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Modulation of sensorimotor gating in prepulse inhibition by conditional brain glycine transporter 1 deletion in mice

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Abstract

Inhibition of glycine transporter 1 (GlyT1) augments *N*-methyl-D-aspartate receptor (NMDAR)-mediated transmission and represents a potential antipsychotic drug target according to the NMDAR hypofunction hypothesis of schizophrenia. Preclinical evaluation of GlyT1 inhibiting drugs using the prepulse inhibition (PPI) test, however, has yielded mixed outcomes. Here, we tested for the first time the impact of two conditional knockouts of GlyT1 on PPI expression. Complete deletion of GlyT1 in the cerebral cortices confers resistance to PPI disruption induced by the NMDAR blocker MK-801 (0.2mg/kg, i.p.) without affecting PPI expression in unchallenged conditions. In contrast, restricting GlyT1 deletion to neurons in forebrain including the striatum significantly attenuated PPI, and the animals remained sensitive to the PPI-disruptive effect of MK-801 at the same dose. These results demonstrate in mice that depending on the regional and/or cell-type specificity, deletion of the GlyT1 gene could yield divergent effects on PPI.

Keywords

attention; glutamate; glycine; NMDA; schizophrenia

INTRODUCTION

Glycine transporters (GlyT1 and GlyT2) are important regulators of extracellular glycine concentration in the central nervous system (Berger et al., 1998; Bergeron et al., 1998; Chen et al., 2003, Smith et al., 1992), which in turn can affect glycinergic inhibitory transmission

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Conflict of interest All the authors declare that they have no conflict of interest.

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as well as glutamatergic excitatory neurotransmission. GlyT1s are of particular relevance for the latter, because they are co-expressed with *N*-methyl-D-aspartate receptors (NMDARs) in glutamatergic synapses in the forebrain. They are thus ideally positioned for the homeostatic control over extracellular glycine availability in the vicinity of the synaptic cleft. They prevent saturation of the glycine_B site located on the NMDAR, binding to which by endogenous ligands (glycine and D-serine) is necessary for activation of the NMDAR by glutamate (Berger et al., 1998; Smith et al., 1992). Through enhancing glycine availability, inhibition of GlyT1 can therefore facilitate NMDAR-mediated neurotransmission in an activity-dependent manner (Clements and Westbrook, 1991; Curras and Pallotta, 1996; Dingledine et al., 1990; Johnson and Ascher, 1987). This has led to the suggestion that GlyT1 inhibition might be a therapeutic target for treating schizophrenia, especially the negative and cognitive symptoms of the disease where NMDAR hypofunction is implicated (Javitt, 2008, 2009; Singer et al., 2007b).

Preclinical evaluation of novel GlyT1 inhibitors relies heavily on the use of a common translational paradigm – prepulse inhibition (PPI) of the acoustic startle reflex (Boulay et al., 2008; Depoortère et al., 2005; Kinney et al., 2003; Lipina et al., 2005; Le Pen et al., 2003; Yang et al., 2010). This refers to the attenuation of the startle reaction to a sudden intense auditory ‘pulse’ stimulus when it is shortly preceded by a weaker non-startling ‘prepulse’ stimulus (Buckland et al., 1969; Hoffman and Searle, 1965; Graham, 1975). PPI is disrupted in schizophrenia patients (Bolino et al., 1994; Braff et al., 1978, 1992, 2001; Csomor et al., 2008; Grillon et al., 1992; Parwani et al., 2000), and PPI deficiency is considered to be a schizophrenia endophenotype (Turetsky et al., 2007). PPI is readily attenuated by different classes of psychomimetic (dopaminergic, glutamatergic and cholinergic) drugs; and clinically effective antipsychotics can antagonize such drug-induced PPI disruption as well as potentiate PPI expression in healthy subjects (for reviews see, Geyer et al., 2001, 2002, Swerdlow et al., 2008).

A number of GlyT1 inhibitors have been shown to be effective in several PPI-disruptive schizophrenia disease models in animals, including acute NMDAR blockade (Lipina et al., 2005, Yang et al., 2010), neonatal lesion of the hippocampus (Le Pen et al., 2003), and naturally low PPI expression in the DBA/2 mouse strain (Boulay et al., 2008; Depoortère et al., 2005; Kinney et al., 2003). Unlike existing antipsychotic drugs such as haloperidol and clozapine (Lipina et al., 2005; McCaughan et al., 1997; Patel et al., 1998; Quagazzal et al., 2001; Singer et al., 2009b; for a review see Geyer et al., 2001), systemic GlyT1 inhibitors do not seem to reliably enhance PPI in non-perturbed animals. Instead, Lipina et al. (2005) reported that ALX-5407 dose-dependently (10–15mg/kg) disrupted PPI in C57BL/6 mice. This effect might indeed be due to elevated systemic glycine levels because a glycine rich diet also attenuated PPI in rats (Waziri and Baruah, 1999). However, at a lower dose (1 mg/kg) ALX5407 can also partially reverse the PPI-disruptive effect of the NMDAR antagonist MK-801 (Lipina et al., 2005). The picture is further complicated by the finding that constitutive heterozygous GlyT1 knockout spared PPI but exacerbated its disruption by MK-801 (Tsai et al., 2004). Thus, whether GlyT1 inhibition increases or decreases PPI appears to depend on the dosage and test condition, i.e. whether PPI was evaluated in the presence of a PPI-disruptive treatment.

Conditional disruption of GlyT1 can achieve selective loss of GlyT1 function in terms of brain region or cell-type that is unattainable by systemic pharmacological manipulations, and is therefore instrumental in investigating the mechanisms contributing to systemic drug effects. We have generated knockout mice lacking GlyT1 either in forebrain neurons (GlyT1^{ΔFB/neuron}; CamKIIαCre:GlyT1^{tm1.2fl/fl}, see Yee et al., 2006) or the cerebrum and hippocampus regardless of cell types (GlyT1^{ΔFB/global}; EMX1Cre/GlyT1 KO, see Singer et al., 2009c), resulting in 35% and 77% reduction of GlyT1-mediated re-uptake, respectively.

Extensive characterization of these two mutant mouse lines has yielded divergent as well as overlapping antipsychotic and pro-cognitive phenotypes (see Table 1), thus strengthening the hypothesis that GlyT1 may be an effective target for treating a wide spectrum of schizophrenia-related behavioural deficits. The two conditional GlyT1 knockout mouse lines have not yet been tested on PPI, and the present study was conducted to fill this lacuna. Following the assessment of baseline PPI expression in these animals, we went on to test if they might be resistant to the PPI-disruptive effects of the NMDAR antagonist MK-801 (0.2 mg/kg).

