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Commentary

Glycine transporter 1 as a potential therapeutic target for schizophrenia-related symptoms: Evidence from genetically modified mouse models and pharmacological inhibition

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ABSTRACT

Schizophrenia is characterized by positive symptoms such as hallucinations, negative symptoms such as blunted affect, and symptoms of cognitive deficiency such as deficits in working memory and selective attention. N-methyl-D-aspartate receptor (NMDAR) hypofunction has been implicated in all three pathophysiological aspects of the disease. Due to the severe side effects of direct NMDAR agonists, targeting the modulatory co-agonist glycine-B site of the NMDAR is considered to be a promising strategy to ameliorate NMDAR hypofunction. To assess the antipsychotic and pro-cognitive potential of this approach, we examine the strategies designed to enhance glycine-B site occupancy through glycine transporter 1 (GlyT1) blockade. Among the existing transgenic mouse models with GlyT1 deficits, the one specifically targeting forebrain neuronal GlyT1 has yielded the most promising data on cognitive enhancement. Parallel advances in the pharmacology of GlyT1 inhibition point not only to an enhancement of attention, learning and memory but also include suggestions of mood enhancing effects that might be valuable for treating negative symptoms. Thus, interventions at GlyT1 are highly effective in modifying multiple brain functions, and dissection of their respective mechanisms is expected to further maximize their therapeutic potential for human mental diseases.

1. Introduction

Treatment of schizophrenia remains far from optimal. While current antipsychotics generally reduce positive symptoms, poor compliance, undesirable side-effects and the limited impact on negative and cognitive symptoms remain a major disabling burden. Hypotheses regarding the pathophysiology of schizophrenia originated in the "dopamine hypothesis" [1] and have advanced to include a role for GABA [2–5] and, in particular, glutamate [6–8]. N-methyl-D-aspartate receptor (NMDAR) antagonists such as ketamine and phencyclidine (PCP) can cause psychotic and cognitive abnormalities reminiscent of schizophrenia [9]. Impaired NMDAR function is readily expected to undermine the formation of neural plastic change such as NMDAR-dependent long-term potentiation (LTP) [10,11]. In addition, NMDAR hypofunction can also reduce the inhibitory activity of critical parvalbumin-positive GABA interneurons, leading to disinhibition of postsynaptic pyramidal cells [12–14]. The resulting dysregulation in neuronal network activity may contribute to the pathophysiology of schizophrenia [15,16].

Treatment of schizophrenia with D-serine, glycine or sarcosine (N-methylglycine) has yielded some therapeutic benefit particularly with regard to negative symptoms [17,18], albeit with limiting side effects. These agents modulate NMDAR functions either directly or indirectly via the co-agonist glycine-B site located on NMDARs. Local glycine concentration at NMDAR-containing synapses is largely regulated by the sodium-dependent glycine transporter 1 (GlyT1) expressed in pre- and post-synaptic terminals of glutamatergic neurons as well as in neighbouring astrocytes [19]. Sarcosine is an endogenous inhibitor of GlyT1 that has been evaluated in several clinical trials as add-on treatment [20–24,73]. Sarcosine confers additional benefits particularly

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against negative and cognitive symptoms, when given as add-on to neuroleptic medication except in the case of clozapine [22,25]. Unequivocal clinical data in support of GlyT1 inhibition as effective monotherapy for schizophrenia are lacking [23,24,26]. For the analysis of GlyT1's physiological role and its impact on a wide spectrum of behavioural parameters, several mouse GlyT1 mutants have been generated. To further explore the potential of GlyT1 as a therapeutic target to ameliorate the symptoms of schizophrenia, a wide variety of GlyT1 inhibitors have been synthesized. Based on preclinical data in animal studies (see Section 3), various GlyT1 inhibitors are promising therapeutic candidates (Tables 2–4), of which some reached the stage of clinical development. The most recent positive clinical interim results were reported in a press release for RG1678 [27, also see, http://www.roche.com/investors/ir_update/inv-update-2009-11-10.htm].

The present review covers three topics. First, it summarizes the lessons learned from GlyT1 mutant animals with regard to behavioural outcome. Second, it summarizes the behavioural readouts of the major GlyT1 inhibitors with regard to their impact on attention, motor behaviour, learning and memory. Third, it summarizes briefly the therapeutic potential of GlyT1 inhibition beyond schizophrenia.

2. Genetic mouse models to study GlyT1

Gene targeting approaches performed during the past 8 years have contributed greatly to our understanding of GlyT1 function on the biochemical, physiological, and behavioural levels. Molecular strategies utilized to date include (i) a complete and global genetic disruption of GlyT1 (i.e. homozygous knockout), (ii) a global ~50% reduction of GlyT1 expression (i.e. heterozygous knockout), and (iii) conditional, regional or cell-type selective disruption of GlyT1 (i.e. in vivo recombination of a floxed GlyT1 allele with a tissue- or cell type-specific Cre-recombinase). Animal models were created in different laboratories using different sources of embryonic stem cells to generate the mutant animals, as well as different strains of mice were used to propagate the mutations. The following section provides an overview of genetic mouse models having been generated to study the impact of changes in GlyT1 expression on various molecular, biochemical, and physiological parameters.

2.1. Homozygous constitutive deletion of GlyT1

Two lines of constitutive GlyT1 knockout mice were created in the laboratories of Heinrich Betz ($Slc6a9^{tm1Betz}$) [28] and Joseph Coyle ($Slc6a9^{tm1Jtc}$) [20]. Unfortunately, both lines were perinatally lethal due to severe respiratory depression, a phenotype that precluded further analysis of behavioural phenotypes.

2.2. Heterozygous constitutive deletion of GlyT1

Heterozygous GlyT1 knockouts, however, survived and were studied in more detail [20,30] (see Section 3). On the physiological level, the mutation ($Slc6a9^{tm1Jtc}$), maintained on a 129/SvEvTac background, led to enhanced NMDAR function in CA1 pyramidal cells; while evoked NMDAR currents could be potentiated with 10 μM glycine or 10 μM D-serine in pyramidal cells of wild-type mice, currents could not further be potentiated in slices from heterozygous GlyT1 knockouts because the glycine-B site was already saturated by an increased glycine tone [20]. These findings were accompanied by faster decay kinetics, reduced ifenprodil sensitivity and increased zinc-induced antagonism of NMDAR currents; in addition, the mutants were characterized by an increase in NMDA/AMPA response-ratio (peak–amplitude ratio of NMDA- to AMPA-receptor evoked excitatory postsynaptic currents (EPSCs) in hippocampal CA1 pyramidal cells) compared to wild-type mice.

A second line of heterozygous GlyT1 knockout mice was created in Meike Pauly-Evers's laboratory based on a pure C57BL/6 background and designated GlyT1tm1.1 ($Slc6a9^{tm1.1Blt}$) [31]. Western blot analysis revealed that whole-brain protein extracts from adult heterozygous mutant mice contained approximately 50% the level of the GlyT1 protein compared to extracts from wild-type mice. While the pattern of immunohistologically stained receptor distribution throughout the brain was the same in heterozygous and wild-type adult mice, the level of immunoreactive protein was significantly and proportionately reduced in all brain regions of the heterozygotes. Electrophysiologically, this mutation resulted in enhanced NMDAR-mediated synaptic currents. In particular, the NMDA/AMPA response ratio in CA1 pyramidal neurons in hippocampal slices from 17 to 24 days old heterozygous mutants was found to be 36% greater than in wild-type slices. In addition, the frequency and amplitude of AMPA-evoked and spontaneous miniature EPSCSs were unaltered indicating that the enhanced NMDA/AMPA current ratio was due to increased NMDAR responsiveness.

