for more than six generations. Each *Cre* line was then bred with *Sp4* heterozygous mice on a Black Swiss background to obtain double heterozygous mice. The double heterozygous mice were finally bred with *Sp4* heterozygous mice on a 129S background to obtain F1 generation mice (Figure 1S). All F1 *Sp4* heterozygous mice were sacrificed, and the remaining four genotypes were used for molecular and behavioral analyses (Supplemental Table I). Mice were housed in a climate-controlled animal colony with a reversed day/night cycle. Food (Harlan TeklabI) and water were available *ad libitum*, except during behavioral testing. Behavioral testing began when mice were 3 months old with PPI and ketamine-induced locomotor activity tests separated by at least 2 weeks. All testing procedures were approved by the UCSD Animal Care and Use Committee (permit number: A3033-01) prior to the onset of the experiments. Mice were maintained in American Association for Accreditation of Laboratory Animal Care–approved animal facilities at UCSD and the local Veteran's Administration Hospital. These facilities meet all Federal and State requirements for animal care.

LacZ Staining

20 μm thick cryostat sections were cut from fresh adult mouse brains. The sections were fixed, permeabilized, and stained as previously described (Zhou et al., 2005).

Western Blot

Total protein was extracted from mouse cortices and striata and Western blot analyses were subsequently performed as described (Ji et al., 2014). Anti-Sp4 antibody was purchased from Santa Cruz Biotechnology (sc-13019, sc-645).

Immunohistochemical Analysis

Adult mice were anesthetized with carbon dioxide, and perfused transcardially with 2% phosphate buffered saline paraformaldehyde. Mouse brains were dissected out and further fixed in 4% paraformaldehyde solution at 4°C for 24 hrs. Paraffin sections were generated. Sequential chromogenic immunohistochemistry was conducted to examine the co-localization of Sp4 protein with Gad67 protein, a marker for GABAergic neurons in mouse cortex, as described by Kim et al. (2012).

Prepulse Inhibition

Startle reactivity and prepulse inhibition (PPI) were measured with startle chambers (SR-LAB, San Diego Instruments) as described by Ji et al. (2013). The background noise level was 65 dB, and the pulse was 120 dB for 40 ms. The prepulse intensities were 69, 73, and 81 dB (4, 8, and 16 dB above the 65-dB background noise, respectively), delivered 80 ms before the pulse. The test session began and ended with five presentations of the "pulse-alone" trial. In between, each acoustic or "no stimulus" (65 dB background noise only) trial type was presented ten times in a pseudo-random order. The amount of PPI was calculated as a percentage score for each acoustic prepulse trial: %PPI = 100% × {1 - [(Startle response for "prepulse + pulse")/(Startle response for "pulse-alone")]}.

Video-Tracking Locomotion Tests

Locomotor activity was measured using the Video-Tracker (VT) system as previously described (Ji et al., 2013). Mice were tested during their dark period with lights on for the entire duration of the test. They were first acclimated to the testing room for 60 min. Then, they were placed into white plastic enclosures ($41 \times 41 \times 34\,cm^3$), surrounded by an opaque plastic curtain. A video camera, mounted 158 cm above the enclosures, generated the signal for the Polytrack digitizer (San Diego Instruments). The position of each animal (x, y; in pixels) was sampled with a frequency of 18.18 Hz, which was used to generate a coordinate file (x, y, t) consisting of the x-location, y-location, and the duration (time t) spent in that location. After acclimation, mice were placed in their enclosures for 30 min of habituation. They then received ketamine (described below) and were placed back in their enclosures for another 60 min.