METHODS

Subjects

A homozygous Glyt1tm1.2fl/fl colony was established and maintained on a pure C57BL/6 background as described before (Gabernet et al., 2005; Yee et al., 2006). The forebrain neuron-selective deletion of GlyT1 in the forebrain was achieved by CamKII α Cre-mediated recombination (see Yee et al., 2006), whereas global (i.e., in neurons and glia) GlyT1 disruption in the cerebral cortices (including the hippocampus) was achieved by EMX1Cre-mediated recombination (see Singer et al., 2009c; Iwasato et al., 2000). Appropriate Glyt1tm1.2fl/fl:Cre+/- mice were mated with Glyt1tm1.2fl/fl control mice to generate the desired mutants and controls as littermates (Yee et al., 2006; Singer et al., 2009c). Animals of both sexes were employed in the present study. Hereafter, the two mutant mouse lines are referred to as “GlyT1 Δ FB/neuron” and “GlyT1 Δ FB/global”, respectively.

The mice were weaned 21 day after birth, and littermates were kept in unisex groups of four to six in Type-III cages (Techniplast, Milan, Italy) housed in a temperature-and humidity-controlled (at 22°C and 55% R. H.) animal vivarium under a reversed light-dark cycle with lights off from 0800–2000hrs. Ad libitum water and food (Kliba 3430, Klibamuhlen, Kaiseraugst, Switzerland) was provided throughout the study.

Behavioural testing began when the animals were approximately 12 weeks old, with all tests conducted in the dark phase of the light cycle. The two mutant mouse lines were evaluated separately against their own littermate controls. The following numbers of animals were included in the four experiments: *Experiment 1*: 24 (9 male and 15 female) GlyT1 Δ FB/neuron mice and 33 (15 male and 18 female) control littermates; *Experiment 2*: 17 (7 male and 10 female) GlyT1 Δ FB/global mice and 24 (13 male and 11 female) littermate controls; *Experiment 3*: 19 (11 male and 8 female) GlyT1 Δ FB/global mice and 15 (7 male and 8 female) littermate controls; *Experiment 4*: 24 male GlyT1 Δ FB/neuron mice. All manipulations and procedures described had been approved by the Swiss Cantonal Veterinary Office; they conformed to the ethical standards stipulated in the Swiss Federal Act on Animal Protection (1978) and Swiss Animal Protection Ordinance (1981), in accordance with the European Council Directive 86/609/EEC (1986).

Open field test of locomotor activity

Subjects of Experiments 1 and 2 were first evaluated for spontaneous locomotor activity in an open field, because activity could be a potential confound in the assessment of PPI. The apparatus consisted of four identical square arenas made from waterproof white plastic laminated wood, each measuring 40 × 40 cm and surrounded by 35 cm high walls, as previously described (Yee et al., 2006). The animals were tested in squads of four (one mouse in each arena), under diffused dim lighting at about 30 lux. They were gently placed in the centre of the appropriate open field and allowed to explore undisturbed for an hour, before being removed and the open fields cleansed with water and dried prior to the next squad. Locomotor activity was indexed by distance travelled, recorded in successive 10-min

bins. Data were collected and processed using the EthoVision® tracking system (Version 3.1, Noldus Technology, Wageningen, Netherlands).

Prepulse inhibition (PPI) of the acoustic startle reflex

The PPI experiment was carried out in four acoustic startle chambers for mice (SR-LAB, San Diego Instruments, San Diego, CA, USA). The design and parameters of the PPI test were adopted from the procedures recommended by Yee et al. (2005), including multiple pulse stimulus intensities. Briefly, a PPI test session lasted approximately 45 min in which a series of four different types of trial were presented to the animals. These included pulse-alone trials, prepulse-plus-pulse trials, prepulse-alone trials, and no-stimulus trials in which no discrete stimulus other than the constant background noise (65 dB_A) was presented. Three pulse stimulus intensities (100, 110 and 120 dB_A), with a duration of 40 ms, and three prepulse stimulus intensities (71, 77 and 83 dB_A), with a duration of 20 ms, were used. The stimulus onset asynchrony between the two stimuli was 100 ms. All stimuli were in the form of white noise, presented against a constant background noise at 65 dB_A.

A session began with the animals being placed in the Plexiglas enclosure inside the startle chamber, positioned under a loudspeaker. They were acclimatised to the apparatus for two minutes before the first trial began. The first six trials consisted of pulse-alone trials, comprising two trials of each of the three possible pulse intensities. These trials served to habituate and stabilize the animals' startle response, and were analysed separately. The animals were subsequently presented with 10 blocks of discrete test trials to assess PPI, with each block being made up of three pulse-alone trials (100, 110 or 120 dB_A), three prepulse-alone trials (71, 77 and 83 dB_A), the nine possible combinations of prepulse-plus-pulse trials (3 levels of prepulse × 3 levels of prepulse), and one no-stimulus trial (i.e., background alone). The 16 discrete trials within each block were presented in a pseudorandom order, with a variable inter-trial interval averaging 15 s (ranging from 10 to 20 s). The session was concluded with a final block of six consecutive pulse-alone trials similar to that administered at the beginning of the test session.

The whole body motion of the subject as measured by the stabilimeter on each and every trial constituted the raw data. This output was referred to as the reactivity score, and was expressed in arbitrary units. As detailed below, the data were categorized into different subsets which were separately analyzed.

- Startle habituation: Comparison of the reactivity scores obtained on the first and last blocks of six pulse-alone trials allowed the assessment of habituation of the startle reaction.
- Reactivity on pulse-alone trials: The pulse-alone trials presented in the middle block of trials were separately analyzed to assess the animals' startle reaction.
- Reaction on prepulse-alone trials: Prepulse-elicited reactivity was assessed by analysing the reaction to the prepulse-alone trials presented in the middle block of trials. The 'no-stimulus' trials were included in this analysis as a baseline condition against which the effect of increasing prepulse intensities could be evaluated.
- Prepulse inhibition: Finally, PPI was indexed by percent inhibition (% PPI). At each pulse intensity, the reduction in startle reaction on prepulse-plus-pulse trials relative to pulse-alone trials was calculated: $[1 - (\text{reaction in prepulse-plus-pulse trials}) / (\text{reaction in pulse-alone trials})] \times 100\%$. This was repeated for each of the nine possible prepulse-pulse combinations.

Drug

Dizocilpine (MK-801; obtained from Sigma, Switzerland) was dissolved in sterile saline solution to achieve a dose of 0.2 mg/kg at a volume of injection of 5 ml/kg and was administered via the intraperitoneal (i.p.) route 15 min prior to testing.