2.3. Conditional deletion of GlyT1 in forebrain neurons

The first conditional GlyT1 knockout mouse was generated in 2005 in collaboration between the laboratories of Detlev Boison (University of Zurich) and Meike Pauly-Evers (Hoffmann LaRoche, Basel) [31]. Using a gene targeting construct comprising 129/JEms DNA that contained two loxP-sites, flanking exons 5 through 11 of the GlyT1 gene, homologous recombination in C57BL/6 ES cells resulted in cells with the correct recombination event. Cells with this "floxed" GlyT1 allele were injected into C57BL/6 blastocysts to finally generate a colony of homozygous $GlyT1tm1.2^{fl/fl}$ mice ($Slc6a9^{tm1.1Bois}$). As expected, animals from this line are viable and do not display any known phenotypic abnormalities.

To avoid any developmental compensatory changes that might be associated with any constitutive gene deletion and to achieve forebrain-specific neuronal recombination of the floxed GlyT1 allele in the late postnatal brain (around postnatal day P21), conditional $GlyT1tm1.2^{fl/fl}$ mice were bred with CamKIIα‴ Cre2834[Tg(Camk2a-cre)2834Lusc/0)] mice [32] that were likewise maintained on a pure C57BL/6 background. The resulting $CamKII\alpha Cre2834{:}GlyT1tm1.2^{fl/fl}$ mice carry a neuron-selective disruption of GlyT1 restricted to the forebrain, and they are henceforth designated as "$GlyT1^{\Delta FB\text{-}neuron}$ mice" in this review. These mutant mice were characterized by 30% reduction of GlyT1 expression in forebrain, which was associated with a 35% reduction of GlyT1-dependent [^{3}H]glycine uptake. Importantly, NMDAR expression was maintained at normal levels excluding any compensatory changes. $GlyT1^{\Delta FB\text{-}neuron}$ mice were further characterized by enhanced NMDAR-mediated synaptic currents. Around P21-30, a substantial increase (+120%) in NMDA/AMPA response ratio was found in the mutants relative to control mice as measured in brain slices [33].

2.4. Conditional deletion of GlyT1 in telencephalon

A similar breeding strategy was used to delete GlyT1 specifically in neurons and glia of the entire dorsal telencephalon. Crossing $GlyT1tm1.2^{fl/fl}$ mice with $Emx1^{Cre/Cre}$ mice [34] that contain a Cre-recombinase gene "knocked in" the endogenous Emx1 locus gives rise to offspring with ubiquitous dorsal telencephalon specific Cre expression in neurons and astrocytes during early embryogenesis: namely, $Emx1Cre{:}GlyT1tm1.2^{fl/fl}$ (designated as "$GlyT1^{\Delta Telenceph}$" here) mice [35]. Compared with

GlyT1$^{\Delta FB\text{-neuron}}$ mice, the GlyT1$^{\Delta Telenceph}$ mice were characterized by a more substantial 79% reduction of GlyT1 expression in forebrain resulting in a 77% reduction of GlyT1-dependent [^{3}H]glycine uptake. However, and in contrast to GlyT1$^{\Delta FB\text{-neuron}}$ mice [33], the global deletion of GlyT1 in the dorsal telencephalon did not affect NMDAR-mediated neurotransmission in hippocampal CA1 pyramidal cells [35].

2.5. Other conditional deletions of GlyT1

Additional conditional GlyT1 knockout lines have recently been established by first generating a floxed GlyT1 knockout mouse (Slc6a9^{tm1Veul}/Slc6a9^{tm1Veul}) using 129P2/OlaHsd ES cells [37]. To generate a neuron-specific knockout of GlyT1, these animals were bred with Tg(Syn1-cre)671Jxm mice that express Cre recombinase under the control of a rat synapsin I promoter [37]. Cre recombinase activity was detected in neuronal cells, including brain, spinal cord and dorsal root ganglia, as early as E12.5, and in neurons in adult animals [36]. In the resulting Slc6a9^{tm1Veul}/Slc6a9^{tm1Veul}Tg(Syn1-cre)671Jxm/? mice, that were maintained on a mixed 129P2/OlaHsd × C57BL/6 × CBA background, GlyT1 specific uptake of glycine by hippocampal membranes was reduced by 50%. In contrast to homozygous constitutive GlyT1 knockouts hypotonia or motor defects were not observed [37].

To generate an astrocyte-specific knockout of GlyT1, Slc6a9^{tm1Veul}/Slc6a9^{tm1Veul} mice were bred with Tg(Gfap-cre)2Brn mice that express Cre recombinase under control of the mouse Gfap promoter [37]. This Cre-transgene was expressed in the developing forebrain and in the spinal ganglia but also in scattered precursor cells located in the external granule layer of the cerebellum [38]. The resulting Slc6a9^{tm1Veul}/Slc6a9^{tm1Veul}Tg(Gfap-cre)2Brn/? mice were maintained on a mixed 129P2/OlaHsd × FVB/N background. 80% of the mutants developed a severe hypotonic phenotype that led to premature death between day 1 and 10 after birth. 20% of the mutants survived, developed no phenotype and had a normal life-span. At the time of writing, there are as yet any behavioural data reported in this new mutant line. It could prove to be a valuable tool to address the phenotypic divergences noted between GlyT1$^{\Delta FB\text{-neuron}}$ and GlyT1$^{\Delta Telenceph}$ mice, which as explained above are differentiated in one key aspect of cell type specificity of gene deletion.

3. Behavioural phenotypes of GlyT1 gene deletion mouse models

The assessment of the behavioural outcome associated with the loss of GlyT1 in highly specific mouse models was expected to yield novel insights into GlyT1 function. Comparisons between models with different patterns of GlyT1 deletion in terms of regional and cell type specificity can achieve a differentiation not readily attainable by pharmacological manipulations alone. Three mouse models of GlyT1 genetic deletion will be reviewed. In the conventional constitutive GlyT1 deletion models, only heterozygous mice were behaviourally analyzed (GlyT1$^{+/-}$ [20]) due to early postnatal lethality of the homozygous deletion (see Section 2). GlyT1$^{+/-}$ mice (based on the constitutive GlyT1 deletion mouse line 2, as described in Section 2.2) are associated with a constitutive reduction of neuronal and glial GlyT1 expression by ~50%, and therefore a global reduction of GlyT1-mediated reuptake of glycine of a similar magnitude [20]. In contrast to the global GlyT1 deficit, two conditional gene deletion models have been developed by our laboratories based on the Cre/loxP recombination system in which GlyT1 deletion was restricted either to the forebrain in a neuron-specific manner (GlyT1$^{\Delta FB\text{-neuron}}$ [33]) or to the telencephalon in a cell type non-specific manner (GlyT1$^{\Delta Telenceph}$ [35]). Because of the preferential expression of GlyT1 by glial cells, the two conditional gene deletion models differed strikingly in terms of the overall reduction of GlyT1-mediated reuptake measured in the forebrain (Fig. 1A). By confining the deletion to neurons, the reduction recorded in GlyT1$^{\Delta FB\text{-neuron}}$ mice was about half of that in GlyT1$^{\Delta Telenceph}$ mice, which is mirrored by a similar differential reduction of GlyT1 protein expression between the two mutant mouse lines (Fig. 1). Another notable difference between GlyT1$^{\Delta FB\text{-neuron}}$ and GlyT1$^{\Delta Telenceph}$ mice is that the genetic deletion of GlyT1 extends to the striatum in the former but not the latter mutant line. Given the diversity of genetic models with neurobiologically relevant distinction, it comes as no surprise that divergences as well as convergences of behavioural effects exist between them (see Table 1). On the physiological level, the relevance of the cellular specificity of GlyT1 deletion was particularly apparent in the observed enhancement of the NMDA/AMPA current ratio. GlyT1$^{\Delta FB\text{-neuron}}$ mice exhibited an increase of >120% [33], and GlyT1$^{+/-}$ mice in the range of +36% to +72% [Refs. 31 and 20, respectively], while the slight increase (+14%) obtained in GlyT1$^{\Delta Telenceph}$ mice was not statistically significant [35].