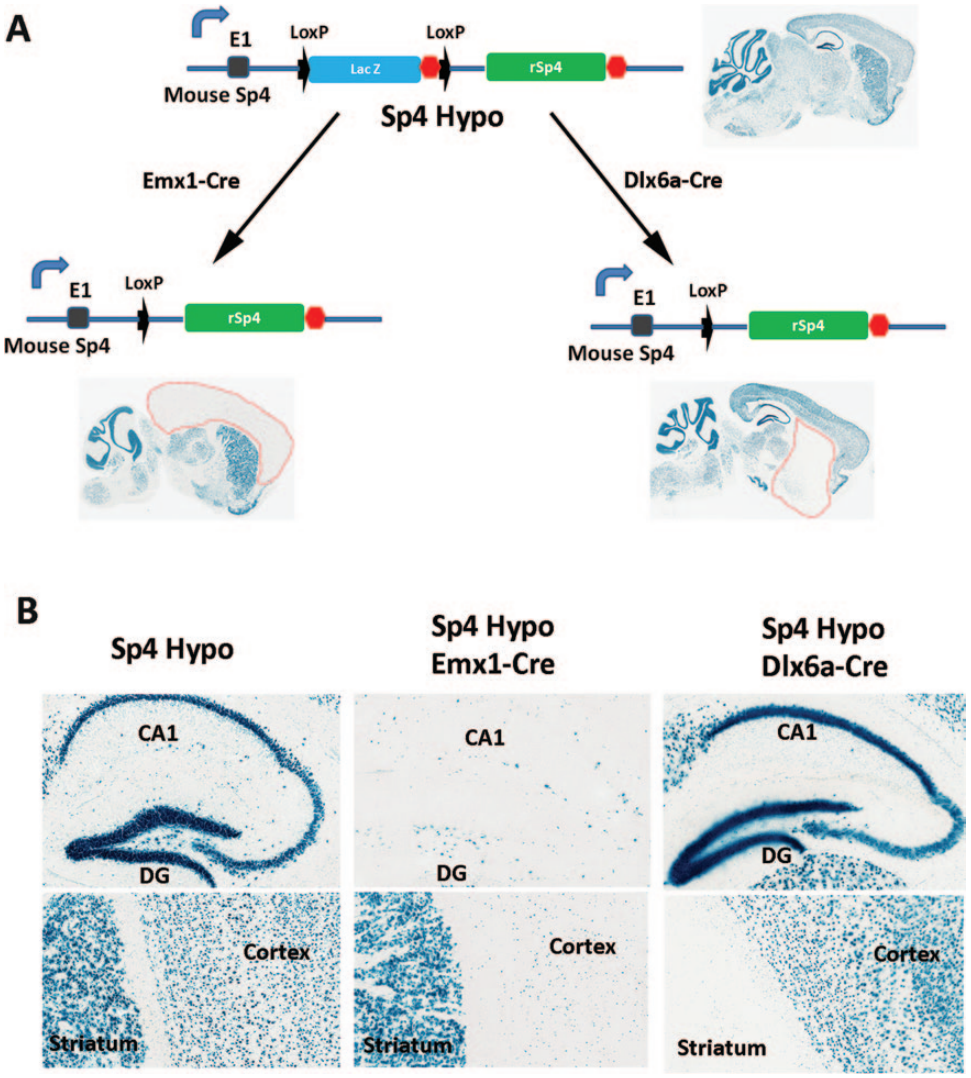

Figure 1. Restoration of rat *Sp4* gene in forebrain excitatory neurons and GABAergic neurons, respectively. (A) A nuclear *LacZ* expression cassette was capped with a splicing acceptor and further flanked by two Lox P sites. The Lox P flanked *LacZ* was inserted in the first intron of mouse *Sp4* gene. Expression of *LacZ* gene revealed that the *Sp4* gene is specifically expressed in neuronal cells in mouse brain. After breeding with *Emx1-Cre* and *Dlx6a-Cre* mouse lines, the *LacZ* gene cassette was excised in forebrain excitatory neurons and GABAergic neurons, respectively. The removal of the *LacZ* cassette allows expression of the downstream rat *Sp4* gene that was also capped with a splicing acceptor to splice with mouse exon 1 to generate a functional full-length Sp4 gene. The absence of blue staining in neuronal cells indicates restoration of the *Sp4* gene. Because mouse cortices predominantly consist of excitatory neurons, *LacZ* blue staining almost completely disappeared in the cortices of *Sp4* hypomorphic mice carrying the *Emx1-Cre* gene. In contrast, mouse striata predominantly contains GABAergic neurons, the *LacZ* blue staining almost completely disappeared in the striata of *Sp4* hypomorphic mice carrying *Dlx6a-Cre* gene. (B) Under higher magnification, neurons that were not derived from the *Emx1* lineage remain blue in the cortex of *Sp4* hypomorphic mice carrying *Emx1-Cre* gene. Many of them are GABAergic neurons.

Ketamine

Ketamine was dissolved in saline and administered i.p. at a volume of 5 ml/kg after 30 min habituation in the VT arena (Brody et al., 2003). The doses of ketamine were determined from the same F1 genetic background mouse in previous studies (Ji et al., 2013). A within-subjects crossover design was used for drug studies, with 2 weeks between drug treatments.

Statistical Analysis

Repeated measures analysis of variance with genotype as a between-subjects factor and drug treatment, block, and prepulse intensity as within-subjects factors were performed on the %PPI data and total distance traveled. Post hoc analyses were carried out using Newman-Keuls or Tukey's test. Alpha level was set to 0.05. No outliers were detected or removed from the analysis. All statistical analyses were carried out using the BMDP statistical software (Statistical Solutions Inc.).