Statistical analysis

All data were submitted to parametric analysis of variance (ANOVA) using genotype (mutant vs. littermate control), sex (male vs. female), and drug (MK-801 vs. saline) as the between-subjects factors. Additional within-subjects factors (e.g., 10-min bins, pulse intensity, prepulse intensity) were included according to the nature of the considered dependent variables. Restricted analyses and Fisher's least significant difference (LSD) *post hoc* comparisons were used to further examine the patterns of significant interactions. Additional analyses of covariance (ANCOVA) were carried out to examine the extent to which a significant effect emerging from an ANOVA of one variable could be explained by another variable. Specifically, the mean startle reactivity was used as a covariate in the analysis of % PPI (see also Yee et al., 2004). In order to improve the distribution and variance homogeneity of the data a logarithmic transformation [$\ln(\text{reactivity score} + e)$] was applied to the mean reactivity scores obtained on pulse-alone and prepulse-alone trials (see Csomor et al., 2008). All statistical analyses were carried out using SPSS for Windows (version 13, SPSS Inc. Chicago IL, USA) implemented on a PC running the Windows XP (SP3) operating system.

RESULTS

EXPERIMENTS 1 and 2

Open field activity

Forebrain neuronal GlyT1 deletion (GlyT1^{ΔFB/neuron}): The activity levels as measured by distance travelled per 10-min bin were highly comparable between GlyT1^{ΔFB/neuron} mice and littermate controls (Figure 1A). Both groups exhibited clear locomotor habituation as activity levels monotonically decreased over time, which was again highly comparable between groups, although male mice tended to habituate faster than female mice. This sex difference was observed in both GlyT1^{ΔFB/neuron} and control mice (data not shown). In support of these interpretations, a $2 \times 2 \times 6$ (Genotype $\times$ Sex $\times$ 10-min bins) ANOVA of the distance travelled per 10-min bin revealed a significant main effect of bins [$F(5,265)=210.83$, $p<0.001$] and a significant sex by bins interaction [$F(5,265)=3.17$, $p<0.05$]. No other effects achieved statistical significance.

Forebrain global GlyT1 deletion (GlyT1^{ΔFB/global}): The comparison between GlyT1^{ΔFB/global} mice and their corresponding littermate controls also yielded no significant group difference (Figure 1B). Again, both the absolute level of activity and the rate of locomotor habituation were comparable between GlyT1^{ΔFB/global} and control mice. A similar sex difference as described above also emerged, with male mice habituating faster than female mice regardless of genotype (data not show). These impressions were confirmed by a $2 \times 2 \times 6$ (Genotype $\times$ Sex $\times$ 10-min bins) ANOVA of the distance travelled per 10-min bin, revealing a significant effect of bins [$F(5,185)=98.53$, $p<0.001$] and its interaction with sex [$F(5,185)=4.48$, $p<0.05$]. No other effects achieved statistical significance.

Thus, neither mutant line exhibited any gross changes in spontaneous locomotor activity. The overall difference in activity levels between the two experiments is likely attributable to uncontrolled differences in apparatus settings despite our efforts to equate them across experiments.

Habituation of the startle response—Comparison between the first and last blocks, both comprising pulse-alone trials, yielded no statistical evidence for the presence of startle habituation (Table 2). Separate $2 \times 2 \times 2 \times 3$ (Genotype $\times$ Sex $\times$ Blocks $\times$ Pulse intensity) ANOVAs conducted for the two experiments only revealed a significant main effect of pulse intensity (GlyT1 $^{\Delta\text{FB}/\text{neuron}}$: $F(2,106)=26.08$, $p<0.001$; GlyT1 $^{\Delta\text{FB}/\text{global}}$: $F(2,74)=85.19$, $p<0.001$).

Reaction to pulse-alone and prepulse-alone trials—Table 3 summarizes the results of Experiments 1 and 2.

Forebrain neuronal GlyT1 deletion (GlyT1 $^{\Delta\text{FB}/\text{neuron}}$): Analysis of the startle reactivity on pulse-alone trials by a $2 \times 2 \times 3$ (Genotype $\times$ Sex $\times$ Pulse intensity) ANOVA yielded no significant difference between genotypes. There was only a significant main effect of pulse intensity [$F(2,106)=113.76$, $p<0.001$] indicating that the startle reactivity generally increased with increasing pulse intensity. Likewise, analysis of prepulse-elicited reactivity on prepulse-alone trials by a $2 \times 2 \times 4$ (Genotype $\times$ Sex $\times$ Prepulse intensity) ANOVA that included no-stimulus trials only revealed a significant main effect of prepulse intensity [$F(3,159)=14.02$, $p<0.005$]. No further significant outcomes were detected.

Forebrain global GlyT1 deletion (GlyT1 $^{\Delta\text{FB}/\text{global}}$): Parallel analyses comparing GlyT1 $^{\Delta\text{FB}/\text{global}}$ and control mice in Experiment 2 also did not reveal any genotype effects (Table 3). Irrespective of genotype or sex, increasing pulse intensity led to stronger reaction to the pulse stimulus [$F(2,74)=121.85$, $p<0.001$], and similarly there was a progressive increase in the direct response to the prepulse stimulus relative to the no-stimulus control condition [$F(3,111)=15.18$, $p<0.001$]. No other effect attained statistical significance.

Prepulse inhibition

Forebrain neuronal GlyT1 deletion (GlyT1 $^{\Delta\text{FB}/\text{neuron}}$): As illustrated in Figure 2A, PPI expression was attenuated in GlyT1 $^{\Delta\text{FB}/\text{neuron}}$ mutants compared with littermate controls. This genotype difference was consistently seen across all prepulse-pulse conditions except at the combination of 77-dB prepulse with 100-dB pulse. A $2 \times 2 \times 3 \times 3$ (Genotype $\times$ Sex $\times$ Pulse intensity $\times$ Prepulse intensity) ANOVA of % PPI revealed a significant main effect of genotype [$F(1,53)=5.40$, $p<0.05$]. The main effect of prepulse intensity was also highly significant [$F(2,108)=66.73$, $p<0.001$] indicating that % PPI generally increased as a function of prepulse intensity. No other effects or interaction terms attained statistical significance.

Forebrain global GlyT1 deletion: In contrast, PPI expression was highly comparable between GlyT1 $^{\Delta\text{FB}/\text{global}}$ mice and their littermate controls across all prepulse-pulse combinations (Figure 2B). Statistical analysis of % PPI yielded only a significant effect of prepulse intensity [$F(2,74)=37.38$, $p<0.001$], with no other effects or interactions achieving statistical significance.