3.1. Learning and memory

Pro-cognitive effects on a variety of memory tests have been reported in the three gene deletion models described above (Sections 2.2–2.4), lending support to the general thesis that GlyT1 inhibition possesses pro-cognitive potential. The first report was based on the GlyT1$^{+/-}$ mice by the Coyle group [20], who showed that retention of spatial reference memory was somewhat enhanced in the classical water maze task. The effect was however relatively weak, being evident only in the form of a slight improvement in search accuracy during the retention probe test. The preference for the target area where the escape platform was previously found was essentially unaffected. This general lack of an effect in the acquisition and retention of spatial reference memory is consistent with the null results also reported in the GlyT1$^{\Delta FB\text{-neuron}}$ and GlyT1$^{\Delta Telenceph}$ mice, which clearly yielded multiple effects on learning and memory. Recognition memory that taxes familiarity judgement of objects is enhanced in both GlyT1$^{\Delta FB\text{-neuron}}$ mice and GlyT1$^{\Delta Telenceph}$ mice in delay-dependent manner, which is suggestive of a stronger memory trace and/or resistance to memory decay. These two mutant lines are further associated with other distinctive memory effects.

Working memory impairment is one of the most prominent schizophrenia-related cognitive impairments and represents a major barrier to effective rehabilitation [43,44]. The impairment affects the short-term storage and expression of relevant information for effective guiding and execution of purposeful goal-directed behaviour. Situations in which the relevant information is constantly changing are particularly taxing, as information requires regular updating and this is especially prone to proactive interference by previously acquired relevant (but now irrelevant) memory traces. In the water maze, when the escape platform location is changed daily but remains fixed across trials within a given day, the animals are required to rapidly learn the new location revealed to them on the first trial to guide the escape from the water in subsequent trials on the same day. GlyT1$^{\Delta Telenceph}$ mice performed better on this test when the retention interval between the first and second trials was extended such that control animals could no longer hold the relevant information [35]. This is suggestive of enhanced working memory retention, and is in agreement with the acute effect of GlyT1 inhibiting drug (see Section 4). No such phenotype however was seen in GlyT1$^{\Delta FB\text{-neuron}}$ mice.

The performance of GlyT1$^{\Delta FB\text{-neuron}}$ mice in such a water maze working memory test was nonetheless significantly altered, suggesting that they might be less prone to the negative impact of proactive interference and were relying less on a working

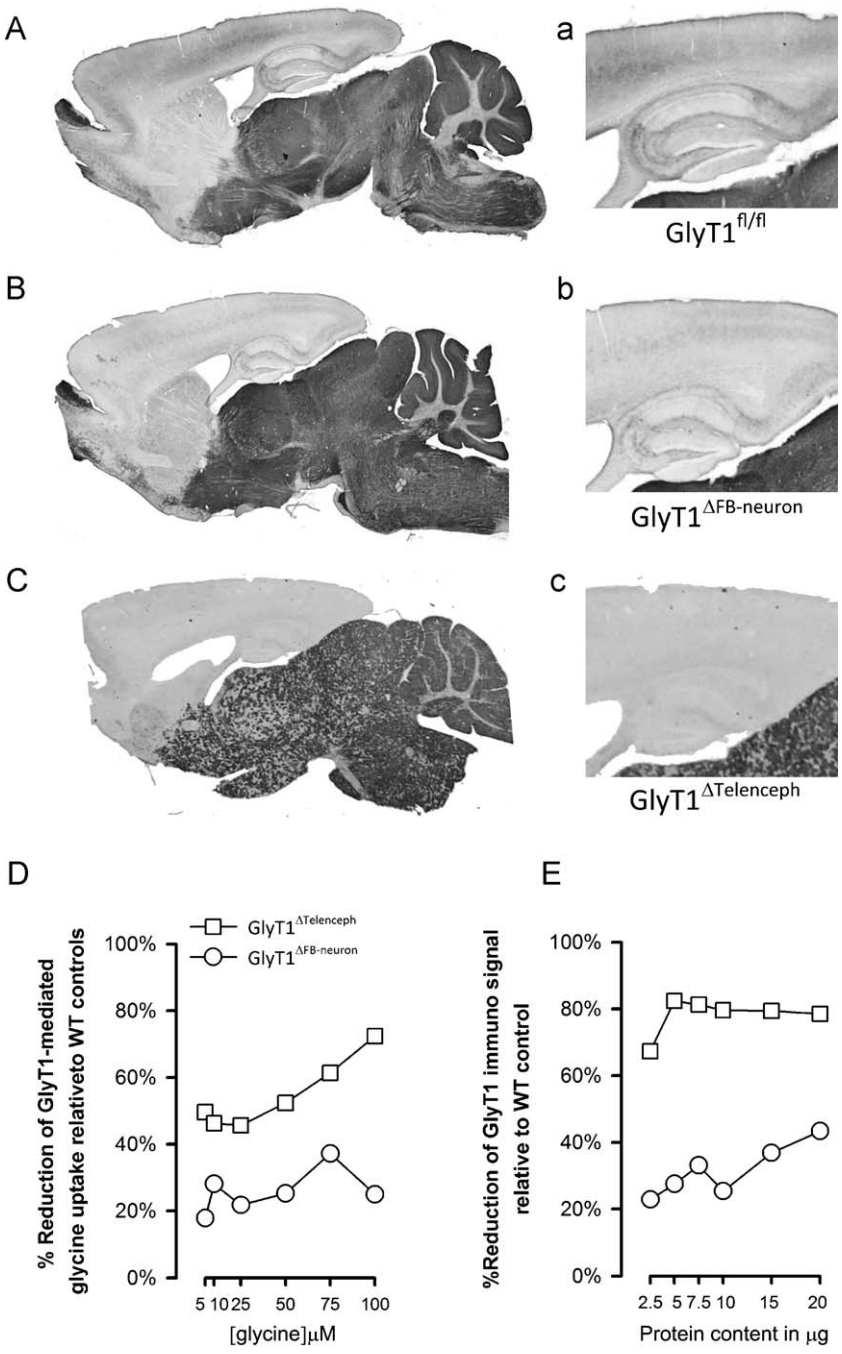

Fig. 1. Comparison of biochemical phenotypes between GlyT1$^{\Delta FB\text{-neuron}}$ mice and GlyT1$^{\Delta Telenceph}$ mice. Immunostained parasagittal sections taken from GlyT1$^{fl/fl}$ control (A), GlyT1$^{\Delta FB\text{-neuron}}$ (B) and GlyT1$^{\Delta Telenceph}$ (C) mice illustrate the loss of GlyT1-immunoreactivity (GlyT1-ir) in the latter two lines. Sagittal sections of 4% paraformaldehyde-fixed brain were stained for GlyT1 (brown) using a primary GlyT1 antibody raised in rabbits [33] and diaminobenzidine (DAB). The partial loss of GlyT1-immunoreactivity was restricted to the forebrain areas in GlyT1$^{\Delta FB\text{-neuron}}$ mice. In contrast, the loss was complete in the telencephalon in the GlyT1$^{\Delta Telenceph}$ line, but these mice were further associated with ectopic punctated foci of GlyT1 lesions in the brainstem. Higher magnification images centering on the hippocampus and overlying cortex are illustrated in (a), (b) and (c), taken from the same section shown in (A), (B) and (C), respectively. Reduction of GlyT1-mediated reuptake (D) and deletion of GlyT1 protein expression in the forebrain region (E), based on results extrapolated from data previously obtained [33,35]. Full descriptions of methods are provided in the relevant papers. The GlyT1$^{\Delta FB\text{-neuron}}$ and GlyT1$^{\Delta Telenceph}$ mouse lines have been reported to be associated with an increase of +120% and +14% in the NMDAR/AMAPR response ratio, respectively [33,35]. The latter increase was not statistically significant [35]. For comparative purpose, the corresponding increase in NMDAR/AMAPR response ratio of the two heterozygous knockout lines developed independently by Tsai et al. [20] and Gabernet et al. [31] are +72% and +36%, respectively.

memory strategy. They thus outperformed control mice in the first trial when the escape platform was freshly changed (and therefore unknown), and did not exhibit a general improvement of escape performance from trial 1 to 2. Their overall escape performance was therefore slightly more efficient than that of littermate controls. Additional working memory tests are clearly warranted to clarify this unique set of findings.