Results

Generation of Neuron-Specific *Sp4* Rescue Mice

Mouse *Emx1* (Briata et al., 1996) and *Dlx5/6* (Robledo et al., 2002) expression are first detected at embryonic day 9.5 (E9.5) and E8.5, respectively. Forebrain excitatory neurons originate from the *Emx1* cell lineage (Gorski et al., 2002), while forebrain GABAergic neurons are derived from the *Dlx5/6* cell lineage (Monory et al., 2006). Since mouse *Sp4* expression starts around E9.5 (Supp et al., 1996), the *Cre* driven by *Emx1* and *Dlx5/6* genes will restore the *Sp4* gene during the entire development of forebrain excitatory

and GABAergic neurons, respectively. In F1 *Sp4* homozygous mice carrying either the *Emx1-Cre* or *Dlx6a-Cre* (*Dlx5/6-Cre*) gene (Figure S1), the nuclear *LacZ* cassette was excised to restore *Sp4* expression in each cell lineage (Figure 1). Consistent with previous reports on *Emx1-Cre* expression (Gorski et al., 2002), introduction of *Emx1-Cre* into *Sp4* heterozygous mice abolished *LacZ* staining in both cortical and hippocampal excitatory neurons (Figure 1A). Under higher magnification, many *LacZ* blue spots remained in both the cortex and hippocampus. They presumably consisted of GABAergic and other types of neurons (Figure 1B). Introduction of *Dlx6a-Cre* into *Sp4* heterozygous mice abolished *LacZ* staining in striatal GABAergic neurons. Because cortical GABAergic neurons are a minority group of neurons scattered across the cortex, the disappearance of *LacZ* staining in these GABAergic neurons cannot be directly visualized in the cortices of *Sp4* heterozygous mice carrying a *Dlx6a-Cre* transgene. However, *Dlx6a-Cre* expression in cortical GABAergic neurons was readily detected in the same *Dlx6a-Cre* mouse line when breeding with *ROSALacZ* (*Gtrosa26*^{tm1Sor}) reporter mice (http://www.informatics.jax.org/recombinase/specificity?id=MGI:3758328&systemKey=4856356).

Western blot provided validation of restoration of the Sp4 transcription factor in the cortex of the [*Sp4* $^{Hypo/Hypo}$; *Emx1*$^{+/+(Cre)}$] rescue mice (Figure 2A). Restoration of the Sp4 transcription factor was also confirmed in the striata of [*Sp4* $^{Hypo/Hypo}$; *Tg(Dlx6a-Cre)*] rescue mice (Figure 2B). An increased level of Sp4 protein was observed in the cortices of GABAergic *Sp4* rescue mice [*Sp4* $^{Hypo/Hypo}$; *Tg(Dlx6a-Cre)*]. Nevertheless, this increase was inconclusive because there are fewer GABAergic neurons than excitatory neurons in mouse cortices. To examine whether Sp4 expression is specifically restored in the cortical GABAergic neurons of *Sp4* rescue mice [*Sp4* $^{Hypo/Hypo}$;