EXPERIMENT 3

The PPI-disruptive phenotype of GlyT1 $^{\Delta\text{FB}/\text{neuron}}$ mice and the null effect in GlyT1 $^{\Delta\text{FB}/\text{global}}$ mice prompted us to further examine whether these mice would nonetheless confer resistance to the disruptive effect of NMDAR antagonist on PPI expression. Our previous studies suggest that MK-801 at a dose of 0.2mg/kg reliably disrupts PPI in C57BL/6 mice. First, a 2×2 (Genotype $\times$ Drug) factorial design was employed to examine GlyT1 $^{\Delta\text{FB}/\text{global}}$ mice.

Habituation of the startle response—Startle habituation was indexed by comparison of the first block against the last block of pulse-alone trials (Table 4). While there was a tendency of startle habituation in the saline-treated mice, MK-801 tended to reverse this effect leading to sensitization. In addition, the drug potentiated the startle response in general. A $2 \times 2 \times 2 \times 2 \times 3$ (Drug $\times$ Genotype $\times$ Sex $\times$ Blocks $\times$ Pulse intensity) ANOVA yielded a significant drug effect [$F(1,26)=5.11$, $p<0.05$] and its interaction with blocks [$F(1,26)=9.28$, $p=0.005$]. A 3-way ANOVA restricted to MK-801 treated animals yielded evidence for a sensitization effect [$F(1,12)=9.82$, $p<0.01$], where an equivalent ANOVA restricted to saline treated animals did not reveal a significant blocks effect [$p=0.14$]. The overall ANOVA also yielded a significant pulse intensity effect as expected [$F(2,52)=13.15$, $p<0.001$], and a significant sex difference with male generally exhibiting a stronger startle response [$F(1,26)=7.01$, $p<0.005$] (data not shown).

Reaction to pulse-alone and prepulse-alone trials—Both startle reaction and prepulse-elicited reactivity were generally enhanced by MK-801 (see Table 5). A $2 \times 2 \times 2 \times 3$ (Drug $\times$ Genotype $\times$ Sex $\times$ Pulse intensity) ANOVA of startle reactivity on pulse-alone trials yielded a main effect of drug [$F(1,26)=6.31$, $p<0.05$], which was further accompanied by a pulse intensity effect [$F(2,52)=25.81$, $p<0.001$]. In addition, there was a significant sex effect [$F(1,26)=7.44$, $p<0.05$], indicating that the startle reaction was generally stronger in male relative to female mice (data not shown).

A $2 \times 2 \times 2 \times 4$ (Drug $\times$ Genotype $\times$ Sex $\times$ Prepulse intensity) ANOVA comparing prepulse-alone reaction and baseline reaction in no-stimulus trials, yielded a significant main effect of drug [$F(1,26)=5.79$, $p<0.05$], and prepulse intensity [$F(3,78)=31.61$, $p<0.001$]. A significant sex difference [$F(1,26)=6.31$, $p<0.05$] was also detected, which again was attributed to the stronger reaction in the male mice regardless of genotypes (data not shown).

Prepulse inhibition—MK-801 was highly effective in disrupting PPI in control mice (Figure 3A), but GlyT1^{ΔFB/global} mice were largely unaffected by MK-801 at this dose (Figure 3B). A $2 \times 2 \times 2 \times 3 \times 3$ (Drug $\times$ Genotype $\times$ Sex $\times$ Pulse intensity $\times$ Prepulse intensity) ANOVA of % PPI yielded a significant drug effect [$F(1,26)=9.35$, $p=0.005$] and drug by genotype interaction [$F(1,26)=6.25$, $p<0.05$]. Post hoc pair-wise comparisons confirmed the presence of a significant drug effect in the controls [$p=0.001$] but not in the mutants [$p>0.5$]. Forebrain global GlyT1 deletion antagonised the PPI-disruptive effect of MK-801, and this antagonism did not depend on pulse or prepulse intensity – no significant higher-order interaction involving both drug and genotype was revealed. The possible confounding effect on startle reaction as such by the drug was excluded by an ANCOVA of % PPI with the mean startle reactivity (ln-transformed) as the covariate, which again yielded a significant drug $\times$ genotype interaction [$F(1,25)=4.84$, $p<0.05$] although the covariate effect was also significant [$F(1,25)=5.77$, $p<0.05$].

EXPERIMENT 4

Next, we examined specifically if GlyT1^{ΔFB/neuron} mice would similarly confer resistance to the PPI disruptive effect of MK-801 at the same dose, even though they showed reduced baseline levels of PPI in Experiment 1. To this end, the reaction to 0.2mg/kg MK-801 was specifically tested in a naïve cohort of male GlyT1^{ΔFB/neuron} mice.

Habituation of the startle response—As illustrated in Table 4, startle habituation was not evident except at the highest pulse stimulus (120 dB_A) in MK-801 treated GlyT1^{ΔFB/neuron} mice. A $2 \times 2 \times 3$ (drug $\times$ block $\times$ pulse intensity) yielded a significant 3-way interaction [$F(2,44)=4.10$, $p<0.05$] supporting this interpretation. Again, MK-801 tended to increase startle reaction but the drug effect failed to attain statistical significance

[$p=0.18$] (Table 4). As expected, startle reactivity monotonically increased as a function of pulse intensity [$F(2,44)=46.26$, $p<0.001$].

Reaction to pulse-alone and prepulse-alone trials—As illustrated in Table 5, the startle reactivity showed the expected increase with increasing pulse intensity [$F(2,44)=43.39$, $p<0.001$]. MK-801 resulted in a non-significant increase in startle reaction to the pulse stimuli [$p=0.13$]. Similarly, direct reaction to the prepulse stimuli increased with increasing prepulse intensity [$F(3,66)=8.19$, $p<0.001$], and was significantly stronger in MK-801 treated mice regardless of genotype [$F(1,22)=5.93$, $p<0.05$].

Prepulse inhibition—PPI in MK-801-treated GlyT1^{ΔFB/neuron} mice was clearly attenuated compared with saline-treated mice (Figure 3C). This drug effect was most pronounced at the lowest prepulse level as it was consistently seen across all three pulse conditions. A $2 \times 3 \times 3$ (drug $\times$ prepulse intensity $\times$ pulse intensity) ANOVA yielded a significant effect of drug [$F(1,22)=5.62$, $p<0.05$] and its interaction with prepulse intensity [$F(2,44)=6.75$, $p=0.005$]. The expected monotonic increase in PPI as a function of prepulse intensity also attained statistical significance [$F(2,44)=48.63$, $p<0.001$]. Taking into account the startle-enhancing effect of MK-801 in an ANCOVA of % PPI with mean startle reactivity as covariate yielded a similar pattern of outcomes.