Our interpretation that GlyT1$^{\Delta FB\text{-neuron}}$ mice might be less susceptible to proactive interference and therefore accorded with more flexible and adaptive behavioural control is supported by another set of experiments [40]. As mentioned above, when the escape platform in the water maze was kept constant, GlyT1$^{\Delta FB\text{-neuron}}$ mice apparently performed normally. But, this was no longer the case when they were subjected to a series of such

Table 1
Comparison of the behavioural phenotypes of three major documented mouse models of GlyT1 genetic deletion.

Summary of behavioural phenotypes in GlyT1 mutant mouse models			
Behavioural functions	Constitutive GlyT1$^{+/-}$	Forebrain neurons GlvT1$^{\Delta FB\text{-neuron}}$	Telecephalon GlYT1$^{\Delta Telenceph}$
Associative learning			
Conditioned freezing	–	Enhanced	Null
Active avoidance learning	–	Enhanced	Null
Conditioned taste aversion	–	Enhanced	Null
Spatial learning			
Working memory	–	?	Enhanced
Reference memory	Weakly enhanced	Null	Null
Recognition memory			
Object recognition	–	Enhanced	Enhanced
Attentional processes			
Latent inhibition (learned inattention)	–	Enhanced	Null
Prepulse inhibition (early sensory gating)	Null	Impaired	Null
MK-801 induced PPI disruption	Sensitized	Null	Resistant
Amphetamine induced PPI disruption	Resistant	–	–
Behavioural flexibility			
2-Choice discrimination reversal	–	Enhanced	–
Spatial reversal learning	–	Enhanced	Null
Motor effects of psychostimulant drugs			
Motor stimulation by PCP or MK-801	Null	Resistant	Resistant
Motor stimulation by amphetamine	Weak resistant	Delayed	Null
Other behaviour			
Accelerating rotarod	–	Null	Null
Elevated pluz maze test of anxiety	–	Null	Null
Spontaneous open field activity and exploration	Null	Null	Null

Constitutive GlyT1$^{+/-}$ mice refer to heterozygous GlyT1 knockout mice generated by Coyle and colleagues [20, i.e., "line 2" as described in Section 2.2]. GlyT1$^{\Delta FB\text{-neuron}}$ mice refer to conditional knockouts with GlyT1 deletion restricted to forebrain neurons [33,39–42] (see Section 2.3). GlyT1$^{\Delta Telenceph}$ mice refer to conditional knockouts with GlyT1 deletion confined to the telencephalon in a cell type non-specific manner [35,41] (see Section 2.4). "Null" indicates that non-significant difference in comparison to controls, whereas "–" denotes that no existing data are available. The additional description in parentheses in the first column provides the psychological/behavioural significance of the named specific test paradigm.

reference memory problems one after another. GlyT1$^{\Delta FB\text{-neuron}}$ mice outperformed controls from the second problem onwards. They were less persistent in searching where the escape platform was previously located, and more readily extending their search to alternative locations. Thus, they seemed to be more responsive to changes to environmental contingency. Such behavioural flexibility has also been confirmed in a non-spatial discrimination learning paradigm that is motivated by appetitive rewards instead of escape from distress [40].

Forebrain neuronal deletion of GlyT1 also uniquely gives rise to a phenotype in Pavlovian associative learning, by which a neutral stimulus (a conditioned stimulus, CS) acquires behavioural significance by virtue of its ability to predict other significant events (unconditioned stimuli, USs). This is a robust phenotype observed across different Pavlovian conditioning paradigms [33], and remains still detectable in aged mice over 20 months old [42]. This effect cannot be accounted for by any non-specific confounding changes in anxiety, spontaneous activity or pain sensitivity (unpublished data). The expression of this relatively simple phenotype is already indicative of enhanced memory retention as well as responsiveness to changes in environmental contingency. These traits can be readily illustrated by one of our previously unpublished experiments in which three weeks separated the conditioning of a tone CS to a shock US and the subsequent

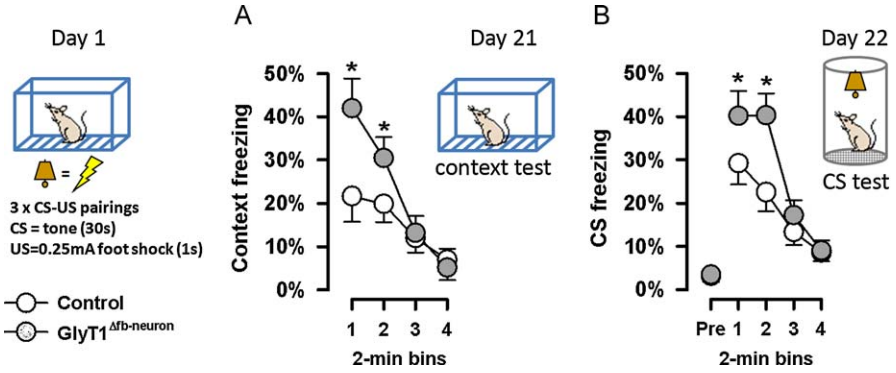

Fig. 2. Long-term retention for memory of Pavlovian associations in the conditioned freezing paradigm. On day 1, adult female GlyT1$^{\Delta FB\text{-neuron}}$ mutant mice ($n = 6$) and littermate controls ($n = 8$) were subjected to three tone-shock pairing as described previously (see [33]). Freezing obtained from day 1 conformed closely to previous published data [33], yielding no difference between mutant and controls (data not shown). The animals were then returned to their home cage until tests of conditioned response. (A) Conditioned freezing to the shocked context was assessed on day 21. A 2 × 4 (genotype × 2-min bins) ANOVA of percent time freezing yielded a significant interaction [$F_{(3,36)} = 3.43$, $p < 0.05$], which was attributed solely to the elevated levels of freezing by mutants specific to the first two bins ($^{*}p < 0.05$ based on post-hoc comparisons). (B) On day 22, animals were exposed to a neutral context. Following a 2-min period of acclimatization (Pre-CS period, 'Pre' in the *x*-axis), the tone stimulus was presented for 8 min (CS-period). A 2 × 4 (genotype × 2-min bins) ANOVA of percent time freezing in the CS-period similarly yielded a significant interaction [$F_{(3,36)} = 3.10$, $p < 0.05$]. GlyT1$^{\Delta FB\text{-neuron}}$ mutant mice again showed an elevated response in the initial 4 min to the CS ($^{*}p < 0.05$, post-hoc comparisons) which rapidly returned to control levels by the final two bins.

retention test of conditioned response (Fig. 2). Animals were first re-exposed to the shocked context to assess the retention of context-US association, followed a day later by a test of conditioned responding to the tone CS now presented in a neutral context. The conditioned response (CR) of interest is freezing – a conditioned fear response to impending danger. The magnitude of the CR to the context as well as to the CS was substantially higher in GlyT1$^{\Delta FB\text{-neuron}}$ mice, suggesting that the memory trace of the Pavlovian association was stronger. Yet, this potentiated response rapidly fell to control levels in the course of the 8-min test, because the CS was evidently no longer predictive of the expected shock. This rapid reduction is indicative of efficient extinction learning. Hence, the increase in freezing did not bring about unnecessary prolongation of the CR.

3.2. Attentional processes

Dysfunction in attention has long been considered a core deficit of schizophrenia by both Kraepelin [45] and Bleuler [46], which might be linked to both positive and cognitive symptoms of the disease. In particular, the inability to tune down attention to irrelevant stimuli might contribute to the sensory flooding linked to florid psychotic symptoms. Two relevant paradigms that are applicable to both humans and rodents in the assessment of such attentional processes are latent inhibition (LI) and prepulse inhibition (PPI). The expression of LI and PPI is impaired in schizophrenia patients [e.g., 47–52], and PPI impairments have also been considered as endophenotypes of the disease because of their occurrence also in patients' healthy relatives [53,54]. Psychostimulant drugs, including amphetamine and NMDAR antagonists, disrupt LI and PPI, and such drug-induced disruptions can be reversed by antipsychotic drugs (for reviews see [55–57]). LI can also be potentiated by antipsychotic drugs under test conditions that yield weak or no LI effect in controls [57].

