Glycine Transporters and Its Coupling with NMDA Receptors

Francisco Zafra, Ignacio Ibáñez, David Bartolomé-Martín, Dolores Piniella, Marina Arribas-Blázquez, and Cecilio Giménez

Abstract Glycine plays two roles in neurotransmission. In caudal areas like the spinal cord and the brainstem, it acts as an inhibitory neurotransmitter, but in all regions of the CNS, it also works as a co-agonist with L-glutamate at N-methyl-D-aspartate receptors (NMDARs). The glycine fluxes in the CNS are regulated by two specific transporters for glycine, GlyT1 and GlyT2, perhaps with the cooperation of diverse neutral amino acid transporters like Asc-1 or SNAT5/SN2. While GlyT2 and Asc-1 are neuronal proteins, GlyT1 and SNAT5 are mainly astrocytic, although neuronal forms of GlyT1 also exist. GlyT1 has attracted considerable interest from the medical community and the pharmaceutical industry since compelling evidence indicates a clear association with the functioning of NMDARs, whose activity is decreased in various psychiatric illnesses. By controlling extracellular glycine, transporter inhibitors might potentiate the activity of NMDARs without activating excitotoxic processes. Physiologically, GlyT1 is a central actor in the cross talk between glutamatergic, glycinergic, dopaminergic, and probably other neurotransmitter systems. Many of these relationships begin to be unraveled by studies performed in recent years using genetic and pharmacological models. These studies are also clarifying the interactions between glycine, glycine transporters, and other co-agonists of the glycine site of NMDARs like D-serine. These findings are also relevant to understand the pathophysiology of devastating diseases like schizophrenia, depression, anxiety, epilepsy, stroke, and chronic pain.

Keywords Glycine • Transport • Glutamate • NMDA receptors • Astrocytes • Schizophrenia • GlyT1

F. Zafra (✉) • I. Ibáñez • D. Bartolomé-Martín • D. Piniella
M. Arribas-Blázquez • C. Giménez
Centro de Biología Molecular Severo Ochoa, Facultad de Ciencias,
Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid,
C / Nicolás Cabrera, 1, 28049 Madrid, Spain

Centro de Investigación Biomédica en Red de Enfermedades Raras and IdiPAZ,
Instituto de Salud Carlos III, Madrid, Spain
e-mail: fzafra@cbm.csic.es

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List of Abbreviation

AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
GlyR	Glycine receptor
GlyT1	Glycine transporter-1
GlyT2	Glycine transporter-2
LTP	Long-term potentiation
NFPS	(±)-N-[3-(4′fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine
NMDA	N-methyl-D-aspartate
NMDAR	NMDA receptor
SCZ	Schizophrenia
SNAT	Sodium-coupled neutral amino acid transporters

1 Introduction

A simple search in PubMed database reveals that one subtype of glutamate receptor, the NMDA receptor (NMDAR), has maintained a remarkable interest for the last 25 years, accounting for about 40,000 references. This is more than double the interest aroused altogether by the two other subtypes of ionotropic glutamate receptors (AMPA and kainate receptors) and quadruplicates the number of references to metabotropic glutamate receptors. The reason for this decided interest may be related to the fact that NMDARs are located at a crossroads where fast excitatory neurotransmission converges with plasticity processes that are on the basis of extraordinary brain properties such as learning, memory, and cognition (Nakazawa et al. 2004; Collingridge et al. 2013). This strategic situation makes NMDARs not only hubs for physiology but also for pathology. Thus, acute overstimulation of NMDARs during ischemic stroke initiates the so-called excitotoxic process that ends up in neuronal death. Chronic excitotoxicity has been hypothesized to play a role in numerous neurodegenerative diseases including Alzheimer's disease, Huntington's disease, or amyotrophic lateral sclerosis. In addition, hypofunction of NMDARs seem to be detrimental, and evidence accumulates that this is associated to schizophrenia (SCZ) and other psychiatric diseases. Accordingly, these receptors have concentrated a lot of effort to develop drugs that might modulate their activity.

2 NMDAR Subunits and the Glycine Binding Site

The atomic structure of NMDAR has been recently resolved at 4 Å resolution (Karakas and Furukawa 2014; Lee et al. 2014). NMDARs are obligatory heterotetramers mainly composed of two GluN1 subunits and two GluN2 and/or GluN3

subunits. There are eight different splice variants of the GluN1 genes, four GluN2 genes (GluN2A-D), and two GluN3 genes (GluN3A-B) (for a review, Paoletti et al. 2013). The subunit composition varies regionally and developmentally. For instance, GluN2A and GluN2B subunits are especially relevant in the adult forebrain and present different function and distribution in neurons. In general GluN2B is expressed at both synaptic and extrasynaptic sites, whereas GluN2A is mainly expressed at the synapse (Lavezzari et al. 2004; Papouin et al. 2012). This subunit composition undergoes developmental variations, and, as the brain develops, synaptic NMDARs containing GluN2A subunits are targeted to synapses, and those with GluN2B subunits, which dominate in the neonatal brain, are displaced to extrasynaptic sites (Williams et al. 1993; Sheng et al. 1994). However, at least in the developing brain, this distribution is dynamic and is subjected to modifications by synaptic activity. For instance, NMDARs containing GluN2B are redistributed away from glutamate synapses through increased lateral diffusion during LTP in immature neurons (Dupuis et al. 2014). Opening of NMDAR channels involves the relief of the Mg^{2+} blockade of the ionic pore by membrane depolarization and results in an influx of calcium ions that activates diverse signal transduction cascades that control strength of neural connectivity or neuroplasticity.

A unique property of NMDARs among ionotropic receptors is that opening the channel requires the presence of a co-agonist. While glutamate binds to a bilobulated cavity located in the extracellular domain of GluN2 subunits, the other ligand, that was identified as glycine by Johnson and Ascher (1987), binds to a similar cavity located in GluN1 or GluN3 subunits (called the glycine-B site, as opposed to the glycine-A site on the strychnine-sensitive glycine receptor GlyR). However, the physiological role of the glycine-B site was largely controversial because the affinity for glycine is very high (in the low micromolar range), and it was thought that the site would be tonically saturated at the concentrations of glycine assumed to be present in the synaptic cleft (Kemp et al. 1988). Nonetheless, subsequent studies revealed that it is not necessarily the case, supporting the existence of subpopulations of NMDARs regulated by glycine in vivo. This is probably dependent on several different factors. Firstly, there are a number of transport systems for glycine that precisely control the glycine concentration in the synaptic and the perisynaptic space (Berger et al. 1998; Bergeron et al. 1998; Li et al. 2009; Wilcox et al. 1996; Chen et al. 2003). Neurons and glial cells express different glycine transporters that are located strategically and regulate the concentration of glycine in the neighborhood of NMDARs in a dynamic manner. Among them, glycine transporter GlyT1 might play a pivotal role, but there are also a number of low-affinity transporters for neutral amino acids, like Asc-1 or SNATs, that might contribute significantly to the process. Secondly, NMDARs have a heterogeneous subunit composition, which determines the affinity for glycine as well as the subcellular localization and developmental stage of the synapses. Heterodimeric NMDARs containing GluN2B subunits display ten times higher affinity for glycine than those containing the GluN2A subunits (EC_{50} ~0.1 μM and 1 μM, respectively). Occupancy of the glycine binding site not only governs the opening of the channel but also increases the affinity of the receptor for glutamate (glutamate and glycine sites are allosterically coupled) and