DISCUSSION

The present study shows that regional disruption of GlyT1 in the brain resulted in appreciable effects on sensorimotor gating in the form of PPI of the acoustic startle response. The effects are independent of sex and are not confounded by phenotypes in spontaneous activity or anxiety (see Singer et al., 2009c; Yee et al., 2006). However, the direction of the effect is critically dependent on the conditional knockout system used (also see Tsai et al., 2004). Near complete deletion of GlyT1 from neurons and glial cells in the cerebral cortex and hippocampus by the EM1-Cre system did not affect baseline PPI (Experiment 2) but conferred resistance to MK-801-induced PPI disruption (Experiment 3). On the other hand, the CamKII α -Cre system that deletes GlyT1 in forebrain principal neurons attenuated baseline PPI expression (Experiment 1); and the mutants remained sensitive to the PPI disruptive effect of MK-801 at 0.2 mg/kg (Experiment 4). This contrasting pattern of outcomes suggests that forebrain global GlyT1 deletion (GlyT1^{ΔFB/global}) is associated with an antipsychotic-like phenotype, whereas forebrain neuronal GlyT1 deletion (GlyT1^{ΔFB/neuron}) gives rise to PPI deficiency – a schizophrenia endophenotype. Hence, the present PPI study does not provide an unequivocal support to the antipsychotic potential of GlyT1 inhibition. Instead, it reinforces the impression from the literature that GlyT1 inhibition can both positively and negatively affect the physiological and psychological processes underlying PPI.

Divergent effects on PPI

The GlyT1^{ΔFB/global} model falls in line with several reports showing that GlyT1 inhibitors (including ALX-5407, sarcosine, ORG 24598, SSR504734, SSR103800) are able to antagonize PPI deficits in a number of schizophrenia disease models, including acute NMDAR blockade (Lipina et al., 2005; Yang et al., 2010), neonatal lesion of the hippocampus (Le Pen et al., 2003), neonatal chronic phencyclidine exposure (Depoortère et al., 2005), and the DBA/2 mouse model (Olivier et al., 2001) with naturally low PPI expression (Boulay et al., 2008; Depoortère et al., 2005; Kinney et al., 2003). These findings are often taken as preclinical support for GlyT1 inhibition as a viable strategy to achieve antipsychotic action. These studies also show that systemic GlyT1 inhibitors do not reliably enhance PPI in non-perturbed animals, which might be considered as an advantage, and

further points to a different mode of action from existing typical and atypical antipsychotics, such as haloperidol and clozapine, which are capable of enhancing PPI in normal subjects (Lipina et al., 2005; McCaughan et al., 1997; Ouagazzal et al., 2001; Patel et al., 1998; Singer et al., 2009b; Swerdlow and Geyer, 1993; for a review see Geyer et al., 2001).

On the other hand, the PPI disruptive phenotype that emerged from the GlyT1^{ΔFB/neuron} model also finds agreement within the existing literature. In rats, sustained elevation in the CNS glycine level by a glycine-rich diet disrupted PPI (Waziri and Baruah, 1999), which is suggestive of a negative relationship between systemic glycine levels and PPI expression. A similar link has also been observed in schizophrenia patients. Heresco-Levy et al. (2007) reported that glycine levels in the cerebrospinal fluid of schizophrenia patients were higher than in healthy controls, and that patients with the highest glycine levels showed the lowest PPI, again indicating a negative correlation between CNS glycine levels with PPI performance. Evidence for PPI disruption by GlyT1 inhibition is also not unprecedented. Lipina et al. (2005) showed that ALX-5407 can attenuate PPI in normal C57BL/6 mice at sufficiently high doses (10–15 mg/kg; but also see Kinney et al., 2003). Moreover, Tsai et al. (2004) reported that heterozygous GlyT1 deletion potentiated the disruptive effect of systemic MK-801 on PPI.

Strengths & limitations of the genetic knockout approach

The above two sets of apparently conflicting data suggest that there are regional impacts from the forebrain on the regulation of PPI by CNS glycine homeostasis. This was apparent when the manipulation was restricted largely to the forebrain, without direct interference to glycinergic mechanisms in the startle circuits involving the cerebellum, brain stem or spinal cord (Lin et al., 1994; Lynch, 2004; Webb and Lynch, 2007). Compared with pharmacological interventions, conditional knockout systems can afford superior specificity in substrate targeting, and the ability to localize this to restricted brain areas. However, as in any gene knockout approach the physiological impact might not be directly comparable to acute pharmacological interventions. The knockout is typically initiated early in life providing the opportunity for potential development and/or compensatory changes that might also differ qualitatively from those resulting from chronic drug treatments since the target gene is deleted. It is known that glycine_B site activation is involved in NMDARs internalization (Martina et al., 2004; Nong et al., 2003), but we have previously ascertained that the level of NMDAR subunit 1 (NR1) protein expression was not altered in either of our KO models (Singer et al., 2009c; Yee et al., 2006). Hence, it is unlikely that the composition or expression of NMDARs had undergone significant compensatory change in either of our KO models, even though secondary changes in other amino acid transporter systems might have occurred. It is also unlikely that a potential sustained elevation of synaptic glycine results in physiological down-regulation of NMDAR-dependent currents due to glycine-mediated NMDAR desensitization given that desensitization of NMDA-induced currents is expected to decrease as the concentration of glycine increases (Lerma et al., 1990; Mayer et al., 1989).

Mechanistic comparison between the two conditional knockout models

The critical determinants whereby GlyT1 disruption or inhibition might give rise to one or the other pattern of outcomes remains essentially unknown. Their eventual resolution would obviously bear relevance to the future design of GlyT1 inhibitors as putative antipsychotic drugs – in maximizing their therapeutic efficacy and minimizing any unwanted side effects. What could be learned from the present dissociation between the GlyT1^{ΔFB/global} and GlyT1^{ΔFB/neuron} models? The two conditional models are differentiated in terms of cell-type specificity, regional selectivity and developmental onset of GlyT1 ablation.