LI occurs when learning about the predictive significance of a stimulus (as in associative learning) is retarded due to prior non-reinforced (i.e., non-consequential) exposures to that stimulus [58]. An animal learns from such non-reinforced pre-exposures that the stimulus in question is irrelevant, and as a result learns to pay less attention to that stimulus. This form of perceptual learning can be considered as learned inattention – learning not to attend. As a consequence, subsequent learning about any predictive significance of that stimulus is more difficult and proceeds more slowly compared with a novel stimulus without such a history of non-reinforcement. LI remained clearly present in GlyT1$^{\Delta FB\text{-neuron}}$ mice even though the pre-exposures were insufficient to induce LI in controls [33] – a phenotype that directly parallels the effects of antipsychotic drugs on LI. Moreover, this matches the effects of GlyT1 inhibiting drugs that reversed the LI-disruptive effect of NMDAR antagonists (see Section 4).

No such phenotype, however, was observed in GlyT1$^{\Delta Telenceph}$ mice [35]. Instead, evidence for an antipsychotic-like phenotype in these mice emerged in the PPI of acoustic startle reflex paradigm. When the startle-eliciting pulse stimulus is shortly preceded by a weak but detectable prepulse stimulus, the resulting startle reflex can be substantially weakened. The diminution of the startle response is attributed to a gating process under higher cortical control. This gating process is triggered by the prepulse stimulus in order to protect itself from interference by the succeeding pulse stimulus [59,60]. GlyT1$^{\Delta Telenceph}$ mice exhibited normal PPI expression and were resistant to the PPI-disruptive effect of the NMDAR antagonist, MK-801 [41]. This drug-induced phenotype is similar to the resistance to amphetamine-induced PPI disruption reported in GlyT1$^{+/-}$ mice [20]. These phenotypes are suggestive of functional antagonism against PPI disturbance arising from NMDAR hypofunction and dopaminergic hyperfunction. However, in contrast to this suggestion, GlyT1$^{+/-}$ mice were at the same time more sensitive to the disruptive effect of MK-801 at 0.32 mg/kg. Similarly intriguing is the reduction in baseline PPI seen in GlyT1$^{\Delta FB\text{-neuron}}$ mice [41]. Both findings are contrary to what might be considered as an antipsychotic-like phenotype, although baseline PPI remained unaffected in GlyT1$^{+/-}$ mutants. Thus, all three models indicate that the regulation of PPI can be affected by GlyT1 down-regulation. However, a satisfactory explanation for the divergent effects on PPI as well as in LI is lacking.

3.3. Psychostimulant-induced motor behaviour

None of the three genetic mouse models of GlyT1 deletion affected spontaneous motor and explorative behaviour in the open field or motor coordination as assessed using the standard accelerating rotarod apparatus (Table 1). Thus, any difference in their motor response to psychostimulant drugs can be interpreted without fear of baseline confounds. Although such tests are hardly cognitive in nature or by design, they nonetheless provide some critical readouts of pharmacologic interaction with specific neurotransmitter systems. Here, in spite of the lack of a complete correspondence between the three genetic mouse models (see Table 1), a general consensus exists pointing to a resistance to the motor stimulant effect of systemic psychomimetic drugs. Both GlyT1$^{\Delta FB\text{-neuron}}$ mice and GlyT1$^{\Delta Telenceph}$ mice responded less to the motor stimulant effects of NMDAR antagonists, although only GlyT1$^{\Delta FB\text{-neuron}}$ mice were in addition showing a delayed response to amphetamine-induced hyperactivity, which might be similar in nature to the weak resistance to amphetamine exhibited by GlyT1$^{+/-}$ mice. By contrast, no alteration to amphetamine response was observed in GlyT1$^{\Delta Telenceph}$ mice, and this might be linked to the sparing of striatal GlyT1 in these animals. This might also explain their normal expression of LI, because LI is critically controlled by limbic striatal interaction [61].

3.4. Comparative behavioural assessment

Despite that only a limited number of GlyT1 knockout mouse lines have undergone comprehensive behavioural and cognitive phenotyping, the overview clearly suggests that GlyT1 down-regulation in the brain is highly effective in altering a variety of brain functions, showing that GlyT1 is an effective target to modulate both glutamatergic (via NMDAR) and dopaminergic neurotransmissions. Support for the latter is provided by the observation that the acute motor response to amphetamine was delayed in GlyT1$^{\Delta FB\text{-neuron}}$ mice [33], and a recent replication has extended the result to a clear reduction in the response (Dubroqua et al., unpublished observation). Independently, heterozygous knockout has also been reported to attenuate the PPI-disruptive effect of amphetamine [20]. The lack of complete alignment in the magnitude, and sometimes direction, of the observed impact on specific behaviour across different genetic models (Table 1) is at first sight perplexing, and prompt for further consideration related to physiological, anatomical and cell-type specificity unique to each genetic manipulation and possible differences in associated adaptive compensations. Their eventual resolution should shed light on the neural mechanisms involved. The recent revelation that hippocampal neural proliferation is elevated in GlyT1$^{\Delta FB\text{-neuron}}$ mice [42] could be of relevance to some of the pro-cognitive effects found in these mice as well as to the suggestion that GlyT1 inhibitors might also possess anti-depressant efficacy [62] (see Section 4).

The GlyT1$^{\Delta FB\text{-neuron}}$ line was by far the most impactful manipulation, which also entailed the more notable increase in NMDA/AMPA response ratio – achieving an increase of 120% [33], in comparison to the non-significant increase of only 14% in the GlyT1$^{\Delta Telencephalon}$ mouse line [35], and 36–72% increase,

respectively, in heterozygous knockout mice [31,20]. However, it is worth pointing out that the apparent increase in NMDA/AMPA ratio reported in GlyT1$^{+/-}$ mice did not seem to stem from an elevation of NMDAR current (see Fig. 3 in [20]). Instead, this change might be the consequence of a reduction of AMPAR current although this possibility was not further tested. Thus, there seems to be a correlation between the effect on NMDAR function and the behavioural phenotype. The greater decrease in GlyT1 level and glycine reuptake, but relatively weak impact on NMDAR function and behaviour, seen in the GlyT1$^{\Delta\text{Telencephalon}}$ mouse line (compared to GlyT1$^{\Delta\text{FB-neuron}}$ mice) therefore deserves further examination. We have speculated elsewhere [35,41] that one possible explanation for the divergent phenotypic impact between GlyT1$^{\Delta\text{FB-neuron}}$ and GlyT1$^{\Delta\text{Telencephalon}}$ mice might be attributed to NMDAR internalization primed by excessive activation of the glycine-B site. Another possibility may point to an increase in glycinergic inhibitory tone involving increased activity of strychnine-sensitive glycine receptors (GlyRs) due to excessive extracellular glycine concentration. This would be expected to result from loss of glial GlyT1 (in both GlyT1$^{\Delta\text{Telencephalon}}$ mice and GlyT1$^{+/-}$ mice) which together with glial-based glycine transporter 2 (GlyT2) contribute to the regulation and termination of glycinergic neurotransmission. Thus, further deletion/inhibition of GlyT1 beyond the level that may saturate the glycine-B site could be counterproductive in terms of overall excitability at the network level via NMDAR internalization and inhibitory glycinergic transmission, resulting in an inverted U-shaped relationship between glycine-B site occupancy and therapeutic potential. However, one should be careful not to dismiss the possibility that the pro-cognitive impact of GlyT1 deletion might also stem from its effect on glycinergic inhibition. Notably, the phenotypes of enhancement in working memory performance and resistance to MK-801-induced PPI disruption in GlyT1$^{\Delta\text{Telencephalon}}$ mice [35,41] as well as that of improved spatial search accuracy in heterozygous GlyT1 knockouts [20] are distinctly lacking in GlyT1$^{\Delta\text{FB-neuron}}$ mice. Thus, the loss of glial-based GlyT1s and the expected impact on glycinergic neurotransmission common to GlyT1$^{\Delta\text{Telencephalon}}$ and GlyT1$^{+/-}$ mice might contribute directly to these cognitively relevant phenotypes.