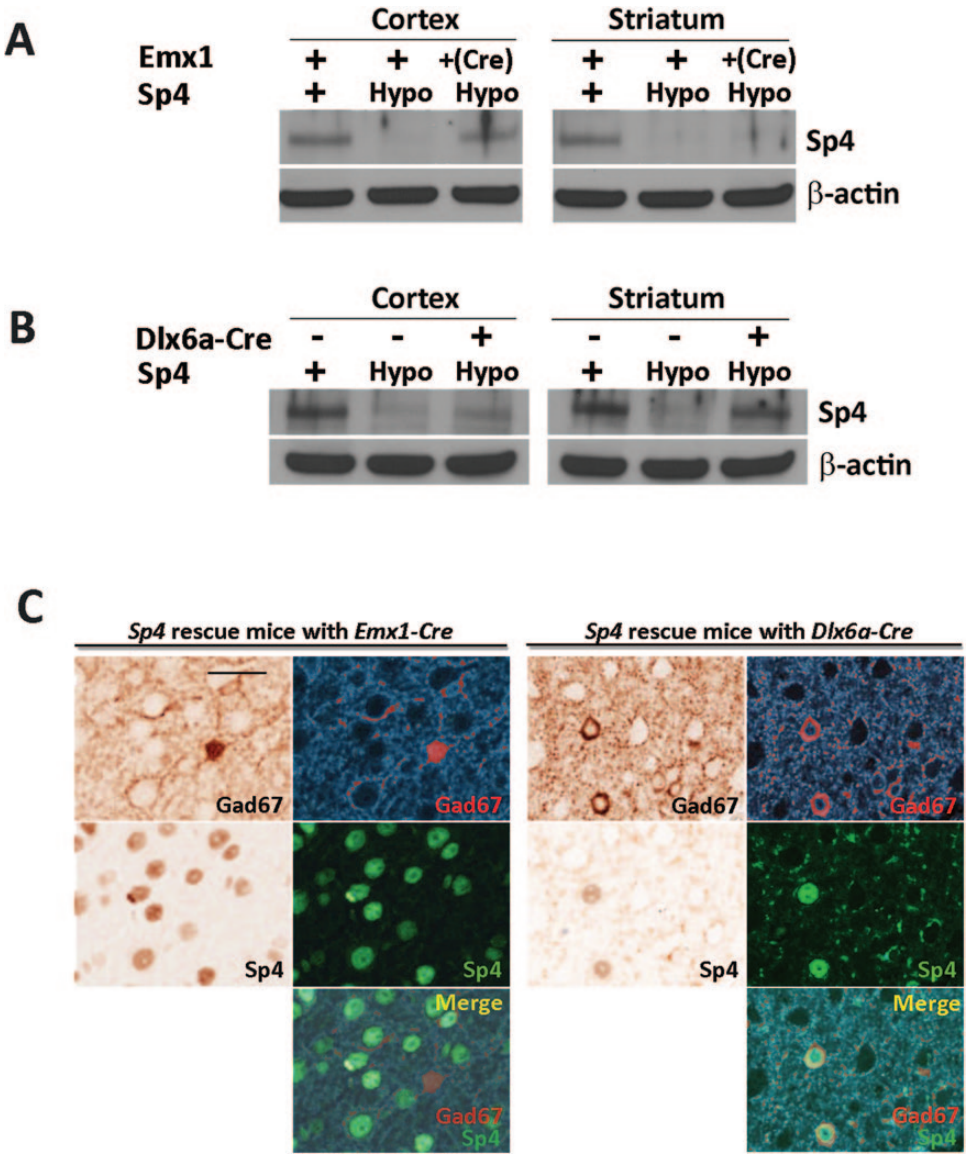

Figure 2. Sp4 expression in neuron-specific *Sp4* rescue mice. (A) Western blot confirmed expression of Sp4 protein in the cortices of rescue mice [*Sp4Hypo/Hypo; Emx1+/+(Cre)*]. (B) Western blot confirmed expression of Sp4 protein in the striata of rescue mice [*Sp4Hypo/Hypo; Tg(Dlx6a-Cre)*]. A low level of Sp4 protein in the cortices of the rescue mice likely comes from restored Sp4 expression in cortical GABAergic neurons. (C) Co-localization between Sp4 and Gad67 proteins was analyzed using sequential chromogenic immunohistochemistry in the cortices of *Sp4* rescue mice with either the *Emx1-Cre* or the *Dlx6a-Cre* gene. In *Sp4* hypomorphic mice carrying the *Emx1-Cre* gene, Sp4 protein expression was restored only in cortical excitatory neurons, but not in Gad67-positive cortical GABAergic inhibitory neurons. In contrast, Sp4 protein expression was only detected in Gad67-positive cortical GABAergic neurons in *Sp4* hypomorphic mice carrying the *Dlx6a-Cre* gene. Expression of Sp4 and Gad67 proteins was pseudocolored into green and red, respectively, and superimposed for their co-localization (Kim et al., 2012). Scale bar: 25 μm.

Tg(Dlx6a-Cre)], we conducted sequential immunohistochemical analyses of Sp4 and Gad67 proteins (Kim et al., 2012). Sp4 protein was detected only in the Gad67-positive GABAergic interneurons in the *Sp4* rescue mice carrying the *Dlx6a-Cre* gene (Figure 2C). There is no detectable Sp4 protein in the surrounding Gad67-negative neurons, which are mostly cortical excitatory neurons. In contrast, most cortical neurons, except Gad67-positive interneurons, express Sp4 protein in the *Sp4* rescue mice carrying the *Emx1-Cre* gene.

Behavioral Characterization of the *Emx1-Cre* Rescue Mice

Deficits in PPI and ketamine-induced hyperlocomotion were examined in the two different neuron-specific *Sp4* rescue mice. In the *Emx1-Cre* rescue cohort, startle reactivity (Supplemental Table II) and PPI (Supplemental Table III and IV) were investigated. *Sp4* hypomorphic mice showed significantly reduced PPI [$F(1,80)=18.23$, $p < 0.0001$; Figure 3A], which was not rescued by restoration of the *Sp4* gene in forebrain excitatory neurons in [*Sp4* $^{Hypo/Hypo}$; *Emx1* $^{+/+(Cre)}$] rescue mice [*Sp4* X *Emx1-Cre* interaction, $F(1,80) < 1$, ns]. No sex effects were observed. We examined the locomotor response of these mice to ketamine (50 mg/kg) using video-tracking (VT) equipment. Before ketamine treatment, there was no significant *Sp4* gene effect on distance traveled during habituation. After ketamine injection, *Sp4* hypomorphic mice exhibited significantly more ketamine-induced hyperactivity compared to wild-type controls {Figure 3B; *Sp4* X ketamine interaction [$F(1,79)= 12.97$, $p = 0.0006$] on distance traveled}. No *Sp4* X *Emx1-Cre* X ketamine interaction was detected [$F(1,79) < 1$, ns].