modulates the receptor function by decreasing its desensitization (Mayer et al. 1984; Lester et al. 1993). In addition, glycine primes NMDARs for endocytosis thereby controlling the levels of the receptor in the cell surface (Nong et al. 2003). Further complexity is added by the fact that D-amino acids, especially D-serine, are almost as effective as glycine in activating the receptor by binding to the glycine-B site (Kleckner and Dingledine 1988; Mothet et al. 2000; Yang et al. 2003; Panatier et al. 2006; Henneberger et al. 2010). Immunohistochemical localization of D-serine demonstrated that this amino acid is mainly localized in astrocytes and that its distribution matches quite extensively with the expression of NMDARs (Schell et al. 1995), thus raising the possibility that D-serine could be the physiological ligand of the glycine-B site. In fact, some studies suggested that D-serine would be the preferred ligand (Mothet et al. 2000). However, subsequent reports have come to draw a more complex picture, where it seems to emerge a regional and temporal differentiation in the preference for one or the other ligand. The extracellular levels of D-serine in the CNS are controlled by two members of the SLC1 family, the Na^+-dependent alanine-serine-cysteine transporters 1 and 2 (ASCT1 and ASCT2) (Martineau et al. 2014). These are expressed by both astrocytes and neurons. Additionally, D-serine can be transported by the neuronal Na^+-independent antiporter alanine-serine-cysteine-1 (Asc-1), a member of the SLC7 family, that can work in the reverse manner to release D-serine and glycine from neurons (Fukasawa et al. 2000; Helboe et al. 2003, Rosenberg et al. 2013). D-serine is also released from astrocytes via mechanisms implicating Ca^{2+} and SNARE-dependent exocytosis (Yang et al. 2003; Henneberger et al. 2010; Martineau et al. 2013) and sustained by the activity of a glia-specific vesicular transporter for D-serine (Martineau et al. 2013; although see Agulhon et al. 2010). Thus, an open question is how dynamics are the fluxes of D-serine in the neighborhood of NMDARs and how they compare with those of glycine to cooperate/compete in determining the responses of the receptor. Glycine appears to be the preferred co-agonist in receptors containing GluN2B subunits, while it would be D-serine for those containing GluN2A (Fossat et al. 2012; Papouin et al. 2012; Le Bail et al. 2015). This might explain the developmental change in the ligand preference described in the connection between the Schaffer collaterals and CA1 pyramidal neurons, turning from glycine to D-serine in parallel to the replacement of GluN2B by GluN2A that occurs between weeks 1 and 3 after birth (Le Bail et al. 2015). Nevertheless exceptions might exist to this rule, like synapses of the hypothalamic supraoptic nucleus that robustly express GluN2B but seem to depend only on D-serine – although inhibitors of glycine transporter were not used in these studies (Panatier et al. 2006; Doherty and Sladek 2011). These observations suggest a complex synapse-specific cross talk between both ligands. As an example, two recent articles show that both, glycine and D-serine, are necessary for induction of LTP in CA1 hippocampal area and dentate gyrus (Le Bail et al. 2015), as well as in the lateral nucleus of the amygdala (LA) (Li et al. 2013). In LA, the prevalence of D-serine or glycine at synaptic NMDARs would be determined by synaptic activity. Ambient D-serine may maintain activation of NMDARs in LA neurons in the absence of evoked synaptic events, while activity-dependent release of glycine from astrocytes is implicated in the activation

of NMDARs during afferent stimulation (Li et al. 2013). In addition, electrophysiological measures both in the inner retina and in the hypoglossal nucleus also suggested that GlyT1 activity keeps glycine levels near NMDARs at sufficiently low concentrations so as to allow D-serine to play a major role as an NMDAR co-agonist (Berger et al. 1998; Stevens et al. 2010). Blockade of GlyT1 with a specific inhibitor increased the extracellular levels of glycine to saturating levels and canceled the effect of added D-serine (Stevens et al. 2010). So, the question is if there are any mechanisms (physiological or pathological) that regulate in a concerted manner the diverse enzymes and transporters that control the fluxes of glycine and D-serine in the synapse to determine the activity profiles of NMDARs (reviewed by Mothet et al. 2015).

3 Regulation of the Extracellular Glycine Concentration by Glycine Transporters

The fluxes of glycine at inhibitory and excitatory synapses are controlled by two glycine transporters, GlyT1 and GlyT2, which belong to the sodium- and chloride-dependent neurotransmitter transporter family and are encoded by genes SLC6A9 and SLC6A5, respectively (Aragón and López-Corcuera 2005). GlyT1 and GlyT2 have different regional and cellular expression patterns in the CNS. Initially, mRNA for GlyT1 was localized at high concentrations in glutamatergic neurons and also in glial cells (Smith et al. 1992; Borowsky et al. 1993; Zafra et al. 1995b). However, early immunohistochemical studies detected the glial protein but failed in recognizing the neuronal forms of GlyT1, probably due to some kind of epitope occlusion of the neuronal protein (Zafra et al. 1995a). The expression in astrocytes is especially high in glycinergic areas, where GlyT1-immunoreactive glial profiles ensheath glycinergic synapses. Its essential role in these synapses is to lower extracellular glycine concentration as shown in GlyT1-deficient mice, where the decay time constant of glycinergic mIPSCs recorded in hypoglossal motoneurons was longer than that in wild-type mice, indicating an increased synaptic concentration of neurotransmitter (Gomeza et al. 2003a). Antibodies developed later allowed the detection of GlyT1 immunoreactivity not only in astrocytes but also in neuronal elements, mainly in glutamatergic terminals along the forebrain. Lower levels of immunoreactivity were also observed in the postsynaptic membrane of asymmetric synapses, forming immunoprecipitable complexes with NMDAR (Cubelos et al. 2005a). As we will discuss later in more detail, this distribution is compatible with a role of GlyT1 in modulating NMDARs. Additionally, GlyT1 has been found not only in the plasma membrane of glutamatergic terminals but also in synaptic vesicles (Cubelos et al. 2014). It is unknown if this localization is related to recent evidence indicating that glycine is released from glutamatergic terminals in hippocampal neurons in a Ca^{2+}-dependent manner upon depolarization (Muller et al. 2013). This release would require an accumulative mechanism of glycine into synaptic vesicles that is

not characterized (VIATT is absent in these terminals). It was suggested that GlyT1 present in vesicular membrane might mediate the accumulation of glycine into the lumen either by a diffusive equilibration process or, even, by some type of uncharacterized active transport (Cubelos et al. 2014). Independently of the Ca^{2+}-dependent release of glycine, presumably vesicular, there is also evidence for a Ca^{2+}-independent release component that might be attributed to the reversal operation of GlyT1 located in the astrocytic or the neuronal membrane (Galli et al. 1993; Luccini et al. 2008). The GlyT1-dependent uptake of glycine is an electrochemical process coupled to the movement of sodium and chloride ions, with a stoichiometry 1 Gly/2 Na^+/1 Cl^-, that under depolarizing conditions might be reverted, allowing the efflux of glycine, either from the glial cells or from the neurons (Roux and Supplisson 2000; Huang et al. 2004; Aubrey et al. 2005). Diverse estimations suggest that GlyT1 activity is not far from equilibrium. Roux and Supplison (2000) calculated, assuming that intracellular glycine is 2 mM in astrocytes (in the hippocampus) and 10 mM in glycinergic neurons, that GlyT1 is close to equilibrium for an extracellular glycine concentration of 100 nM and a resting potential of −70 mV. Non-vesicular glycine release via GlyT1 reversal may occur under high-frequency stimulation (HFS) and probably ischemia, conditions that depolarize the astrocyte. HFS triggers AMPA receptor activation in astrocytes and an increase in intracellular [Na^+], which combined with plasma membrane depolarization may be sufficient to induce the reversion of GlyT1 during HFS (Attwell et al. 1993; Rose and Ransom 1996; Roux and Supplisson 2000; Marcaggi and Attwell 2004; Huang et al. 2004). Similarly, during ischemia, glial cells are depolarized up to −50 mV and intracellular [Na^+] may rise up to 39 mM (Attwell et al. 1993), conditions that might allow the reversion of GlyT1. Indeed, this seems to be the case in hypoxic retina (Hanuska et al. 2016) and in hippocampal brain slices in various cell-damaging conditions (Saransaari and Oja 2001). Although the reversal of GlyT1 under physiological condition has less experimental support, a recent article shows the existence of dopamine-induced release of glycine from cortical astrocytes in primary culture that is blocked by a specific GlyT1 inhibitor (Shibasaki et al. 2016). However, if this also occurs in native tissue is insufficiently documented. As we will discuss later, there is other transporters for glycine that might contribute to glycine release.

The other glycine transporter, GlyT2, is a neuronal protein, associated only to glycinergic neurons in the spinal cord, the cerebellum, and diverse nuclei of the brainstem, like the lateral superior olive, the inferior colliculi, and the dorsal and ventral cochlear nuclei, among others (Zafra et al. 1995a, b; Friauf et al. 1999). Minor populations of glycinergic interneurons immunoreactive for GlyT2 have been also described in the hippocampus (Danglot et al. 2004; Song et al. 2006), supporting the existence of functional glycinergic synapses in this region. Electron microscopy shows that GlyT2 is enriched in presynaptic terminals containing high concentrations of glycine. Within glycinergic boutons, GlyT2 immunostain was associated with the plasma membrane but often appeared as discrete clumps, generally excluded from the region of the active sites of synapses, suggesting that it may

be excluded from synaptic clefts (Spike et al. 1997). Importantly, while most of the transporters of this family have a stoichiometric coupling of two sodium ions transported with every glycine molecule (including GlyT1), GlyT2 is coupled to the electrochemical movement of three sodium ions, favoring the maintenance of a high concentration gradient along the presynaptic membrane and supplying enough glycine for presynaptic vesicle refilling, a process that seems necessary to preserve quantal glycine content in synaptic vesicles (Rousseau et al. 2008; Pérez-Siles et al. 2012; Apostolides and Trussell 2013). GlyT2 activity dysfunctions reduce presynaptic glycine release and cause a significant decrease of inhibitory glycinergic neurotransmission that results in spasticity (James et al. 2012). Nevertheless, some evidence suggests that GlyT2 also participates in reuptake of glycine from the synaptic cleft, and the blockade of the transporter function could enhance glycinergic inhibitory neurotransmission in some situations, although to a lesser extent than GlyT1: for instance, the pharmacological blockade of GlyT2 in lamina X neurons of rat spinal cord slices increases glycinergic neurotransmission in the spinal cord (Bradaïa et al. 2004). Similarly, the GlyT2 inhibitor Org 25543 increases the extracellular glycine concentration, as detected by microdialysis perfusion of the lumbar dorsal spinal cord of rats (Whitehead et al. 2004), suggesting that in caudal regions of the CNS, glial GlyT1 and neuronal GlyT2 closely cooperate in the regulation of extracellular glycine at inhibitory synaptic sites.