Another worthwhile direction to advance would be to further dissect the behavioural relevance of neuronal versus glial GlyT1. The newly generated mutant mouse line with glia-specific GlyT1 deletion [37] (see Section 2.5) could be instrumental in this direction. In addition, a region-specific knockout would further increase our understanding of the regulation of cognitive function by GlyT1-related mechanisms at the circuitry level. In addressing the divergent impacts on sensorimotor gating function (as exemplified by the PPI paradigm) between GlyT1$^{\Delta\text{FB-neuron}}$ and GlyT1$^{\Delta\text{Telencephalon}}$ mice, we have previously emphasized the relevance of striatal GlyT1, which was deleted in GlyT1$^{\Delta\text{FB-neuron}}$ mice but spared in GlyT1$^{\Delta\text{Telencephalon}}$ mice. The striatum is a critical component of the brain circuitry underlying PPI. The PPI-impairing phenotype uniquely seen in GlyT1$^{\Delta\text{FB-neuron}}$ mice [41] might stem from an impact on striatal function. Interestingly, PPI was both positively and negatively modulated by constitutive heterozygous GlyT1 deletion – it offered resistance against amphetamine-induced PPI disruption but exacerbated the disruption induced by the NMDAR blocker MK-801 [20]. Hence, while the expression of PPI (in itself or under drug challenge) is altered in all three mutant mouse lines, its precise regulation by striatal and cortical/hippocampal GlyT1-related mechanism remains to be fully elucidated. Cell-type and region-specific gene deletion models as well as local pharmacological interventions would be necessary.

However, further advances in the specificity of molecular intervention alone require parallel development of preclinical translational behavioural models, which is lacking in particular with respect to negative symptoms of schizophrenia. Current drug-induced disease models typically rely on acute treatment of psychomimetic drugs, such as amphetamine or PCP. The "disease state" induced is therefore reflexive of the transient pharmacodynamic of dopamine and NMDA receptors rather than the structural changes in brain circuitry believed to underlie the disease itself. Extension to amphetamine withdrawal model or subchronic PCP model [63–66] that are known to induce more permanent functional alteration at the brain circuitry level would yield valuable data when examining the impact of molecular interventions. In addition, combining genetic models with early environmental interventions, such as maternal infection [67,68], isolation rearing or early life stress [69,70] can also assess the potential drug target in terms etiological mechanisms and prevention of disease progression, as well as shedding lights on critical gene–environmental interactions.

4. Psychopharmacological evaluation of GlyT1 inhibitors in animal models

The therapeutic potential of GlyT1 inhibitors for schizophrenia pharmacotherapy was first suggested in the 1990s based on the reported ability of the GlyT1 inhibiting glycine analogue glycyldodecylamide (GDA) to antagonize PCP induced hyperactivity more efficiently than glycine itself [71,72]. As a result, the pharmaceutical industry has developed a variety of selective and high-affinity GlyT1 inhibitors currently at various stages of evaluation [73–76]. As a proof-of-mechanism on the cellular level, several GlyT1 inhibitors have been shown to elevate glycine levels in the brain [e.g., 27,62,77], potentiate NMDAR-mediated transmission and synaptic plasticity [e.g., 78–80], and antagonize the behavioural effects of NDMAR antagonists in animals (Tables 2–4). In addition, a large body of preclinical evidence was reported on antipsychotic and pro-cognitive effects of various compounds (Tables 2 and 3). With the advent of a novel assay for detection of psychoactive GlyT1 inhibitors (prevention of glycine-B site antagonist-induced hyperlocomotion such as RO4840695 and RO4543338 [81]), the pace of drug discovery will surely be hastened in the near future.

4.1. Effects on learning and memory

GlyT1 inhibitors have been shown to affect learning and memory across multiple cognitive domains. Several compounds (NFPS, PF-3463275, SSR103800, and SSR504734) effectively reversed cognitive deficits induced by acute or neonatal NMDAR antagonist treatment including impairments on reference memory, object as well as social recognition memory, and working memory (see Table 2). This might predict antipsychotic efficacy against the cognitive dimension of schizophrenia given that similar cognitive deficits are consistently seen in schizophrenia such as impaired visual object memory, face recognition memory and working memory [44,82]. In addition, NFPS and SSR504734 were able to enhance social recognition and working memory in non-perturbed animals [83,84], thus extending the use of GlyT1 inhibitors as potential cognitive enhancers for non-clinical purposes. Intra-amgydalar injection of NFPS also facilitated conditioned fear extinction learning in a potentiated startle paradigm [85]. This effect is reminiscent of the effects of forebrain neuronal GlyT1 deletion on conditioned fear expression (see Section 2 and Fig. 1). Such an acceleration of fear reduction might be beneficial to treat anxiety disorders and GlyT1 inhibitor treatment in conjunction with exposure therapy has been proposed as therapeutically beneficial in post-traumatic stress disorder [85]. Anxiolytic effects have also been reported for SSR504734 which reduced ultrasonic distress calls in maternally separated rat pups (see Table 5). However, the same drug was ineffective in the standard elevated plus maze test of anxiety [86],

Table 2
Summary of the effects of GlyT1 inhibitors on learning and memory functions.

Learning and memory					
Paradigms	Effects	Compound	Dose-range	Species	Reference
Associative learning	Enhancement of fear extinction learning in the potentiated startle paradigm	NFPS	Intra-amgydalar (5 μg/side)	Rat	[85]
	Reduction of acquisition and expression of contextual conditioning	SSR504734	3–30 i.p.	Rat	[87]
Working memory	Enhancement of continuous delayed alternation in normal animals	SSR504734	3–10 i.p.	Mouse	[84]
	Reversal of ketamine-induced deficits on a delayed response task	PF-3463275	0.01–0.17 s.c	Rhesus monkey	[88]
	No effect on MK-801-induced working memory deficits in the radial arm maze	NFPS	10 s.c.	Rat	[89]
Reference memory	Reversal of impaired spatial reference memory following MK-801 pre-treatment	NFPS	10 s.c	Rat	[89]
Recognition memory	Enhancement of social recognition memory in normal animals	NFPS	0.1–1 i.p.	Rat	[83]
	Reversal of impaired social recognition memory in the neonatal PCP model	SSR103800	0.1–10 p.o	Rat	[62]
		SSRS04734	1–10 i.p.	Rat	[77,90]
	Reversal of MK-801-induced impairments on object recognition memory	NFPS	0.1–1 i.p.	Rat	[83]
	Reversal of MK-801-induced impairments on object recognition memory	NFPS	0.3–1 i.p.	Rat	[91]
	Reversal of impaired object recognition memory induced by subchronic PCP	NFPS	0.3–3 i.p. per day for 2 weeks	Mouse	[92]
	Reversal of impaired object recognition memory in PCP-sensitized animals	SSR103800	3 p.o.	Rat	[62]

indicating that the possible regulation of anxiety-like behaviour by GlyT1 remains to be further clarified. Nevertheless, the anxiolytic-like or fear-reducing effects of SSR504734 might have contributed to the rather intriguing finding that this drug reduced contextual fear conditioning following exceptionally distressful electric shocks in successions (2.5 mA for 30 s [87]) – a finding contrary to the effect seen in the genetic model of GlyT1 deletion in forebrain neurons (see Section 3).