The CamKII α -Cre system restricts the gene deletion to principal forebrain neurons (Schweizer et al., 2003, Tsien et al., 1996) whereas the EMX1-Cre system induces deletion in neurons as well as astrocytes (Gulisano et al., 1996; Iwasato et al., 2000). Predictably the overall impact on GlyT1-mediated glycine re-uptake in the brain was substantially more effective in GlyT1 Δ FB/global mice (77% reduction) compared with GlyT1 Δ FB/neuron mice (35% reduction) (Singer et al., 2009c, Yee et al., 2006). For comparison, the constitutive heterozygous knockout model developed by Tsai et al. (2004) disrupted forebrain glycine reuptake by about 50%. Thus, the GlyT1 Δ FB/global model might derive its unique phenotype on PPI as a result of its superior inhibition of global forebrain glycine reuptake. Does this indicate that strong glycine uptake inhibition would necessarily be more beneficial in conferring resistance to PPI-disruptive treatment? A systemic dose response analysis of ALX-5407 (identical to (R)-NFPS) on PPI expression in normal C57BL/6 mice clearly showed that the drug disrupted PPI at sufficiently high doses (10–15 mg/kg) (see Table 1 of Lipina et al., 2005), although at such doses ALX-5407 could enhance PPI in DBA/2 mice – a mouse strain with low baseline PPI and startle reactivity (Kinney et al., 2003). In the same study, Lipina et al. (2005) identified that lower doses of ALX-5407 (1mg/kg) were sufficient to confer resistance to the strong PPI-disruptive effect of MK-801 (1 mg/kg). It is conceivable that the PPI-disruptive effect of ALX-5407 at high doses might involve effects on the brain stem startle circuit that is normally regulated by inhibitory glycinergic mechanisms (Fendt et al., 2001, see also Waziri and Baruah, 1999, and further discussion below). In support of this, a clear dose-dependent monotonic increase in startle magnitude was also reported (see Table 1 of Lipina et al., 2005). In contrast, neither conditional knockout model examined here had affected the baseline startle response. Therefore, the present findings may reflect more accurately the regulation of PPI by forebrain GlyT1 modulation.

As a consequence of extending the deletion of GlyT1 to astrocytes, the GlyT1 Δ FB/global model may be expected to produce physiological effects beyond NMDAR-dependent glutamatergic transmission, namely, on the inhibitory glycinergic neurotransmission via strychnine-insensitive glycine receptors (GlyRs). It is because extracellular glycine at glycinergic synapse is mainly regulated by neuronal GlyT2 and astrocytic GlyT1 (Aragón and López-Corcuera, 2005; Betz et al., 2006), and the latter is disrupted in GlyT1 Δ FB/global but not GlyT1 Δ FB/neuron mice. Reducing glycine reuptake at glycinergic synapses would increase neuronal inhibition via GlyRs (Zhang et al., 2008; Tsai et al., 2004; Perry et al., 2008), especially in the hippocampus where GlyR assumes an important role in suppressing excitation (Keck and White, 2009). This might buffer against the impact of GlyT1 inhibition on NMDAR-mediated excitatory current. Indeed, hippocampal NMDAR currents are enhanced in GlyT1 Δ FB/neuron mice (Yee et al., 2006) but not GlyT1 Δ FB/global mice (Singer et al., 2009c). The balance of excitatory and inhibitory activity in the hippocampus appears to be critical for normal PPI expression. Excessive NMDAR activation (Zhang et al., 1999, 2002) or blockade of GABA_A receptor mediated inhibition (Bast et al., 2001) in the ventral hippocampus disrupted PPI. A partial reduction of α 5 subunit-containing GABA_A receptor in the hippocampus that located extrasynaptically also disrupted PPI (Hauser et al. 2005). Thus, GlyT1 Δ FB/neuron conditional knockout might disrupt PPI due to excessive hippocampal excitation resulting from NMDAR activation. In this respect, it is worth noting that the psychostimulant effects of NMDAR antagonists have been attributed partly to reduced GABA-ergic inhibition especially in the prefrontal cortices (Carlsson et al., 2006; Deutsch et al., 2001). NMDAR blockade by phencyclidine or ketamine has been shown to increase overall neuronal activity in the prefrontal cortex (Breier et al., 1997) by down-regulation of inhibitory interneurons activity (Behrens et al., 2007). Furthermore, prefrontal GABA_B receptor activation was effective in attenuating phencyclidine-induced PPI deficit (Fejgin et al., 2009). One may therefore speculate that any elevation of glycinergic inhibition in the prefrontal cortex that forebrain global GlyT1 deletion (GlyT1 Δ FB/global)

might produced could be contributing to its antagonistic effect against MK-801-induced PPI disruption (Experiment 3). Speculation on any contribution from glycinergic inhibition in our present knockout models certainly deserves further testing, e.g., by examining the impact of forebrain glial-specific knockout (which unfortunately is not yet available) and forebrain GlyT2 knockout.

The critical difference in regional specificity concerns the striatum, which is affected by forebrain neuronal GlyT1 deletion (Schweizer et al., 2003) but not forebrain global GlyT1 deletion (Briata et al., 1996). The striatum is a critical structure in the control of PPI (Koch and Schnitzler, 1997; Kodosi and Swerdlow, 1995). The impacts of selectively blocking the glycine_B site in the dorsal and ventral striatum on PPI expression have been examined in the rat by local infusion of the glycine_B site antagonist 7-chlorokynurenate (7-CLKYN; Kretschmer and Koch, 1997). This compound impaired PPI when infused into the ventral striatum (nucleus accumbens) but not the dorsal striatum (caudate nucleus). Hence, an opposite effect might emerge by enhancing accumbal glycine_B site occupancy, as would be expected in GlyT1^{ΔFB/neuron} mice. However, the regulation of PPI by local accumbal NMDAR activity is far from straightforward. Accumbal NMDA infusion tended to disrupt PPI, whereas MK-801 increased PPI (Reijmers et al., 1995). With this consideration, it might not be surprising that forebrain neuronal GlyT1 deletion could disrupt PPI, and that forebrain global GlyT1 deletion could confer resistance to MK-801-induced PPI disruption without altering striatal GlyT1 expression.

Finally, the early embryonic onset of Emx1-Cre mediated GlyT1 deletion in precursor cells of neuronal and astrocytic lineages (Yoshida et al. 1997) implies that cortical development in the GlyT1^{ΔFB/global} mice occurs in the absence of GlyT1. In contrast, ablation of GlyT1 in GlyT1^{ΔFB/neuron} mice occurs around postnatal day 21 when brain maturation in rodents is largely complete (Tsien et al., 1996). This important difference in the timing of GlyT1 deletion may also be linked to the differential PPI phenotypes of the two mutant mouse lines. If so, it would further hint at a novel developmental role of GlyT1 by which cognitive functions may be altered. In light of the suggestion that disturbance in early brain development may be involved in the etiology of schizophrenia (Fatemi and Folsom, 2009; Rapoport et al., 2005), it might be worthwhile to further investigate the developmental role of embryonic and early postnatal GlyT1 expression.

In summary, one may tentatively speculate that effective inhibition of both neuronal and astrocytic GlyT1 confined to the cerebral cortex, including the hippocampus but not the striatum, would perhaps maximize the effect against sensorimotor gating deficits against systemic NMDAR blockade. Such selectivity might be achievable by targeting distinct GlyT1 isoforms with differential expression in different tissues and cell lines (Adams et al., 1995; Borowsky and Hoffman, 1998; López-Corcua et al., 2001).