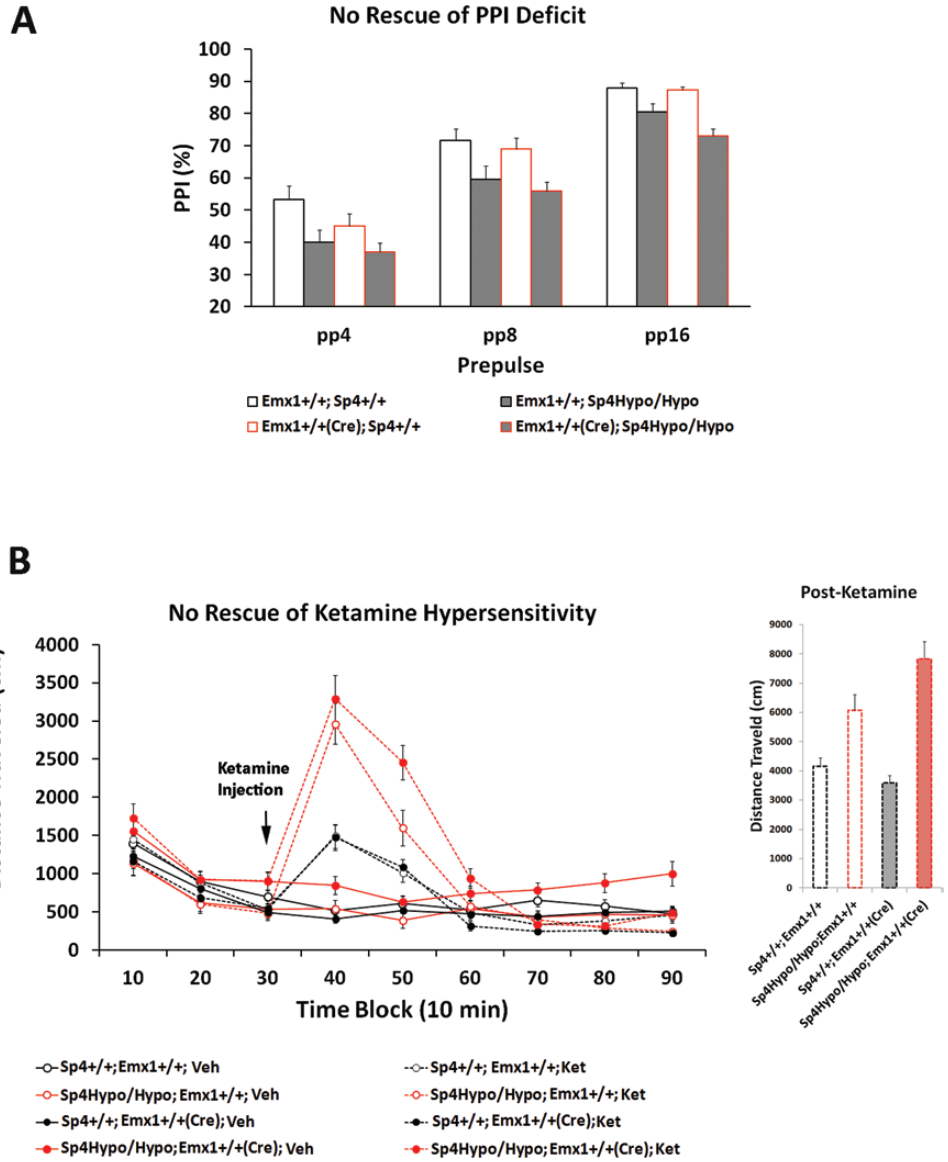

Figure 3. Characterization of rescue mice with restoration of *Sp4* gene only in forebrain excitatory neurons. Male and female mice were balanced in each genotype. There were 18 wild-type (*Sp4+/+; Emx1+/+*), 20 *Sp4* hypomorphic (*Sp4Hypo/Hypo; Emx1+/+*), 19 wild-type carrying *Emx1-Cre* (*Sp4+/+; Emx1+/+(Cre)*), and 27 rescue mice (*Sp4Hypo/Hypo; Emx1+/+(Cre)*) for behavioral analyses. (A) Prepulse inhibition was conducted in these mice. No sex effect was found. *Sp4* hypomorphic mice displayed prepulse inhibition of startle (PPI) deficits across three different prepulse intensities compared to wild-type mice, regardless of the presence or absence of the *Emx1-Cre* gene. (B) All mice were habituated for 30 min before injection of ketamine (50 mg/kg). Injection of ketamine significantly increased locomotor activity in all four genotypes. A significantly larger increase of locomotor activity was observed in *Sp4* hypomorphic mice, with or without the *Emx1-Cre* gene, than in wild-type mice {*Sp4* X ketamine interaction [$F(1,79)= 12.97$, $p = 0.0006$] on distance traveled}. However, there was no *Sp4* X *Emx1-Cre* X ketamine interaction. Error bar: standard error of the mean.

The rescue mice [*Sp4* $^{Hypo/Hypo}$; *Emx1* $^{+/+(Cre)}$] displayed similar ketamine-induced hyperlocomotion as the (*Sp4* $^{Hypo/Hypo}$; *Emx1*$^{+/+}$) *Sp4* hypomorphic mice after ketamine injection, suggesting that there was no rescue of ketamine hypersensitivity.