4.2. Effects on attentional processes

As mentioned in Section 3, schizophrenia-related dysfunction in attention is typically evaluated using the LI and PPI paradigms, and PPI disease models in animals are commonly used as preclinical screen for antipsychotic drug activity [55,93,94]. GlyT1 inhibitors have exerted PPI-corrective efficacy in various PPI deficiency models including acute NMDAR antagonism, neonatal PCP treatment, prenatal hippocampal lesion, and naturally low levels of PPI in the DBA mouse strain (see Table 3). By contrast, Lipina and colleagues [95] have showed that ALX-5407 (also known as 'NFPS', i.e., N[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine) dose-dependently attenuated PPI in normal C57BL/6 mice – a finding that is consistent with the observation of reduced basal PPI in forebrain neuronal GlyT1 knockout mice ([41]; see Section 3). This may imply a negative impact of GlyT1 inhibition on PPI under non-challenged conditions, since sustained elevation of CNS glycine levels by a glycine-rich diet was also reported to disrupt PPI in rats [96]. A possible link between increased glycine levels and PPI disruption is further supported by clinical data showing a negative correlation between CNS glycine

Table 3
Summary of the effects of GlyT1 inhibitors on attentional functions by the latent inhibition (LI) and prepulse inhibition (PPI) paradigms.

Attentional processes					
Paradigms	Effects	Compound	Dose-range	Species	Reference
Early sensory gatting: PPI	Reduction of baseline PPI in normal animals	ALX-5407	1–10 i.p.	Mouse	[95]
	Partial reversal of PPI deficiency induced by neonatal lesion of the ventral hippocampus	ORG 24598	3–10 i.p.	Rat	[104]
	Enhancement of PPI in PPI-deficient mGluR5 knockouts	Sacrosine	100–300 i.p.	Mouse	[105]
	Improvement of innate PPI deficiency in the DBA mouse strain	ALX-5407	3–20 i.p.	Mouse	[106]
		Merck (S)	3–10 i.p.		
		Roche-7	10–30 i.p.		
		Sacrosine	300–3000 i.p.		
		NFPS	1–10 i.p	Mouse	[80]
		SSR103800	3–30 i.p.	Mouse	[62]
		SSR504734	15–30 i.p.	Mouse	[77]
	Reversal of ketamine-induced PPI deficits	Sacrosine	100 i.p.	Rat	[107]
	Reversal of MK-801-induced PPI deficits	ALX-5407	1 i.p.	Mouse	[95]
Learned inattention: LI	Enhancement of LI, and reversal of MK-801-induced abnormally persistent LI in the conditioned emotional response (CER) paradigm	ALX-5407	1 i.p.	Mouse	[95]
		SSR504734	1–10 i.p.	Rat	[103]
	Enhancement of LI, and reversal of both amphetamine-induced LI disruption and MK-801-induced persistent LI in the CER paradigm	SSR103800	1–10 i.p.	Rat	[103]

Compound ALX-5407 is structurally identical to (+)-NFPS (i.e., N[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine). The term 'NFPS' typically refers to the racemate of both enantiomers (+/−)-NFPS (see [80]), but also interchangeably with 'ALX-5407' by some authors (e.g., [108]). In this review, we adopted our terminology in accordance with the relevant references.

Table 4
Summary of the motor effects of GlyT1 inhibiting drugs, and their interaction with psychostimulant drugs.

Locomotor activity and psychostimulant-induced motor effects					
Paradigms	Effects	Compound	Dose-range	Species	References
Locomotor activity	Enhancement of baseline locomotor activity	ALX-S407	3–10 i.p. 10–30 p.o.	Mouse Rat	[106] [109]
		Merck (S)-13 h LY236S109	3–10 i.p. 3–10 p.o.	Mouse Rat	[106] [109]
	Reduction of baseline locomotor activity	Roche-7 Sarcosine	30–300 i.p. 1000–6000 i.p.	Mouse	[106]
	No effect on baseline locomotor activity	SSS504734	3–10 i.p.	Mouse	[86]
	Failure to reverse the hyperlocomotor effect of amphetamine	NFPS	2–30 i.p.	Rat	[118]
	Rerversal of amphetamine-induced hyperlocomotion	Org 24461	2–30 i.p.	Rat	[118]
	Exacerbation of amphetamine-induced hyperlocomotion	S5R504734	3–10 i.p.	Mouse	[86]
	Locomotor activity Reversal of motor hypersensitivity to amphetamine following neonatal PCP	SSH504734	0.1–3 i.p.	Rat	[77]
	Reversal of MK-801-Induced hyperlocomotion	SSR103800 SSR504734	10–30 p.o. 3–30 p.o. 3–10 i.p.	Mouse Mouse	[62,119] [77]
	Reversal of PCP-induced hypo-locomotion	GDA NFPS Org 24461 SSR504734	10–100 i.p. 100 i.p. M0 if 2–30 i.p. 3 p.o. 3–10 i.p.	Mouse Rat Rat Mouse	[71] [118] [118] [62] [86]
	Exacerbation of apomorphine-induced hyper-locomotion	SSR504734	3–10 i.p.	Mouse	[86]
Climbing and stereotypic behaviour	No effect on apomorphine-induced climbing and stereotypy	NFPS Org 24461	10 p.o.	Rat	[118]
Motor coordination	Reduced motor performance on the rotarod	AUS4O7 LY2365109	10–30 p.o.	Rat	[109]

concentration and PPI performance in schizophrenic patients [97]. Thus, ambient glycine concentration appears to be critical in the regulation of PPI in both healthy subjects and schizophrenia patients but the underlying mechanisms are currently largely unknown.

In schizophrenia patients, the abnormal expression of LI can assume one of two forms: LI can be reduced in comparison to healthy controls (see Section 3) or it can be abnormally persistent in spite of manipulations that would minimize LI expression in controls [98]. While the LI disruption is thought to reflect an inability to ignore irrelevant stimuli, abnormally persistent LI has been linked to impairment in shifting attention [98]. These are hypothesized to be linked to the positive and cognitive/negative symptoms of schizophrenia, respectively. Accordingly, amphetamine-induced LI disruption is a disease model of positive symptoms [99–102] whereas MK-801-induced persistent LI represents a model of negative symptomatology [57,102]. The ability of a compound to reverse these drug-induced LI aberrations is taken as indication for therapeutic potential against positive and negative symptoms, respectively. Amongst the GlyT1 inhibitors evaluated in these LI models, SSR504734 and SSR103800 reversed MK-801-induced persistent LI [95,103], while SSR103800 additionally countered the LI-disruptive effect of amphetamine. Thus, SSR103800 has been suggested to be effective against positive as well as negative/cognitive symptoms of schizophrenia. Furthermore, both drugs potentiated LI in non-drugged animals suggesting that GlyT1 inhibition may also alter attention in healthy subjects. Few attempts thus far have been made to examine the

Table 5
Summary of the effects of GlyT1 inhibiting drugs in anxiety-like and depression-related paradigms.

Emotional and affective mental processes					
Paradigm	Effects	Compound	Dose-range	Species	Reference
Depressive-related behaviour	Reduction of immobility time in the forced-swimming test	SSR103800	0.3–3 mg/kg p.o.	Rat	[62]
	Reduction of tonic immobility defensive- and despair-related behaviors in the tonic immobility test	SSR103800	1–30 mg/kg p.o.	Gebril	[62]
Anxiety-related behaviour	Reduction of ultrasonic vocalization distress calls in rat pups	SSR504734	1–10 mg/kg s.c.	Rat	[71]
	No effect on fear of elevated open places in plus maze test	SSR504734	3–10 mg/kg i.p.	Mouse	[84]
Alcoholism	Reduction of alcohol intake and preference	Org 25935	6 mg/kg/day i.p. chronic	Rat	[122]
	Reduction of compulsive and relapse-like alcohol drinking	Org 25935	3–6 mg/kg/day i.p. for 5	Rat	[123]
Neuropathic pain	Anti-nociceptive effects in a chronic construction injury model	ALX 5407	10–100 μg i.t.	Rat	[125]
	Antiallodynia effects in different neuropathic pain models	ORG25935 Sarcosine	3–300 ng i.t. or l0 μg–1 mg/kg i.v.	Mouse	[126]
	Reduction of thermal and mechanical hypersensitivity in the Seltzer and streptozotocin-injected diabetic pain models	NFPS Sarcosine	3–300 ng i.t. 3–300 ng i.t.	Mouse	[127]
Seizures	Raising the electroshock-induced seizure threshold in the maximal electroshock threshold (MEST) test	NFPS SSR 504734 Lu AA21279 SB-710622 Org 25925 GSK931145	3.2–32 mg/kg i.p. 10–30 mg/kg i.p. 10–30 mg/kg i.p. 3.2–32 mg/kg i.p. 1–30 mg/kg i.p. 0.1–10 mg/kg i.p.	Rat	[128]
	Raising the threshold of tonic seizure in the pentylenetetrazole (PTZ) i.v. infusion test	Sarcosine	400–800 mg/kg i.p.	Mouse	[129]

effects of GlyT1 inhibition in other attentional domains, such as sustained attention or vigilance, which are also known to be seriously affected in schizophrenia, yet they are clearly warranted.