Sensorimotor gating in GlyT1^{ΔFB/neuron} mice

In contrast to GlyT1^{ΔFB/global} mice, baseline PPI was already reduced in GlyT1^{ΔFB/neuron} mice. In the context of a potential negative correlation between CNS glycine levels and PPI performance in schizophrenia patients (Heresco-Levy et al., 2007) and the occurrence of PPI deficits in rats maintained on a glycine-rich diet (Waziri and Baruah, 1999), GlyT1^{ΔFB/neuron} mice might provide a genetic model for such PPI deficiency with a specific emphasis on forebrain neuronal GlyT1. This, however, does not preclude the possibility of an opposite effect in other PPI disruption models. Indeed, PPI deficits induced by dopaminergic agonism would be particularly interesting given that forebrain neuronal GlyT1 deletion (GlyT1^{ΔFB/neuron}) can delay (Yee et al., 2006) or even attenuate (Dubroqua S, Boison D, Feldon J, & Yee BK, unpublished data) the motor stimulant effect of acute amphetamine – a phenotype that is absent in GlyT1^{ΔFB/global} mice (Singer et al., 2009c). The DBA/2 mouse

model with intrinsic PPI deficiency is another possibility given that dopaminergic dysfunction might be one physiological correlate of this model (e.g. Cabib et al., 2002; Puglisi-Allegra and Cabib, 1997; Puglisi-Allegra et al., 1990), although its unique sensitivity to detect atypical antipsychotic drugs relative to other mouse strains (e.g., C57BL/6) has been questioned (Singer et al., 2009b).

The GlyT1^{ΔFB/neuron} mice remained sensitive to MK801-induced disruption of PPI (Experiment 4). However, this does not permit us to decide if forebrain neuronal GlyT1 deletion (GlyT1^{ΔFB/neuron}) had altered the magnitude (in either direction) of the response to MK-801 relative to their own littermate controls. A more comprehensive design with littermate controls and multiple doses would be necessary to ascertain this point. However, the present outcome suggests that it is unlikely that GlyT1^{ΔFB/neuron} mice would reveal an antipsychotic phenotype in a PPI-disruption model based on NMDAR hypofunction. Nonetheless, impairment in baseline PPI remains a possible concern that deserves serious consideration in the future.

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Figure 1.

Open field activity recorded over a period of 60 min is expressed as distance travelled per 10-min bin. Neither GlyT1 Δ FB/neuron mice (A) nor GlyT1 Δ FB/global mice (B) differed in their spontaneous locomotor activity in comparison to their respective control littermates. All values refer to mean $\pm$ standard error.

Figure 2.

Prepulse inhibition (PPI) was indexed by percent inhibition (% PPI), calculated relative to the appropriate pulse-alone trials in which no prepulse stimulus was presented. % PPI was expressed here as a function of prepulse intensity, and separately at each pulse intensity. The bar plots represent mean % PPI levels of the mutants and controls, respectively. (A) and (B) depict the effect of forebrain neuronal GlyT1 deletion (GlyT1^{ΔFB/neuron}, Experiment 1) and forebrain global GlyT1 deletion (GlyT1^{ΔFB/global}, Experiment 2) on PPI expression, respectively. * denotes a significant main effect of genotype ($p < 0.05$) based on the overall ANOVA. All values refer to mean $\pm$ standard error.

Figure 3.

The impact of MK-801 on prepulse inhibition (PPI) was examined in GlyT1 Δ FB/global mice in comparison to littermate controls (Experiment 3), and in GlyT1 Δ FB/neuron mice (Experiment 4). The results of Experiment 3 are summarized in (A) and (B), and of Experiment 4 in (C). % PPI was expressed here as a function of prepulse intensity (averaged across the three pulse intensities). * denotes that % PPI was significantly reduced in the MK-801 relative to the saline condition ($p < 0.05$). All values refer to mean $\pm$ standard error.

Table 1

Major behavioural and electrophysiological effects associated with either neuronal or global GlyT1 disruption in the forebrain, based on our previous findings

Phenotypic features	GlyT1 ^{ΔFB/neuron}	GlyT1 ^{ΔFB/global}	References
<i>Electrophysiology</i>			
NMDAR-evoked currents	Enhanced	Not affected	1,2
AMPA-evoked currents	Not affected	Not affected	1,2
<i>Associative learning</i>			
Conditioned freezing	Enhanced	Unchanged	1,2
Active avoidance learning	Enhanced	Unchanged	1,2
Conditioned taste aversion	Enhanced	Unchanged	1,2
<i>Spatial learning</i>			
Working memory	Reduced proactive interference	Facilitation	3,2
Reference memory	Not affected	Not affected	3,2
<i>Recognition memory</i>			
Spontaneous object recognition	Enhanced	Enhanced	4,2
<i>Selective attention</i>			
Latent inhibition	Enhanced	Not affected	1,2
<i>Cognitive flexibility</i>			
2-choice discrimination reversal	Facilitated reversal learning	Facilitated reversal learning *	1,6
Reversal learning in water maze	Facilitated reversal learning	Not affected	1,2
<i>Reaction to psychostimulant drugs</i>			
Dizocilpine (MK-801)	Reduced	n/a	5,2
Phencyclidine (PCP)	Reduced	Reduced	1,2
Amphetamine	Delayed response	Not affected	1,2
<i>Sensorimotor gating</i>			
Prepulse inhibition (PPI)	Attenuated	Not affected	Present study
MK-801-induced PPI disruption	Not abolished	Abolished	

[1] Yee et al., 2006;

[2] Singer et al., 2009c;

[3] Singer et al., 2009a;

[4] Singer et al., 2007a;

[5] Möhler et al., 2008;

[6] unpublished data (Dubroqua et al.).

“n/a” denotes data not yet available. Common to both mutations was enhanced object recognition memory, and resistance to NMDAR antagonist-induced hyperlocomotion. While forebrain global GlyT1 deletion (GlyT1 Δ FB/global) was uniquely associated with enhanced working memory, only forebrain neuronal GlyT1 deletion (GlyT1 Δ FB/neuron) potentiated aversive Pavlovian conditioning, the latent inhibition effect, facilitated reversal learning and affected the hyperlocomotor response to systemic amphetamine.

Summary of the reactivity (ln-transformed) scores obtained in the first and last block of pulse-alone trials used to index startle habituation in Experiments 1 and 2. All values (in arbitrary units) are presented as mean ± standard error. “Mean” refers to the average scores collapsed across the three pulse intensities.