Together, these studies indicate that neuronal GlyT1 has an optimal distribution to regulate the binding of glycine to NMDARs in forebrain regions, while GlyT2 and the glial GlyT1 are better situated to participate in inhibitory glycinergic neurotransmission in caudal areas. Nevertheless, in these caudal regions (and probably other areas), where there is a coexistence of neurons expressing NMDAR and GlyR, this division of labor cannot be so strict. It is known the existence of a cross talk is between these receptors. Glycine released from glycinergic terminals might reach nearby glutamatergic synapses by spillover under some circumstances, overcoming the barrier imposed by glycine transporters to the diffusion of the neurotransmitter (Ahmadi et al. 2003). Normally, glial processes surrounding glutamatergic terminals also contain high levels of GlyT1. These transporters are properly positioned to allow control of the glycine fluxes and NMDA-mediated neurotransmission. Reverse transport of glycine through the glial GlyT1 might also play a role at inhibitory synapses. It has been hypothesized that at times of low-synaptic activity, glycine concentration in the synaptic cleft would decrease allowing the reversal operation of GlyT1 and the release of glycine which is taken up by GlyT2 on postsynaptic neurons for packaging into vesicles (Aubrey et al. 2005). However, the rigorous testing of these hypotheses and an accurate measurement of glycine fluxes between glia and neurons clearly require new tools that enable real-time determination of the oscillations in the concentration of glycine in synaptic and extrasynaptic sites in response to different physiological or pathological stimuli. Examples of these tools might be fluorescent probes with similar characteristics to those already available to measure glutamate fluxes (Marvin et al. 2013).

4 Pharmacological and Genetic Models to Study GlyT1 Function

4.1 GlyT1 Inhibitors

The above-described localization experiments suggested a number of potential roles for glycine transporters. However, as it has been pointed in the preceding paragraphs, definitive proofs about the real physiological meaning of these proteins were only obtained after developing specific inhibitors and genetically modified mice. Of course the idea of developing compounds that increase the availability of glycine at glutamatergic synapses (and perhaps at glycinergic ones) was appealing since it might provide novel therapeutic avenues to treat cognitive impairments in a number of psychiatric conditions and perhaps be relevant in the treatment of pain, epilepsy, or even be useful in enhancing specific cognitive functions in healthy subjects. In theory, these compounds would have lower excitotoxicity than direct NMDAR agonists, and, moreover, they may offer greater spatial and temporal selectivity, since selective inhibition of GlyT1 may result in potentiation of NMDAR only in those specific brain regions activated by social stimuli and cognitive challenges where synaptically released glutamate and pharmacologically increased glycine meet each other. Nevertheless, a potential drawback of this strategy is the enhancement of the inhibitory glycinergic neurotransmission with affectation of motor and sensorial pathways regulated by GlyRs. Even so, there are situations where the stimulation of GlyRs could be therapeutically favorable as might be the case for treating neuropathic pain.

A detailed and updated review about GlyT1 pharmacology has been published recently (Cioffi and Guzzo 2016). In brief, early work had shown that sarcosine (N-Methyl-glycine) inhibits the high-affinity glial transporter of glycine (Zafra and Giménez 1989), and, later, Liu et al. (1993) showed that this compound inhibits GlyT1 but not GlyT2. Thus, sarcosine was used as a lead compound to develop the first generation of GlyT1 inhibitors. These include compounds like (±)-N-[3-(4′fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS) (the R-enantiomer is named ALX5407) that showed a non-competitive binding with high affinity and slow dissociation, leading to prolonged elevation in synaptic glycine concentrations that were able to activate GlyRs and probably were the cause of decreased motor and respiratory activity when administered in vivo to rodents. Several sarcosine derivatives were developed by Organon (Org 24461, Org 24598, and others), Pfizer (N-[3-phenyl-3(4′-(4-toluoyl)phenoxy)-propyl]sarcosine or NPTS), Lundbeck (2-arylsulfanylphenyl-1-oxyalkylamino derivatives), Merck (indandione sarcosine derivatives), and Amgen (benzhydryl piperazine analogue AMG 747), among others. Several of these inhibitors increased glycine levels in the CSF without having the negative motor effects of NFPS but keeping the positive effects in animal models of schizophrenia. Four of them were assayed in clinical trials, being Org 25935 as the most advanced one. In preclinical assays Org 25935 attenuated the scopolamine-induced deficits in the object retrieval/detour task or the ketamine-

induced working memory deficits in monkeys, with an inverted U-shaped dose-response curve indicating that the maximum efficacy is achieved at submaximal occupancy levels (Castner et al. 2014). In humans, it was well tolerated and reduced ketamine-induced psychomimetic and perceptual alterations. However, in clinical trials Org 25935 did not differ significantly from placebo in reducing negative symptoms of SCZ or improving cognitive functioning when administered as adjunctive treatment to atypical antipsychotics (Schoemaker et al. 2014).

After the sarcosine derivatives, a second generation of non-sarcosine-based compounds was developed by several companies, and some of them entered into clinical trials for SCZ. Chemically, they can be divided into several categories: methylphenidate-derived (SSR504734, SSR103800, GSK1018921, GSK931145), alkyl and heteroaromatic substituted sulfonamides and sulfones (ACPPB, DCCCyB, and several others), heteroaryl amides (PF-03463275 and others), benzoylpiperazines (several compounds by Hoffmann-La Roche, including bitopertin), benzoylisoindolines (derived from benzoylpiperazines), and several others (Cioffi and Guzzo 2016). Most of the assayed compounds in these categories are competitive inhibitors, although bitopertin is a non-competitive one. Thus, although it was suggested that competitive inhibition of GlyT1 might impart pharmacological advantages, the mechanism of action of bitopertin, which is the most advanced compound in clinical trials, questions this hypothesis (Mezler et al. 2008). In general, all these compounds were designed and assayed for improved selectivity for GlyT1 over GlyT2, better water solubility or shorter residence time on the transporter, aspects that were expected to improve their therapeutic outcome.

4.2 Pharmacological Models

The availability of all these compounds has allowed answering the question of whether GlyT1 is capable of controlling the activity of NMDARs. In fact, the results show that this is the case, by controlling both the channel opening and those processes triggered by the gating (e.g., the long-term potentiation, LTP, or the long-term depression, LTD). And this is a general phenomenon throughout many regions of the brain, as evidenced by multiple observations from rat cortical and hippocampal neurons, hypoglossal motoneurons, or spinal cord lamina X neurons. In all cases, blockade of GlyT1 promotes an elevation of glycine levels and this impacts in NMDAR activity (Chen et al. 2003; Kinney et al. 2003; Martina et al. 2004; Lim et al. 2004; Bradaïa et al. 2004; Zhang et al. 2008, 2014). This is finally reflected in an effect on learning and memory in diverse experimental paradigms. Thus, a number of first and second generation of GlyT1 inhibitors reversed many of the cognitive deficits observed in animal models of acute or neonatal NMDAR antagonist treatment (phencyclidine, MK801, ketamine) including impairments on reference memory, object and social recognition memory, and working memory. As we will discuss below, this is especially relevant in the context of psychotic diseases. And also in normal rats, GlyT1 inhibitors improved working memory and social

recognition, suggesting that these compounds might be useful as enhancers of cognitive functions in healthy humans (Singer et al. 2009a).

GlyT1 inhibitors have been also instrumental in clarifying basic mechanism of the brain circuitry. In this sense, two seemingly contradictory functions of glycine and GlyT1, both in excitatory and inhibitory neurotransmission, could be more tightly linked than it could be suspected, in areas where NMDARs and GlyRs coexist, through a GlyT1-mediated control of the overall excitability of neural networks. The link is provided by the magnitude of the glycine concentration in the synaptic cleft. For instance, Zhang et al. (2014) altered the concentrations of glycine by controlling the doses and the time of incubation of hippocampal slices with the GlyT1 inhibitor NFPS. While moderate levels of glycine promoted LTP, higher doses induced LTD. These changes in synaptic plasticity were dependent on trafficking of NMDARs to (LTP) and from (LTD) the membrane. In this way, low levels of glycine seem to act mainly on the glycine-B site of NMDARs to induce LTP by promoting the insertion of NMDAR in the synapse (by uncharacterized trafficking mechanisms). However, when levels of glycine get higher and cross the threshold of the NMDAR, endocytosis mechanism (Nong et al. 2003) and glycine may activate GlyRs. As a result, the inhibitory effect may be stronger than the excitatory one, and the net effect mediated by glycine is a depression of the NMDA response (Zhang et al. 2014). Another consequence in this scenario of a stepwise increase in the concentrations of extracellular glycine is the triggering of homeostatic mechanisms in the neuronal network to avoid runaway excitation after LTP induction. In hippocampal slices this compensatory mechanism was dependent on GlyT1 located in glial cells since fluoroacetate, a glia-specific metabolic inhibitor, blocked the effect as also did strychnine, indicating its dependence on hippocampal GlyRs (Zhang et al. 2008). Similarly, during the induction and expression of NMDAR-dependent LTP in pyramidal neurons of the visual cortex, GlyT1 controls the activity of extrasynaptic GlyRs, and this resulted in a shunting inhibition of afferent inputs which thus displayed a depression (a LTD-like effect) at the soma after dendritic integration. In this case, it seems that the NMDAR co-agonist is synaptic D-serine rather than glycine, illustrating again the complex cross talk between D-serine and glycine (Meunier et al. 2016).