4.3. Effects on spontaneous and psychostimulant-induced motor behaviour

At higher doses, GlyT1 inhibitors can produce motor and respiratory deficits which are lethal as exemplified by homozygous GlyT1 knockout mice (see Section 2). These adverse effects are likely due to increased glycine levels at inhibitory GlyRs located in the brainstem, cerebellum and spinal cord [109,110]. Therefore some safety concerns have been raised concerning the clinical use of GlyT1 inhibitors. It has been hypothesized that the severity of these effects depends on the chemotype and/or the mode of inhibition, such that irreversible, sarcosine-based compounds were proposed to exert the most severe side effects [109]. This hypothesis has been questioned by a more recent study showing that induction of motor dysfunction, as measured by locomotor hyperactivity, was independent of chemotype and mode of inhibition [106] (see also Table 4). A recent study [106] reported that the target residence time, i.e., the dissociative half-life of the compound–target complex, seems to be the key mechanistic determinant. It was found that compounds with a short residence time such as Roche-7 did not produce locomotor hyperactivity. Importantly, Roche-7 retained the PPI-enhancing effect when evaluated in the DBA mouse strain with intrinsic low-PPI function (Table 3), suggesting that this drug's short residence time does not weaken its antipsychotic potential. Nevertheless, residence time seems to be a critical factor of current interest for the separation of toxic and therapeutic effects of GlyT1 inhibitors.

Regardless of chemotype and mode of inhibition, GlyT1 inhibitors reliably antagonize the motor-stimulant effect of NMDAR antagonists such as MK-801, PCP and ketamine, and such efficacy is thus a consistent effect of this class of drugs (Table 4). NMDAR antagonist-induced hyperlocomotion has been extensively used as a pharmacological model of the positive and negative symptoms of schizophrenia [111,112]. However, existing studies evaluating the efficacy of GlyT1 inhibitors to antagonize the motor-stimulant effect of amphetamine – a pharmacological model considered to approximate more closely positive symptoms attributable to dopaminergic hyper-function [113,114], yielded mixed outcomes. Org 24461 and SSR504734 were found to reverse and exacerbate the amphetamine effect on activity, respectively, while NFPS was reported to have no effect (see Table 4). This inconsistency might reflect a differential impact on the dopaminergic neurotransmitter systems between different types of GlyT1 inhibitors. SSR504734 has been shown to increase extracellular dopamine levels in the prefrontal cortex [77] and facilitate glutamate-dependent dopamine release in the nucleus accumbens without affecting basal levels of accumbal dopamine [115]. It is not known whether these effects are unique to SSR504734 and might even contribute to the pro-cognitive effects of this drug given the beneficial effects of dopamine releasers against some cognitive deficits of schizophrenia [116,117]. However, the potential risk of an exacerbation of the psychotic symptoms by SSR504734 has not been supported in other animal models of dopaminergic dysfunction. On the contrary, SSR504734 has been reported to reduce the motor hypersensitivity to amphetamine induced by neonatal PCP treatment, reverse amphetamine-induced LI disruption [103] and potentiate the hypo-locomotor effect of apomorphine, all of which exemplify its antipsychotic potential [86] (see Tables 3 and 4). Further investigation of the impact of GlyT1 inhibitors on the dopaminergic system would certainly be very instructive in clarifying the contribution of any interaction with dopamine to their antipsychotic and/or pro-cognitive potential.

4.4. Therapeutic effects beyond schizophrenia

As summarized in Table 5, GlyT1 inhibition has also been indicated in conditions beyond the control of schizophrenia symptoms. GlyT1 inhibitors might exert some beneficial effects in anxiety disorders and major depression as supported by the anxiolytic and anti-depressive profiles of SSR103800 and SSR504734 in animal models [62,77]. A more systematic evaluation would be highly desirable especially because of the possible comorbidity between depression and schizophrenia [120,121]. Another suggested field of application is alcoholism. Recent data suggest that repeated administrations of the GlyT1 inhibitor Org25935 decreases alcohol consumption and preference [122] and reduces compulsive and relapse-like alcohol drinking in rats [123]. Such effects might be further linked to the effects of GlyT1 down-regulation on learning and memory functions, e.g., extinction learning, as reviewed above. In addition, recent data suggested an involvement of GlyT1 in the regulation of pain signal transmission in the spinal cord and several GlyT1 inhibitors have been shown to be anti-nociceptive in animal models of neuropathic pain [124]. Lastly, GlyT1 inhibitors have also been shown to increase seizure threshold and are therefore potentially anticonvulsive [128,129]–an effect that is likely attributable to the facilitation of glycinergic neuronal inhibition.

5. Conclusions

The extensive characterization of the GlyT1 mutant mouse models available to date and the diversity of pharmacological studies on GlyT1 inhibitors *in vivo*, identify GlyT1 as a control element of multiple behavioural and cognitive functions that are related directly or indirectly to schizophrenia symptoms. The premise behind such manipulations of GlyT1 is to enhance activity-dependent NMDAR function. It should however not be overlooked that GlyT1 inhibition may also affect glycinergic inhibition. Although GlyT2 is more strongly linked to the neuronal re-uptake of glycine in glycinergic synapses [28,29,130,131], astrocytic GlyT1 is also relevant in the control of glycinergic inhibitory activity as demonstrated in *in vitro* studies [110,132]. *In vivo*, overall deletion of GlyT1 in glia but not in neurons, caused severe motor impairment in the first postnatal week which was attributed to excessive glycinergic inhibition [37]. Indeed, the balance between potentiation of NMDAR-mediated neuronal excitation and possible potentiation of glycinergic inhibition by GlyT1 inhibiting drugs may critically determine the final behavioural outcomes. Future, region-specific mutant mouse lines would be highly instructive in further delineating the control of specific behaviour. Pharmacological assessment of existing GlyT1 inhibiting drugs highlighted the possibility that GlyT1 might also be a drug target to ameliorate depression and obsessive compulsive disorder [62], seizures [128,129] neuropathic pain [124], in addition to the expected positive effects on learning and memory. The continued development of conditional GlyT1 knockout mouse models in combination with novel GlyT1 inhibitors are expected to unravel the full potential of this therapeutic strategy for mental disorders. This approach is further complemented by alternative pharmacological strategies to indirectly modulate glycine-B site activation [86,87], for example, with its partial agonist cycloserine which was effective in modifying Pavlovian learning [133,134], and by focusing on the other endogenous ligand of the glycine-B site, D-serine, which enjoys superior specificity for the glycine-B site without any cross-over effect on strychnine-sensitive GlyRs. Manipulation of the regulatory enzyme of D-serine, D-amino acid oxidase, can similarly modulate glycine-B site occupancy and therefore affect NMDAR function and associated behaviours. It is conceivable that coordinated polytherapy targeting on related

mechanisms may maximize therapeutic potential and minimize unwanted side effects and toxicity.

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