Table 2

Pulse Intensity	Experiment 1: GlyT1 ^{ΔFB/neuron}			Experiment 2: GlyT1 ^{ΔFB/global}		
	Mutant		Control	Mutant		Control
	<i>First Block</i>	<i>Second Block</i>	<i>First Block</i>	<i>First Block</i>	<i>Second Block</i>	<i>Second Block</i>
<i>100dB_A</i>	3.29±0.14	3.10±0.14	3.24±0.11	3.05±0.14	3.15±0.15	3.31±0.12
<i>110dB_A</i>	3.86±0.13	3.74±0.12	3.90±0.11	3.90±0.15	3.82±0.15	4.01±0.13
<i>120dB_A</i>	4.29±0.16	4.11±0.17	4.14±0.13	4.18±0.21	4.10±0.18	4.26±0.18
Mean	3.81±0.12	3.65±0.13	3.76±0.10	3.71±0.14	3.69±0.14	3.86±0.12
						3.85±0.14

Table 3

Summary of the reactivity scores (ln-transformed) obtained on pulse-alone, prepulse-alone and “no-stimulus” trials in Experiments 1 and 2, taken from the middle block of trials. All values (in arbitrary units) are presented as mean $\pm$ standard error. “No-stimulus” refers to trials in which no discrete stimulus except the background noise (at 65dBA) was presented.

	Experiment 1: GlyT1^{ΔFB/neuron}		Experiment 2: GlyT1^{ΔFB/global}	
	Mutant	Control	Mutant	Control
Pulse-alone trials:				
100dBA	3.13 $\pm$ 0.12	3.19 $\pm$ 0.10	3.23 $\pm$ 0.11	3.37 $\pm$ 0.09
110dBA	3.84 $\pm$ 0.12	3.92 $\pm$ 0.10	3.91 $\pm$ 0.12	4.10 $\pm$ 0.10
120dBA	4.22 $\pm$ 0.15	4.20 $\pm$ 0.13	4.28 $\pm$ 0.16	4.35 $\pm$ 0.13
Prepulse-alone trials:				
No-stimulus	2.18 $\pm$ 0.07	2.09 $\pm$ 0.05	2.34 $\pm$ 0.07	2.28 $\pm$ 0.06
71dBA	2.29 $\pm$ 0.07	2.14 $\pm$ 0.06	2.39 $\pm$ 0.07	2.29 $\pm$ 0.05
77dBA	2.25 $\pm$ 0.09	2.24 $\pm$ 0.07	2.46 $\pm$ 0.07	2.40 $\pm$ 0.06
83dBA	2.38 $\pm$ 0.11	2.55 $\pm$ 0.09	2.57 $\pm$ 0.08	2.60 $\pm$ 0.06

Summary of the reactivity scores (ln-transformed) obtained in the first and last block of pulse-alone trials used to index startle habituation in Experiments 3 and 4. All values (in arbitrary units) are presented as mean ± standard error. “Mean” refers to the average scores collapsed across the three pulse intensities.

Table 4

Saline	Pulse Intensity	Experiment 3: GlyT1 ^{AFB/global}			Experiment 4: GlyT1 ^{AFB/neuron}		
		Mutant		Control	Mutant		
		First Block	Last Block	First Block	First Block	Last Block	F Last Block
	100dB _A	3.99±0.25	3.49±0.28	3.74±0.27	4.11±0.30	3.22±0.20	3.52±0.27
	110dB _A	4.02±0.25	3.83±0.29	4.43±0.28	3.93±0.32	4.34±0.22	4.31±0.22
	120dB _A	4.37±0.19	3.99±0.20	4.69±0.21	4.18±0.22	4.27±0.23	4.62±0.22
	Mean	4.13±0.19	3.77±0.22	4.29±0.21	4.07±0.24	3.95±0.19	4.15±0.19
MK-801	100dB _A	4.40±0.26	4.45±0.29	3.92±0.30	4.53±0.33	3.68±0.20	3.91±0.27
	110dB _A	4.27±0.26	4.92±0.30	4.03±0.30	4.66±0.35	4.33±0.22	4.52±0.22
	120dB _A	4.66±0.20	4.68±0.21	4.47±0.23	5.03±0.24	5.21±0.23	4.71±0.22
	Mean	4.44±0.20	4.68±0.23	4.14±0.23	4.74±0.26	4.41±0.19	4.38±0.19

Table 5

Summary of the reactivity scores (ln-transformed) obtained on pulse-alone, prepulse-alone and “no-stimulus” trials in Experiments 3 and 4, taken from the middle block of trials. All values (in arbitrary units) are presented as mean $\pm$ standard error. “No-stimulus” refers to trials in which no discrete stimulus except the background noise (at 65dB_A) was presented.

		Experiment 3: GlyT1 ^{ΔFB/global} × MK801			Experiment 4: GlyT1 ^{ΔFB/neuron} × MK801		
		Saline		MK-801	Saline		MK-801
		Mutant	Control	Mutant	Control	Mutant	Mutant
Pulse-alone trials:							
100dB _A	4.01 $\pm$ 0.22	4.24 $\pm$ 0.24	4.57 $\pm$ 0.23	4.38 $\pm$ 0.26	3.60 $\pm$ 0.24	3.83 $\pm$ 0.24	
110dB _A	4.05 $\pm$ 0.22	4.47 $\pm$ 0.20	4.89 $\pm$ 0.19	4.90 $\pm$ 0.21	4.22 $\pm$ 0.17	4.75 $\pm$ 0.17	
120dB _A	4.22 $\pm$ 0.15	4.80 $\pm$ 0.17	4.94 $\pm$ 0.16	4.94 $\pm$ 0.18	4.74 $\pm$ 0.19	5.13 $\pm$ 0.19	
Prepulse-alone trials:							
No-stimulus	2.41 $\pm$ 0.12	2.29 $\pm$ 0.14	2.80 $\pm$ 0.13	2.55 $\pm$ 0.15	2.35 $\pm$ 0.08	2.67 $\pm$ 0.08	
71dB _A	2.47 $\pm$ 0.13	2.42 $\pm$ 0.14	2.72 $\pm$ 0.13	2.58 $\pm$ 0.15	2.41 $\pm$ 0.09	2.77 $\pm$ 0.09	
77dB _A	2.74 $\pm$ 0.15	2.55 $\pm$ 0.16	3.16 $\pm$ 0.15	2.85 $\pm$ 0.18	2.47 $\pm$ 0.16	3.03 $\pm$ 0.16	
83dB _A	3.29 $\pm$ 0.25	3.22 $\pm$ 0.27	3.75 $\pm$ 0.26	3.46 $\pm$ 0.30	2.86 $\pm$ 0.22	3.16 $\pm$ 0.22	