The glutamatergic system also interacts at different levels with the dopaminergic system. Recent observations support a role of GlyT1 in this cross talk, evidenced by the GlyT1 inhibitor ACPPB. Using a model of unilateral 6-OHDA-induced lesions that spared the mesoaccumbens projection, ACPPB promoted dopaminergic reinnervation of the dorsal striatum and normalized 6-OHDA-induced lateralization of sensorimotor behavior (Schmitz et al. 2013). Both effects were dependent on the presence of NMDARs in dopamine neurons. An important consequence of these findings is that if functional sprouting could be induced in dopamine axons in areas that are spared from denervation in Parkinson's disease (i.e. the caudate nucleus, medial portions of the putamen, and the nucleus accumbens), GlyT1 inhibitors might point toward future therapeutic treatments for these patients.

However, all these observations rely on the pharmacological manipulation of GlyT1 and other proteins of the system. In view of the important role of this trans-

porter in regulating the excitatory/inhibitory balance of the neuronal networks, the question is whether the activity of GlyT1 can be modulated under physiological or pathological states by endogenous mechanisms. As discussed above, an increase of the glycine level could occur under some pathophysiological states, such as seizure and ischemia. Due to the dissipation of ionic gradients, the activity of GlyTs is downregulated or even reversed in ischemia (Huang et al. 2004; Baker et al. 1991). GlyT1 can also be silenced by certain regulatory factors like Zn^{2+} (Ju et al. 2004) and protons (Aubrey et al. 2000), both of which are stored in transmitter vesicles, and transient changes in extracellular pH or Zn^{2+} occur during synaptic transmission (Qian and Noebels 2005; Krishtal et al. 1987). Also, the rate of glycine reuptake by GlyT1 can be downregulated by intracellular factors and signaling pathways such as arachidonic acid (Zafra et al. 1990; Pearlman et al. 2003), protein kinase C activation (Gomeza et al. 1995; Sato et al. 1995; Fernández-Sánchez et al. 2009), Ca^{2+}/calmodulin-dependent enzymes (Lopez-Colome and Gadea 1999) or GSK3-β (Jimenez et al. 2015). Additionally, the intracellular membrane trafficking of GlyT1 can be regulated by the SNARE protein syntaxin-1A, which decreases the concentration of GlyT1 protein on the plasma membrane (Geerlings et al. 2000), or by interactions with the exocyst (Cubelos et al. 2005b). There are also complex interactions with other neurotransmitters or neuromodulators, like the purinergic system that enhances the activity of GlyT1 (Jimenez et al. 2011). Accordingly, the function of GlyT1 seems to be effectively regulated under physiological and pathological conditions, and, thus, the concentration of extracellular glycine can be tightly controlled. The balance between stimulatory and inhibitory signaling pathways will finally determine the activity of the transporter, and, consequently, the glycine concentration might fluctuate in the synaptic cleft under physiological conditions. Because of this, GlyT1 remains a target of substantial pharmacological interest for intervening in diseases associated with dysfunction of either NMDARs or GlyR.

4.3 Genetic Models

Additional source of information on the physiological and pathological role of glycine transporters are the various genetically modified mice that have been produced for more than one decade, since the leading work of H. Betz and collaborators (Gomeza et al. 2003a, b). These advances have been extensively reviewed by Mohler et al. (2011). Relative to GlyT1, perhaps the most relevant aspect that cannot be addressed by pharmacological models is the dissection of the physiological role of glial and neuronal forms of the transporter. The three initial genetic models were unable to deal with this question since they were obtained by global deletion of the Slc6a9 (GlyT1) gene, and, moreover, they were neonatally lethal (Gomeza et al. 2003a; Coyle and Tsai 2004; Gabernet et al. 2005). However, heterozygous were viable and at least two lines (GlyT1tm1.1$^{+/-}$ and GlyT1$^{+/-\ Tsai}$) displayed altered NMDAR responses that were compatible with the saturation of de glycine-B site when GlyT1 was reduced to about a half, including an enhanced NMDA/AMPA

response ratio, a resistance to the disruptive effect of amphetamine on prepulse inhibition and a tendency for improved memory retention (Tsai et al. 2004b; Gabernet et al. 2005; Martina et al. 2005). Heterozygous mice also show morphologic and physiologic alterations including an increased number of synapses and an enhanced neuronal excitability, changes that might be attributed to the chronic high levels of glycine in glutamatergic synapses (Bakkar et al. 2011).

Later, the first conditional GlyT1 mouse was generated and termed GlyT1tm1.2$^{fl/fl}$, which contained two pLox sites flanking exons 4 and 11 of Slc6a9 (Yee et al. 2006). These floxed mice were bred with transgenic mice containing Cre recombinase under the CaMKII promoter and resulted in the ablation of GlyT1 in forebrain neurons. As a consequence, both the concentration and the activity of GlyT1 decreased by approximately 30% in forebrain at postnatal day 21. The diminished GlyT1-selective glycine uptake was accompanied by an important increase in the NMDA/AMPA response ratio. Another conditional strain designed to suppress the expression of GlyT1 simultaneously in neurons and astrocytes of the forebrain was obtained by breeding the GlyT1tm1.2$^{fl/fl}$ with the Emx1Cre/Cre mice (EMX/GlyT1-KO) (Singer et al. 2009b). The simultaneous disruption of GlyT1 in neurons and glia resulted in a near-complete absence of response to the acute phencyclidine challenge. This suggests that NMDAR function in EMX/GlyT1-KO mice is altered (being more resistant to systemic pharmacological blockade of NMDAR by phencyclidine), presumably due to increased levels of synaptic glycine. This observation is in good agreement with the finding that GlyT1 inhibitors are highly effective in attenuating the motor stimulant effect of NMDAR blockers (Harsing et al. 2003; Depoortere et al. 2005; Boulay et al. 2008; Singer et al. 2009b). Surprisingly, the EMX/GlyT1-KO mice did not show alterations in the NMDA-mediated EPSC in the hippocampus. The reason for this difference with the neuronal model is unclear, but several possibilities were suggested. For instance, a more drastic increase in the extracellular glycine in the neuronal/glial-deficient mice might have primed NMDAR for endocytosis (Nong et al. 2003). Also, a tonic stimulation of inhibitory GlyRs in the glial/neuronal depleted model might alter the activity of hippocampal network, indirectly compensating the NMDAR current alterations observed in the neuronal model (Singer et al. 2009b). Additional differences between these mice were found in their capability for associative learning that was potentiated in the neuronal model but not in the neuronal/glial. Differences were also observed in the working memory paradigms that were not affected in the neuronal model in contrast to the promnesic effects observed in mutant mice with GlyT1 deletion extended to cortical glial cells (Dubroqua et al. 2012), suggestive of an important and specific role of glial cells in regulating cognitive functions. Both models, however, displayed a similarly increased recognition memory in tests for object familiarity judgment. Interestingly this was also observed in a pharmacological model using GlyT1 inhibitors (Depoortere et al. 2005; Boulay et al. 2008; Karasawa et al. 2008). In general, the different models display a wide spectrum of procognitive effects that support the idea that GlyT1 is a promising target for the treatment of cognitive

symptoms in psychotic diseases. An additional conditional strain was developed again in the lab of H. Betz to suppress the expression of GlyT1 in neurons or in astrocytes (Eulenburg et al. 2010). The targeting vector was designed to enable Cre recombinase-mediated inactivation of the GlyT1 gene through deletion of exons 3 and 4, and these mice were bred with two strains of transgenic mice expressing Cre recombinase. One was under the control of the neuron-specific synapsin 1 promoter, the other under the control of the mouse glial fibrillary acidic protein (GFAP) promoter. The neuronal line did not show motor or respiratory deficits, and the authors conclude that in caudal regions of the CNS, neuronal GlyT1 does not contribute significantly to the regulation of inhibitory glycinergic neurotransmission. However, most of the mice of the glial line developed a strong hypotonic phenotype, which finally resulted in premature death between postnatal day 1 and 10, indicating that glial GlyT1 is the major player in regulating glycinergic neurotransmission. Unfortunately there are no reports related to NMDA-dependent behaviors in these mice. Interestingly, few mice of the glial strain survived and developed to adult age, indicating that Glyt1 is essential in the perinatal period but not in adults where perhaps GlyT1 function could be assumed by other glycine transporters.