Behavioral Characterization of the *Dlx6a-Cre* Rescue Mice

In the *Dlx6a-Cre* rescue cohort, startle reactivity (Supplemental Table V) and PPI (Supplemental Table VI and VII) were investigated. *Sp4* hypomorphic mice exhibited reduced PPI [F(1,85) = 9.73, p = 0.002; Figure 4A]. However, no *Sp4* X *Dlx6a-Cre* interaction was detected [F(1,85) = 1.38, ns], with the [*Sp4* $^{Hypo/Hypo}$; *Tg(Dlx6a-Cre)*] rescue mice exhibiting similar PPI deficits to *Sp4* hypomorphic mice. The response to ketamine was subsequently examined with the VT locomotion test. No significant *Sp4* effect was observed during habituation. After ketamine injection, a significant *Sp4* X ketamine interaction [F(1,85) = 14.54, p < 0.001] was observed (Figure 4B). Interestingly, there was a significant *Sp4* X *Dlx6a-Cre* X ketamine interaction [F(1,85)=7.44, p = 0.008].

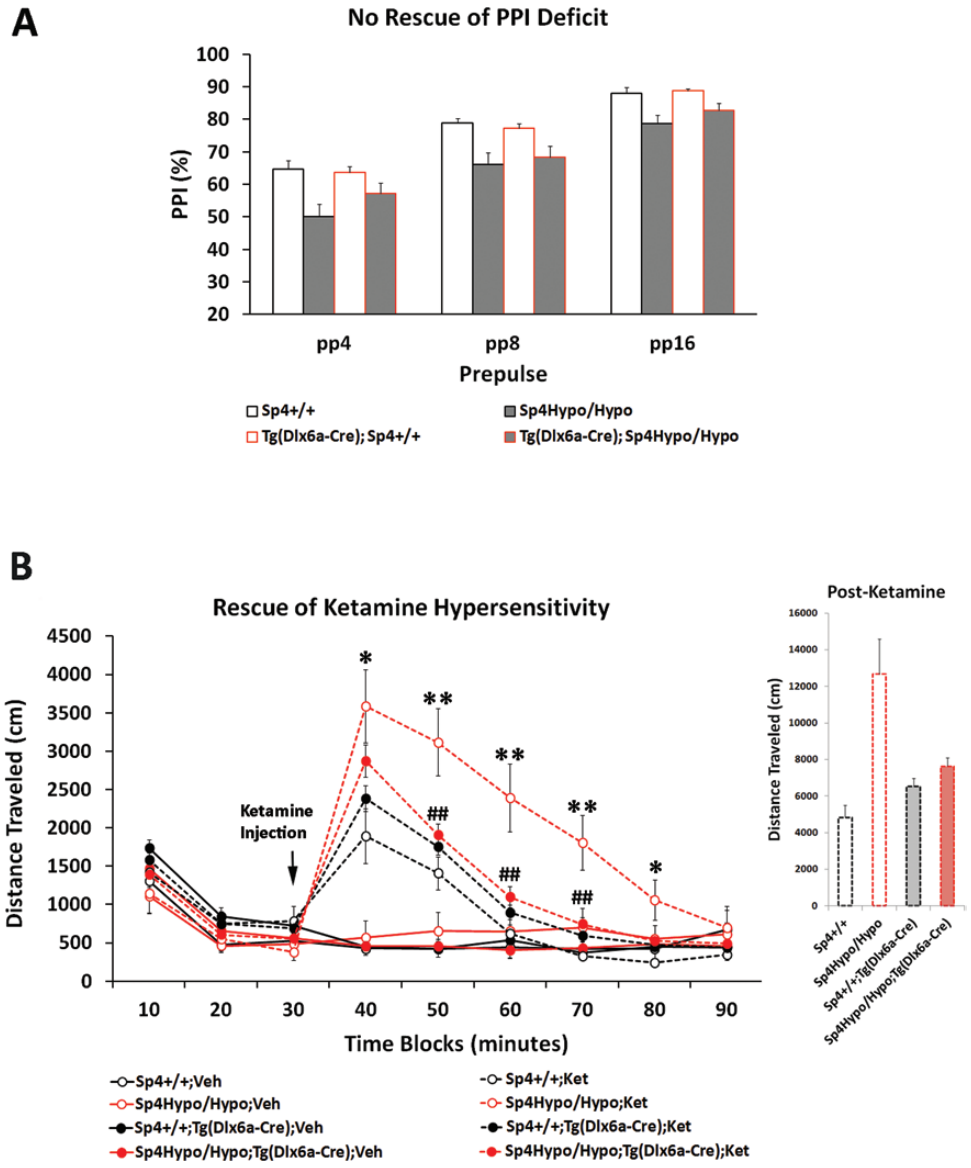

Figure 4. Prevention of ketamine-induced hyperlocomotion in rescue mice with restoration of *Sp4* gene only in forebrain GABAergic neurons. Male and female mice were balanced in each genotype. There were 9 wild-type (*Sp4+/+*), 13 *Sp4* hypomorphic (*Sp4Hypo/Hypo*), 46 wild-type carrying *Dlx6a-Cre* [*Sp4+/+; Tg(Dlx6a-Cre)*], and 21 rescue mice [*Sp4Hypo/Hypo; Tg(Dlx6a-Cre)*] for behavioral analyses. Our breeding generated fewer wild-type and *Sp4* hypomorphic mice. More rescue mice and the control wild-type mice carrying the *Dlx6a-Cre* transgene were produced. (A) Prepulse inhibition was examined in all four genotypes of mice. There was no sex effect on prepulse inhibition of startle (PPI). *Sp4* hypomorphic mice displayed PPI deficits across three different prepulse intensities compared to wild-type mice. No *Sp4* X *Dlx6a-Cre* interaction was observed. (B) After 30 min habituation in VT chamber, mice were injected with ketamine (50 mg/kg). As expected, *Sp4* hypomorphic mice displayed significantly more locomotor activity after ketamine injection [F(1,85)=14.54, p < 0.001]. *Post hoc* analysis revealed more distance traveled in the *Sp4* hypomorphic mice than wild-type mice at 10 ($^{*}p$ < 0.05), 20 ($^{**}p$ < 0.01), 30 ($^{**}p$ < 0.01), 40 ($^{**}p$ < 0.01), and 50 min ($^{*}p$ < 0.05) post–ketamine injection. A significant *Sp4* X *Dlx6a-Cre* X ketamine interaction was found [F(1,85)=7.44, p = 0.008]. Post hoc analysis revealed significant differences in the distance traveled between the *Sp4* hypomorphic mice in the absence or presence (rescue) of the *Dlx6a-Cre* transgene at 20 ($^{\#}p$ < 0.01), 30 ($^{\#}p$ < 0.01), and 40 ($^{\#}p$ < 0.01) min post-ketamine injection. The rescue mice [*Sp4 Hypo/Hypo*; *Tg(Dlx6a-Cre)*] displayed no difference in locomotor activity from the wild-type mice carrying the *Dlx6a-Cre* gene, suggesting an almost complete rescue of the excessive response to ketamine. Error bar: standard error of the mean.

The rescue mice [*Sp4* $^{Hypo/Hypo}$; *Tg(Dlx6a-Cre)*] displayed the same time-course pattern of locomotive hyperactivity as the control mice [*Sp4* $^{+/+}$; *Tg(Dlx6a-Cre)*] after ketamine injection, suggesting that restoration of *Sp4* in forebrain GABAergic neurons is sufficient to rescue ketamine-induced hyperlocomotion in *Sp4* hypomorphic mice.