5 Other Glycine Transporters

The preceding paragraphs summarize an important set of evidence supporting an unquestionable role of GlyT1 in the regulation of NMDARs and GlyRs, especially when GlyT1 operates in the forward direction. Less support has the idea that under physiological conditions GlyT1 might contribute to a fast non-vesicular release of glycine. If this has to occur, glycine might reach glutamatergic synapses by spillover from neighboring glycinergic synapses (Berger and Isaacson 1999; Turecek and Trussell 2001; Ahmadi et al. 2003). However, this might be an alternative in caudal areas of the brain but it seems unlikely in forebrain areas where glycinergic terminals are sparse or absent. A more likely source of glycine would be the reversal operation of a transporter with some electrochemical characteristics better suited to work in the efflux mode. The brain contains several amino acid transporters that belong to the SLC7 and SLC38 family and that might participate in the control of the neuronal-glial fluxes of glycine and other amino acids. Some of them, especially the already mentioned Asc-1 and SNAT5, might fulfill these requirements, showing potential reversion under physiological conditions.

Asc-1 is a plasma membrane antiporter present in neurons that has high affinity for small neutral amino acids, such as glycine, L-serine, D-serine, alanine, and cysteine (Fukasawa et al. 2000; Helboe et al. 2003). It has a widespread distribution throughout the brain, and it is located exclusively in presynaptic terminals. Data obtained with Asc-1 knock-out (KO) mice indicate that this protein is the main D-serine transporter in the brain (Rutter et al. 2007), able to secrete D-serine in an

exchange reaction with other endogenous substrates. In addition, Acs-1 can also extrude glycine affecting NMDAR responses at low-frequency stimulation in hippocampal slices (Rosenberg et al. 2013). However, a recent article reveals that Asc-1 participates mainly in glycinergic transmission in the spinal cord (Safory et al. 2015). The KO mice show a marked decrease in glycine concentrations in the brain and spinal cord along with impairment of glycinergic inhibitory transmission and a hyperekplexia-like phenotype that results in postnatal death, but it is rescued by replenishing brain glycine levels (Xie et al. 2005; Safory et al. 2015). If these KO mice have phenotypes associated to NMDAR, malfunction was not reported, and it is possible that the strong glycine-related phenotype obscures more subtle changes in NMDAR-dependent processes mediated by D-serine or glycine via the Asc-1 transporter.

Another potential mediator of the glycine efflux is SNAT5 (also known as SN2), a member of the gene family SLC38 (Mackenzie and Erickson 2004) that includes diverse transporters for neutral amino acids and notably for glutamine. Some of the members of this family might take part of the glutamate-glutamine cycle between neurons and glial cells. SNAT5 recognizes not only glutamine but also glycine and some other neutral amino acids, including alanine, serine, histidine, or asparagine. Indeed, in vitro studies have shown that glycine is one of the preferred substrates (Nakanishi et al. 2001). Studies on SNAT5 are handicapped by the absence of both, specific inhibitors and animal models, but immunohistochemical and electrophysiological studies indicate that this is a protein with appropriate characteristics to regulate NMDARs. Light and electron microscopy shows that SNAT5 is a glial protein enriched in glutamatergic areas, where immunoreactive processes ensheath glutamatergic terminals (Cubelos et al. 2005c). Moreover, this expression pattern emerges during the postnatal development in parallel to the expression of essential proteins of the glutamatergic system like the vesicular glutamate transporter vGLUT1 and the glial glutamate transporter GLT-1,and to the functional maturation of these synapses (Rodriguez et al. 2014). The electrophysiological characterization indicates that transport mechanism involves Na^+ co-transport and the simultaneous exchange of H^+, resulting in an electroneutral movement of glycine across the astrocyte membrane. Furthermore, SNAT5 mediates the glycine-gated uncoupled flow of H^+ that, together with the coupled one, seems to favor the release of glycine rather than its uptake during neural activity (Hamdani et al. 2012). Neuronal activation results in accumulation of Na^+ and glutamate in perisynaptic glial cells (Chaudhry et al. 1995). In addition, the extracellular concentration of K^+ increases, which activates Na^+/HCO_3^- cotransporters at astrocytic membranes. This further increases the intracellular sodium concentration and the pH (Brookes 2000). The outwardly directed glycine gradient and the inwardly directed gradient for H^+ can now override the gradient of Na^+ so that SNAT5 can readily release glycine. Indeed, the Km of SNAT5 for glycine (about 7 mM) better concurs with the cytoplasmic concentrations of glycine (from 4 up to 11 mM in cultured primary astrocytes) than with the extracellular one, implicating a preference for release mode of SNAT5 (Verleysdonk et al. 1999; Hamdani et al. 2012).

6 GlyT1 as a Target for Psychiatric and Neurologic Diseases

6.1 Schizophrenia

SCZ is a severe chronic and disabling brain disorder affecting approximately 1% of the world population. Clinical symptoms fall into three broad categories: positive, negative, and cognitive symptoms. Current antipsychotic medication primarily improves positive symptoms basically by acting on D_2 dopamine receptors. However, these treatments have a limited value for the other categories of symptoms. An imbalance in the complex, interrelated chemical signaling that allows brain cells to communicate with each other is assumed to underlie SCZ, a disease with a slow gestation along the neurodevelopmental process that usually manifests itself in adolescence and early youth. NMDARs play a key role for shaping neuronal connections during brain development, and evidence has accumulated indicating that hypofunction of NMDARs underlies a number of alterations observed in schizophrenia (review by Moghaddam and Javitt 2012; Coyle 2006, 2012). Initially, this hypothesis is derived from the observation that various antagonists of the NMDARs, like ketamine, phencyclidine, or MK801, mimic numerous symptoms of SCZ in healthy adults and potentiate the positive, negative, and cognitive symptoms in patients. Based on these observations, administration of these NMDAR antagonists has been widely used to induce NMDAR hypofunction in animals as a pharmacological model of SCZ, and several laboratories have demonstrated schizophrenia-like changes on a number of behavioral measures relevant to positive, negative, and cognitive symptoms (Wiescholleck and Manahan-Vaughan 2013). Additionally, different KO mice also model to some extent the disease. Mice that express mutated GluN1 subunits with lowered glycine affinity display cognitive and learning defects including non-habituating hyperactivity, increased stereotyped behavior, disruptions of nest-building activity, and poor performance in the Morris water maze (Ballard et al. 2002). Also transgenic mice expressing reduced levels of the GluN1 subunit display behavioral abnormalities similar to those observed in pharmacologically induced models of SCZ (Mohn et al. 1999), and mice lacking the GluN2A subunit exhibit an increased spontaneous locomotor activity in novel environments and an impairment of latent learning in a water-finding task besides deficit in hippocampal LTP and spatial learning (Miyamoto et al. 2001). These behavioral phenotypes resemble some of the positive and negative symptoms displayed by SCZ patients thereby supporting the hypothesis (Coyle 2012; Ramsey 2009). Further support to the hypothesis is provided by recent large-scale, genome-wide investigations that have recognized that SCZ is a heterogeneous disease entity involving a large number of genes and noncoding risk loci. These studies identified several genes encoding synaptic proteins including NMDAR-associated downstream and upstream signaling proteins that play a central role in the pathogenesis of SCZ (Fromer et al. 2014; Schizophrenia Working Group 2014; Peykov et al. 2015; Balu and Coyle 2015).

Consequently, the stimulation of the glycine-B site on the NMDARs has been considered to be an effective way of indirectly enhancing NMDAR function avoiding excitotoxicity. Indeed, GlyT1 inhibitors exhibit antipsychotic activity in several animal models, as we have exposed in previous paragraphs (Alberati et al. 2012; Boulay et al. 2008; Depoortère et al. 2005; Harada et al. 2012; Chaki et al. 2015). Moreover, sarcosine has been proven to alleviate both negative symptoms and cognitive dysfunction, in addition to positive symptoms, when administered as an adjunctive therapy in small-scale clinical trials (Tsai et al. 2004a). Also, the addition of the second-generation GlyT1 inhibitor bitopertin (RG1678) to standard antipsychotics resulted in a significant reduction of negative symptoms in a randomized, double-blind study in patients with predominant negative symptoms (Umbricht et al. 2014). However, other trials could not replicate these results, together with the negative results mentioned above, for Org 25935 produced a substantial reduction of expectations (Schoemaker et al. 2014). Indeed, a phase III clinical trial carried out by Hofmann-LaRoche for bitopertin failed to reach its endpoints to improve negative symptoms, and the assay was stopped. Consequently, the efficacy of GlyT1 inhibitors against negative symptoms and several cognitive domains needs further investigations. Excellent reviews on the different GlyT1 inhibitors assayed in preclinical and clinical studies have been published (Harvey and Yee 2013; Singer et al. 2015). The neural mechanisms underlying the improvement of cognitive and social deficits caused by GlyT1 inhibitors in animal models are not fully understood but probably involve complex interaction between the glutamatergic and the dopaminergic systems, with additional intervention of the glycinergic and GABAergic systems in diverse areas of the forebrain. For instance, the GlyT1 inhibitor SSR504734 potentiates dopaminergic signaling since it increases the release of dopamine in the prefrontal cortex and in the nucleus accumbens (Depoortère et al. 2005; Leoneti et al. 2006). At least in the nucleus accumbens, these dopamine-enhancing effects could result from increased glycinergic inhibition, indicating that in the effect of GlyT1 inhibitors there are more underlying factors than just the potentiation of NMDARs (Lidö et al. 2011).

6.2 Drug Addiction

One of the many derivatives of the pathological manifestations of SCZ is the frequency with which the schizophrenic patient is addicted to different drugs of abuse including cocaine or ethanol, with a prevalence between three to five times higher than in healthy controls (Coyle 2006). NMDARs also have been implicated in the aberrant regulation of synaptic plasticity that is critical for substance abuse and addiction since glutamatergic inputs from cortical and subcortical regions modulate the mesolimbic dopamine system thereby regulating aspects of drug-seeking

behaviors (see Carlezon and Thomas 2009, for a review). Indeed, these behaviors have been modified in animal models by treatments with the partial glycine-B site agonist D-cycloserine or D-serine (Paolone et al. 2009; Kelamangalath and Wagner 2010). Consistently, GlyT1+/-heterozygote mice, with overactive NMDARs, show some phenotypes consistent with that idea (Puhl et al. 2015). Indeed, inhibitors of GlyT1 (Org 25935 and Org 24598) are effective in reducing relapse-like compulsive drinking and alcohol preference in rodents (Molander, et al. 2007; Vengeliene et al. 2010; Lidö et al. 2011). However, the results in humans have been disappointing, and phase II clinical trials were stopped before conclusion (Bejczy et al. 2014).

Therefore, all these assays in humans either for the treatment of SCZ or for drug addictions illustrate the difficulties of the task, probably due to difficulties in setting the adequate doses for a system affecting simultaneously to excitatory and inhibitory neurotransmission and the involvement of multiple circuits in the regulation of these psychiatric dysfunctions. Also it is difficult to predict the response of these systems to long-term treatments that might result in a complex remodeling of the NMDAR system. Further complexity is added by the fact that frequently the clinical assays have been performed with GlyT1 inhibitors as a coadjuvant therapy with dopaminergic antipsychotics. Nevertheless, still there are evidence for a beneficial influence of sarcosine in humans (Lane et al. 2008; Singh and Singh 2011; Strzelecki et al. 2015), thereby maintaining the hope that one day both the right type of inhibitor and form of administration will be found to achieve a significant therapeutic effect.

6.3 Depression

A consequence derived from recent genetic studies is that genetic risk does not map neatly on psychiatric clinical diagnoses, which is perhaps not surprising given the degree of genetic complexity and the continuous nature of many psychiatric traits. Therefore, there is evidence for shared genetic risk between SCZ, bipolar disorder, autism spectrum disorders, intellectual disability, and attention-deficit hyperactivity disorder. Consequently all these diseases might have a glutamatergic substrate that might be rescued by GlyT1 inhibitors. Indeed, several studies performed in animal models of depression support that GlyT1 inhibitors display an antidepressant effect (Boulay et al. 2008; Depoortère et al. 2005), and in a recent double-blinded trial performed in 40 patients with major depression, sarcosine was found to result in greater improvements in several scores than citalopram (Huang et al. 2013). Although these observations are apparently contradictory to the findings that ketamine, a NMDAR antagonist, is a potent antidepressant (Krystal et al. 2013), a recent study proves that the antidepressant effect of ketamine is NMDAR independent (Zanos et al. 2016).

6.4 Anxiety

Treatment of anxiety with GlyT1 inhibitors has been also considered as a therapeutic possibility. In principle, NMDAR activation induces anxiety-like behavior in mice (Miguel and Nunes-de-Souza 2008), and this seems contradictory to the mode of action of these compounds. However, SSR504734, a GlyT1 inhibitor, has anxiolytic actions since it attenuates both the acquisition and the expression of contextual conditioned fear in rats (Nishikawa et al. 2010) and decreases maternal separation-induced ultrasonic vocalization (USV) in rat pups (Depoortère et al. 2005). However, these effects were reversed by administration of strychnine indicating that they are mediated by GlyRs instead of NMDARs. Indeed, GlyT1-induced decreases in USV were not reversed by administration of the glycine-B antagonist L-687,414 (Komatsu et al. 2015). An anxiolytic effect of GlyT1 inhibitors was also observed by infusion in the amygdala of NFPS, potentiating the fear extinction in a paradigm of conditioned fear in rats. Experimental data in this case suggest that the molecular mechanism acts via an enhancement of NMDA-mediated AMPA receptor endocytosis in the amygdala (Mao et al. 2009).

6.5 Ischemia and Exotoxicity

The control of NMDAR activation is crucial for neuronal function and viability. Overstimulation of NMDAR triggers excitotoxic cell death processes. However, this is critically dependent on the NMDAR subunit composition. Activation of GluN2B-containing NMDAR have been more associated with a death signal than GluN2A-containing NMDAR, inducing Ca^{2+} accumulation, mitochondrial swelling, and neuronal degeneration (Martel et al. 2012). Interestingly, sublethal doses of NMDA activate a neuroprotective mechanism named brain preconditioning. Thus, the question is whether a moderate increase in the NMDAR activity by GlyT1 inhibitors might emulate the effect of low doses of NMDA. Recent evidence indicates that this might be the case since both sarcosine and NFPS induced preconditioning in vivo. The preconditioning protocol by GlyT1 inhibitors reduced the expression of GluN2B subunits, whereas did not change the expression of GluN1 or GluN2A (Pinto et al. 2014, 2015).

6.6 Neuropathic Pain

Neuropathic pain is another pathological condition in which glycine transporters have attracted considerable interest as targets for drug intervention. In this case it comes into play the balance between excitatory and inhibitory neurotransmission in the spinal cord. The pain information is transported from the periphery to the

thalamus through spinal centers using, among others, glutamatergic mechanisms. But GABAergic and glycinergic interneurons of the spinal cord filter and modulate the flow of information. An imbalance between spinal inhibitory and excitatory neurotransmission leads to increased responses to noxious stimuli (Costigan et al. 2009). A reduction of inhibitory neurotransmission as well as an exaggeration of excitatory processes in the spinal cord contributes to the development of increased pain sensitivity. In the spinal cord, it is evident that GlyT1 plays an essential role in controlling both the receptor activity of strychnine-sensitive glycine (mainly GlyR3a) and, secondly, the diffusion or spillover of glycine to the glycine-B site of NMDARs. GlyT1 inhibitors, Org 25935, sarcosine, and NFPS108, contribute to pain relief in different model of neuropathic pain, probably by potentiating the activity of GlyRs in the spinal cord (Tanabe et al. 2008; Morita et al. 2008; Barthel et al. 2014). Recent observations also involved GlyT1 in the mode of action of lidocaine, an anesthetic that has been used to treat neuropathic pain. The lidocaine metabolite, N-ethylglycine, was shown to be a specific inhibitor of GlyT1 that in rodent models of inflammatory and neuropathic pain resulted in an efficient amelioration of hyperalgesia and allodynia without affecting acute pain. N-ethylglycine reduced the increase in neuronal firing of wide-dynamic-range neurons caused by inflammatory pain induction. This effect probably was due to an enhancement of the spinal inhibition, secondary to the increase of glycine concentration at glycinergic inhibitory synapses (Werdehausen et al. 2015). A similar GlyR-mediated mechanism is involved in pain relief in a mouse model of bone cancer (Motoyama et al. 2014). These studies reinforce the idea that GlyT1 substrates may be useful therapeutic agents in chronic pain states involving spinal disinhibition. It is unclear whether spillover of glycine to NMDARs after treatments with glycine transporter inhibitors has positive or negative effects on suppression of pain. Thus, Morita et al. (2008) reported a lag time of 1–2 h after administration of GlyT1 inhibitors before developing the anti-allodynia effect, a lag that was suppressed by administration of glycine-B site antagonists and, therefore, attributable to NMDAR operation. But perhaps a permanent exposure to GlyT1 inhibitor could decrease the amount of NMDARs by priming their endocytosis and decreasing the glutamatergic signaling that might reinforce the pain signal (Nong et al. 2003; Barthel et al. 2014).