Discussion

Genetic manipulations are powerful tools for disentangling complex phenotypic profiles. We previously demonstrated that a full restoration of the *Sp4* gene in the whole animal by crossing with a protamine-cre mouse line rescued the complete phenotypic profile seen in *Sp4* hypomorphic mice (Zhou et al., 2005). Conventional and conditional gene-knockout strategies can determine whether a gene is necessary for a particular phenotype in a specific type of cells. Conditional rescue of a phenotype in knockout mice via restoration of the gene in only one specific cell type can determine whether the gene in that specific cell type is necessary and sufficient for controlling the phenotype. Many behaviors are complex, and likely modulated by interactions between different types of neurons from different brain regions. Given that different types of neurons are involved in the modulation of behavior, it is not surprising that *Sp4* gene restoration in only one specific type of neuron may not be sufficient to rescue the full behavioral profile. Indeed, we failed to rescue deficient PPI in *Sp4* hypomorphic mice regardless of whether the *Sp4* gene was restored in forebrain excitatory neurons or GABAergic neurons, although PPI was rescued previously when the *Sp4* gene was rescued in the whole animal (Zhou et al., 2005). Importantly, however, we found that restoration of the *Sp4* gene in GABAergic neurons was sufficient to rescue ketamine-induced hyperlocomotion in the *Sp4* hypomorphic mice.

Sp4 hypomorphic mice (*Sp4* $^{Hypo/Hypo}$) from the *Dlx6a-Cre* rescue cohort displayed a prolonged response to ketamine relative to the response in wild-type control mice. However, *Sp4* hypomorphic mice (*Sp4* $^{Hypo/Hypo}$; *Emx1*$^{+/+}$) from the *Emx1-Cre* rescue cohort had a similar time-course response to ketamine with their sibling wild-type mice. The prolonged response to ketamine in the *Sp4* hypomorphic mice from the *Dlx6a-Cre* rescue cohort is a more typical response of *Sp4* hypomorphic mice according to our previous studies (Ji et al., 2013). Why did the same dosage of ketamine not elicit a prolonged response to ketamine in the *Sp4* hypomorphic mice from the *Emx1-Cre* rescue cohort? We speculate that there may be still subtle differences between the genetic backgrounds of the *Emx1-Cre* and *Dlx6a-Cre* mouse lines despite the fact that both *Emx1-Cre* and *Dlx6a-Cre* mouse lines were backcrossed for more than six generations. The genetic background of the *Emx1-Cre* rescue cohort may have been overall less sensitive to ketamine than that of the *Dlx6a-Cre* rescue cohort. We consider that different time-course responses to ketamine may be generated by differential ketamine sensitivities between mouse cohorts or different dosages of ketamine. Indeed, increasing dosages of ketamine produced more locomotor activity and prolonged responses (Irifune et al., 1991). This observation may explain why the same dosage of ketamine generated different time-courses in the locomotor responses between the two different mouse cohorts.