6.7 Epilepsy

Epileptic seizures are a major neurological disorder with a particular high incidence in children. In addition to GABA, the glycinergic system is crucially involved in the regulation of neuronal excitability. Inhibition of glycine receptors can evoke epileptiform discharges in the adult and in the immature brain (Straub et al. 1997; Chen et al. 2014). Relative to the involvement of glycine transporters in epileptogenesis, Socala et al. (2010) found that sarcosine exhibits anticonvulsive activity. However, due to the high doses of sarcosine (800–1000 mg/kg) required for effective activity, the possibility of taking sarcosine as a potential antiepileptic drug may remain

elusive. Moreover, it is worth noting that in addition to antagonizing the glycine transporter, sarcosine also directly potentiates NMDAR function as a co-agonist. However, a couple of studies support the idea that pharmacological manipulation of GlyT1 might constitute a valuable treatment for epilepsy. Shen et al. (2015), using two different rodent models of temporal lobe epilepsy (TLE), demonstrated robust overexpression of GlyT1 in the hippocampal formation, suggesting dysfunctional glycine signaling in epilepsy. In support of a role of dysfunctional glycine signaling in the pathophysiology of epilepsy, both the genetic deletion of GlyT1 in hippocampus and the GlyT1 inhibitor LY2365109 increased seizure thresholds in mice. Importantly, chronic seizures in the mouse model of TLE were robustly suppressed by systemic administration of the GlyT1 inhibitor LY2365109. A second study (Zhao et al. 2016) showed that an effective inhibitor of GlyT1, termed M22, elevated the tonic seizure threshold in the mouse model of maximal electroshock seizure threshold and did not impair motor function. Given that current epilepsy treatment is limited by poor responses to available antiepileptic drugs and limited tolerance due to major cognitive side effects, both studies conclude that the GlyT1 inhibitors have potential as new anticonvulsive drugs or as the lead compounds for antiepileptic drugs development.

7 Conclusions nd Prospects

For nearly 25 years, experimental evidence has accumulated which clearly contradicts the initial proposals indicating that the glycine-B site on NMDARs was chronically saturated. Data obtained from pharmacological models and from genetically modified mice indicate that this is not the case as the glycine transporter GlyT1 occupies a strategic position, especially in glial cells, where its expression is higher but also in glutamatergic terminals. This gives the ability to modulate the glycine concentration in the vicinity of NMDARs. Studies have shown that inhibitors of GlyT1 are able to affect various psychical and neurological functions and could be useful in treating deleterious conditions such as schizophrenia, depression, or anxiety, but also pain or epilepsy, despite preliminary clinical studies in humans have been rather disappointing. Perhaps the evolution of the chemical properties of these inhibitors or the discovery of new signaling pathways that may modulate the activity or gene expression (e.g., through specific microRNAs or epigenetic mechanisms) could provide new pharmacological tools to modify the activity of GlyT1 and the associated functions.

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References

Agulhon C, Fiacco TA, McCarthy KD. Hippocampal short- and long-term plasticity are not modulated by astrocyte Ca^{2+} signaling. Science. 2010;327(5970):1250–4.

Ahmadi S, Muth-Selbach U, Lauterbach A, Lipfert P, Neuhuber WL, Zeilhofer HU. Facilitation of spinal NMDA receptor currents by spillover of synaptically released glycine. Science. 2003;300(5628):2094–7.

Alberati D, Moreau JL, Lengyel J, Hauser N, Mory R, Borroni E, et al. Glycine reuptake inhibitor RG1678: a pharmacologic characterization of an investigational agent for the treatment of schizophrenia. Neuropharmacology. 2012;62(2):1152–61.

Apostolides PF, Trussell LO. Rapid, activity-independent turnover of vesicular transmitter content at a mixed glycine/GABA synapse. J Neurosci. 2013;33(11):4768–81.

Aragón C, López-Corcuera B. Glycine transporters: crucial roles of pharmacological interest revealed by gene deletion. Trends Pharmacol Sci. 2005;26(6):283–6.

Attwell D, Barbour B, Szatkowski M. Nonvesicular release of neurotransmitter. Neuron. 1993 Sep;11(3):401–7.

Aubrey KR, Mitrovic AD, Vandenberg RJ. Molecular basis for proton regulation of glycine transport by glycine transporter subtype 1b. Mol Pharmacol. 2000;58(1):129–35.

Aubrey KR, Vandenberg RJ, Clements JD. Dynamics of forward and reverse transport by the glial glycine transporter, glyt1b. Biophys J. 2005;89(3):1657–68.

Baker AJ, Zornow MH, Scheller MS, Yaksh TL, Skilling SR, Smullin DH, et al. Changes in extracellular concentrations of glutamate, aspartate, glycine, dopamine, serotonin, and dopamine metabolites after transient global ischemia in the rabbit brain. J Neurochem. 1991;57(4):1370–9.

Bakkar W, Ma CL, Pabba M, Khacho P, Zhang YL, Muller E, et al. Chronically saturating levels of endogenous glycine disrupt glutamatergic neurotransmission and enhance synaptogenesis in the CA1 region of mouse hippocampus. Synapse. 2011;65(11):1181–95.

Ballard TM, Pauly-Evers M, Higgins GA, Ouagazzal AM, Mutel V, Borroni E, et al. Severe impairment of NMDA receptor function in mice carrying targeted point mutations in the glycine binding site results in drug-resistant nonhabituating hyperactivity. J Neurosci. 2002;22(15):6713–23.

Balu DT, Coyle JT. The NMDA receptor 'glycine modulatory site' in schizophrenia: D-serine, glycine, and beyond. Curr Opin Pharmacol. 2015;20:109–15.

Barthel F, Urban A, Schlösser L, Eulenburg V, Werdehausen R, Brandenburger T, et al. Long-term application of glycine transporter inhibitors acts antineuropathic and modulates spinal N-methyl-D-aspartate receptor subunit NR-1 expression in rats. Anesthesiology. 2014;121(1):160–9.

Bejczy A, Nations KR, Szegedi A, Schoemaker J, Ruwe F, Söderpalm B. Efficacy and safety of the glycine transporter-1 inhibitor org 25935 for the prevention of relapse in alcohol-dependent patients: a randomized, double-blind, placebo-controlled trial. Alcohol Clin Exp Res. 2014;38(9):2427–35.

Berger AJ, Isaacson JS. Modulation of motoneuron N-methyl-D-aspartate receptors by the inhibitory neurotransmitter glycine. J Physiol Paris. 1999;93(1-2):23–7.

Berger AJ, Dieudonné S, Ascher P. Glycine uptake governs glycine site occupancy at NMDA receptors of excitatory synapses. J Neurophysiol. 1998;80(6):3336–40.

Bergeron R, Meyer TM, Coyle JT, Greene RW. Modulation of N-methyl-D-aspartate receptor function by glycine transport. Proc Natl Acad Sci U S A. 1998;95(26):15730–4.

Borowsky B, Mezey E, Hoffman BJ. Two glycine transporter variants with distinct localization in the CNS and peripheral tissues are encoded by a common gene. Neuron. 1993;10(5):851–63.

Boulay D, Pichat P, Dargazanli G, Estenne-Bouhtou G, Terranova JP, Rogacki N, et al. Characterization of SSR103800, a selective inhibitor of the glycine transporter-1 in models predictive of therapeutic activity in schizophrenia. Pharmacol Biochem Behav. 2008;91(1):47–58.

Bradaïa A, Schlichter R, Trouslard J. Role of glial and neuronal glycine transporters in the control of glycinergic and glutamatergic synaptic transmission in lamina X of the rat spinal cord. J Physiol. 2004;559(Pt 1):169–86.