Ketamine acts primarily, although not exclusively (Kapur and Seeman, 2002), as a noncompetitive antagonist of NMDA receptors that are present in both excitatory and GABAergic neurons in multiple brain regions. Cortical GABAergic inhibitory neurons have been suggested to be the primary targets of NMDA receptor antagonists (Moghaddam et al., 1997; Homayoun and Moghaddam, 2007). Our studies suggest that the mouse *Sp4* gene is essential for forebrain GABAergic neurons to dampen locomotor responses to ketamine. Reduced *Sp4* expression may impair the ability of GABAergic neurons to control locomotor hyperactivity in *Sp4* hypomorphic mice after ketamine injection (Ji et al., 2013). In contrast, the *Sp4* gene expression appears dispensable in excitatory neurons in controlling the locomotor response to ketamine. Since the genetic restoration of the *Sp4* gene in *Dlx6a-Cre* lineage cells starts from early embryogenesis, our studies cannot differentiate whether altered development and/or function of forebrain GABAergic neurons are responsible for ketamine hypersensitivity in *Sp4* hypomorphic mice. Although we did not find gross abnormalities in the brains of *Sp4* hypomorphic mice (Zhou et al., 2005; Ji et al., 2013), we cannot rule out subtle structural alterations in GABAergic neurons. As for molecular mechanisms, the NMDA receptor has been proposed as the primary target of ketamine. The protein, but not mRNA, of Nmdar1, the obligatory subunit of NMDA receptors, was down-regulated in the brains of *Sp4* hypomorphic mice (Zhou et al., 2010; Sun et al., 2015). Those findings, however, came from mouse brain tissue that contained different types of neurons and glial cells, and hence do not necessarily exclude the *Nmdar1* gene as a direct target of the Sp4 transcription factor in a small group of specific neurons. There is still a possibility that the Sp4 transcription factor may directly regulate mRNA expression of the *Nmdar1* gene in some forebrain GABAergic neurons (Krainc et al., 1998). Forebrain GABAergic neurons consist of striatal neurons and different cortical GABAergic neurons. It remains to be investigated which subset of GABAergic neurons is responsible for the rescue effects observed here. Investigation of molecular mechanisms can eventually be conducted after identification of the critical GABAergic neurons. It should be kept in mind that other receptors and channels, in addition to NMDA receptors, may also mediate the effects of ketamine in *Sp4* hypomorphic mice (Jamie Sleigha et al., 2014). In the future, restoration of the *Sp4* gene in forebrain GABAergic neurons via temporal and region-specific (e.g. cortex or striatum) control of Cre-mediated DNA recombination may clarify the role of development of GABAergic neurons and functions of regional GABAergic neurons in controlling locomotor responses to ketamine.

Cortical GABAergic inhibitory interneurons have been proposed to be the primary targets of NMDA receptor antagonists (Moghaddam et al., 1997; Homayoun and Moghaddam, 2007) and contribute to the pathogenesis of schizophrenia (Lewis et al., 2005; Lewis and Moghaddam, 2006). Abnormalities in GABA receptors have been reported in the brains of patients with schizophrenia, bipolar disorder, or major depression (Fatemi et al., 2013). Ketamine produces schizophrenia-like behavioral phenotypes in healthy people and exacerbates symptoms in schizophrenia patients. Our studies suggest that SP4, deleted in some patients with schizophrenia, could be a missing piece that links a genetic susceptibility gene with ketamine hypersensitivity and abnormal function of GABAergic neurons in schizophrenia. The influence of the *Sp4*-GABAergic-ketamine pathway may not be limited to the regulation of locomotor activity in response to ketamine. Given that ketamine functions as a potent antidepressant, it will be interesting to investigate whether the *Sp4*-GABAergic-NMDA pathway may also be involved in the regulation of depressive behaviors. Indeed, our most recent studies suggested that *Sp4* hypomorphic mice displayed depressive-like behaviors (Young et al., 2015). In the future, such depressive-like phenotypes will be examined after ketamine treatment in *Sp4* hypomorphic mice with restoration of the *Sp4* gene in GABAergic neurons.

Supplementary Material

For supplementary material accompanying this paper, visit http://www.ijnp.oxfordjournals.org/

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Statement of Interest

In the past three years, Dr Geyer has received consulting compensation from Abbott, Dart, Lundbeck, Neurocrine, Omeros, Otsuka, and Sunovion, and holds an equity interest in San Diego Instruments. Dr Young has received consulting compensation from Cerca Insights, Lundbeck Ltd, Omeros, and Amgen. Dr Powell held service contracts with Servier and ACADIA pharmaceuticals. Dr Risbrough has received research funding from Johnson and Johnson, Omeros, and Sunovion.

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