Brookes N. Functional integration of the transport of ammonium, glutamate and glutamine in astrocytes. Neurochem Int. 2000;37(2-3):121–9.
Carlezon WA Jr, Thomas MJ. Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis. Neuropharmacology. 2009;56(Suppl 1):122–32.
Castner SA, Murthy NV, Ridler K, Herdon H, Roberts BM, Weinzimmer DP, et al. Relationship between glycine transporter 1 inhibition as measured with positron emission tomography and changes in cognitive performances in nonhuman primates. Neuropsychopharmacology. 2014;39(12):2742–9.
Chaki S, Shimazaki T, Karasawa J, Aoki T, Kaku A, Iijima M, et al. Efficacy of a glycine transporter 1 inhibitor TASP0315003 in animal models of cognitive dysfunction and negative symptoms of schizophrenia. Psychopharmacology. 2015;232(15):2849–61.
Chaudhry FA, Lehre KP, van Lookeren Campagne M, Ottersen OP, Danbolt NC, Storm-Mathisen J. Glutamate transporters in glial plasma membranes: highly differentiated localizations revealed by quantitative ultrastructural immunocytochemistry. Neuron. 1995;15(3):711–20.
Chen L, Muhlhauser M, Yang CR. Glycine tranporter-1 blockade potentiates NMDA-mediated responses in rat prefrontal cortical neurons in vitro and in vivo. J Neurophysiol. 2003;89(2):691–703.
Chen R, Okabe A, Sun H, Sharopov S, Hanganu-Opatz IL, Kolbaev SN, et al. Activation of glycine receptors modulates spontaneous epileptiform activity in the immature rat hippocampus. J Physiol. 2014;592(10):2153–68.
Cioffi CL, Guzzo PR. Inhibitors of glycine transporter-1: potential therapeutics for the treatment of CNS disorders. Curr Top Med Chem. 2016;16(29):3404–37.
Collingridge GL, Volianskis A, Bannister N, France G, Hanna L, Mercier M, et al. The NMDA receptor as a target for cognitive enhancement. Neuropharmacology. 2013;64:13–26.
Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. Annu Rev Neurosci. 2009;32:1–32.
Coyle JT. Substance use disorders and Schizophrenia: a question of shared glutamatergic mechanisms. Neurotox Res. 2006;10(3-4):221–33.
Coyle JT. NMDA receptor and schizophrenia: a brief history. Schizophr Bull. 2012;38(5):920–6.
Coyle JT, Tsai G. The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia. Psychopharmacology. 2004;174(1):32–8.
Cubelos B, Giménez C, Zafra F. Localization of the GLYT1 glycine transporter at glutamatergic synapses in the rat brain. Cereb Cortex. 2005a;15(4):448–59.
Cubelos B, Giménez C, Zafra F. The glycine transporter GLYT1 interacts with Sec3, a component of the exocyst complex. Neuropharmacology. 2005b;49(6):935–44.
Cubelos B, González-González IM, Giménez C, Zafra F. Amino acid transporter SNAT5 localizes to glial cells in the rat brain. Glia. 2005c;49(2):230–44.
Cubelos B, Leite C, Giménez C, Zafra F. Localization of the glycine transporter GLYT1 in glutamatergic synaptic vesicles. Neurochem Int. 2014;73:204–10.
Danglot L, Rostaing P, Triller A, Bessis A. Morphologically identified glycinergic synapses in the hippocampus. Mol Cell Neurosci. 2004;27(4):394–403.
Depoortère R, Dargazanli G, Estenne-Bouhtou G, Coste A, Lanneau C, Desvignes C, et al. Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. Neuropsychopharmacology. 2005;30(11):1963–85.
Doherty FC, Sladek CD. NMDA receptor subunit expression in the supraoptic nucleus of adult rats: dominance of NR2B and NR2D. Brain Res. 2011;1388:89–99.
Dubroqua S, Serrano L, Boison D, Feldon J, Gargiulo PA, Yee BK. Intact working memory in the absence of forebrain neuronal glycine transporter 1. Behav Brain Res. 2012;230(1):208–14.
Dupuis JP, Ladépêche L, Seth H, Bard L, Varela J, Mikasova L, et al. Surface dynamics of GluN2B-NMDA receptors controls plasticity of maturing glutamate synapses. EMBO J. 2014;33(8):842–61.

Eulenburg V, Retiounskaia M, Papadopoulos T, Gomeza J, Betz H. Glial glycine transporter 1 function is essential for early postnatal survival but dispensable in adult mice. Glia. 2010;58(9):1066–73.

Fernández-Sánchez E, Martínez-Villarreal J, Giménez C, Zafra F. Constitutive and regulated endocytosis of the glycine transporter GLYT1b is controlled by ubiquitination. J Biol Chem. 2009;284(29):19482–92.

Fossat P, Turpin FR, Sacchi S, Dulong J, Shi T, Rivet JM, et al. Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. Cereb Cortex. 2012;22(3):595–606.

Friauf E, Aragón C, Löhrke S, Westenfelder B, Zafra F. Developmental expression of the glycine transporter GLYT2 in the auditory system of rats suggests involvement in synapse maturation. J Comp Neurol. 1999;412(1):17–37.

Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, et al. De novo mutations in schizophrenia implicate synaptic networks. Nature. 2014;506(7487):179–84.

Fukasawa Y, Segawa H, Kim JY, Chairoungdua A, Kim DK, Matsuo H, et al. Identification and characterization of a Na(+)-independent neutral amino acid transporter that associates with the 4F2 heavy chain and exhibits substrate selectivity for small neutral D- and L-amino acids. J Biol Chem. 2000;275(13):9690–8.

Gabernet L, Pauly-Evers M, Schwerdel C, Lentz M, Bluethmann H, Vogt K, et al. Enhancement of the NMDA receptor function by reduction of glycine transporter-1 expression. Neurosci Lett. 2005;373(1):79–84.

Galli A, Mori F, Bargellini M, Coppini L. Sodium-dependent release of exogenous glycine from preloaded rat hippocampal synaptosomes. J Neural Transm Gen Sect. 1993;93(3):167–79.

Geerlings A, López-Corcuera B, Aragón C. Characterization of the interactions between the glycine transporters GLYT1 and GLYT2 and the SNARE protein syntaxin 1A. FEBS Lett. 2000;470(1):51–4.

Gomeza J, Zafra F, Olivares L, Giménez C, Aragón C. Regulation by phorbol esters of the glycine transporter (GLYT1) in glioblastoma cells. Biochim Biophys Acta. 1995;1233(1):41–6.

Gomeza J, Hülsmann S, Ohno K, Eulenburg V, Szöke K, Richter D, Betz H. Inactivation of the glycine transporter 1 gene discloses vital role of glial glycine uptake in glycinergic inhibition. Neuron. 2003a;40(4):785–96.

Gomeza J, Ohno K, Hülsmann S, Armsen W, Eulenburg V, Richter DW, et al. Deletion of the mouse glycine transporter 2 results in a hyperekplexia phenotype and postnatal lethality. Neuron. 2003b;40(4):797–806.

Hamdani el H, Gudbrandsen M, Bjørkmo M, Chaudhry FA. The system N transporter SN2 doubles as a transmitter precursor furnisher and a potential regulator of NMDA receptors. Glia. 2012;60(11):1671–83.

Hanuska A, Szénási G, Albert M, Koles L, Varga A, Szabo A, et al. Some operational characteristics of glycine release in rat retina: the role of reverse mode operation of glycine transporter type-1 (GlyT-1) in ischemic conditions. Neurochem Res. 2016;41(1-2):73–85.

Harada K, Nakato K, Yarimizu J, Yamazaki M, Morita M, Takahashi S, et al. A novel glycine transporter-1 (GlyT1) inhibitor, ASP2535 (4-[3-isopropyl-5-(6-phenyl-3-pyridyl)-4H-1,2,4-triazol-4-yl]-2,1,3-benzoxadiazol e), improves cognition in animal models of cognitive impairment in schizophrenia and Alzheimer's disease. Eur J Pharmacol. 2012;685(1-3):59–69.

Harsing LG Jr, Gacsalyi I, Szabo G, Schmidt E, Sziray N, Sebban C, et al. The glycine transporter-1 inhibitors NFPS and Org 24461: a pharmacological study. Pharmacol Biochem Behav. 2003;74(4):811–25.

Harvey RJ, Yee BK. Glycine transporters as novel therapeutic targets in schizophrenia, alcohol dependence and pain. Nat Rev Drug Discov. 2013;12(11):866–85.

Helboe L, Egebjerg J, Møller M, Thomsen C. Distribution and pharmacology of alanine-serine-cysteine transporter 1 (asc-1) in rodent brain. Eur J Neurosci. 2003;18(8):2227–38.

Henneberger C, Papouin T, Oliet SH, Rusakov DA. Long-term potentiation depends on release of D-serine from astrocytes. Nature. 2010;463(7278):232–6.

Huang H, Barakat L, Wang D, Bordey A. Bergmann glial GlyT1 mediates glycine uptake and release in mouse cerebellar slices. J Physiol. 2004;560(Pt 3):721–36.
Huang CC, Wei IH, Huang CL, Chen KT, Tsai MH, Tsai P, et al. Inhibition of glycine transporter-I as a novel mechanism for the treatment of depression. Biol Psychiatry. 2013;74(10):734–41.
James VM, Gill JL, Topf M, Harvey RJ. Molecular mechanisms of glycine transporter GlyT2 mutations in startle disease. Biol Chem. 2012;393(4):283–9.
Jiménez E, Zafra F, Pérez-Sen R, Delicado EG, Miras-Portugal MT, Aragón C, et al. P2Y purinergic regulation of the glycine neurotransmitter transporters. J Biol Chem. 2011;286(12):10712–24.
Jiménez E, Núñez E, Ibáñez I, Zafra F, Aragón C, Giménez C. Glycine transporters GlyT1 and GlyT2 are differentially modulated by glycogen synthase kinase 3β. Neuropharmacology. 2015;89:245–54.
Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature. 1987;325(6104):529–31.
Ju P, Aubrey KR, Vandenberg RJ. Zn^{2+} inhibits glycine transport by glycine transporter subtype 1b. J Biol Chem. 2004;279(22):22983–91.
Karakas E, Furukawa H. Crystal structure of a heterotetrameric NMDA receptor ion channel. Science. 2014;344(6187):992–7.
Karasawa J, Hashimoto K, Chaki S. D-Serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. Behav Brain Res. 2008;186(1):78–83.
Kelamangalath L, Wagner JJ. D-serine treatment reduces cocaine-primed reinstatement in rats following extended access to cocaine self-administration. Neuroscience. 2010;169(3):1127–35.
Kemp JA, Foster AC, Leeson PD, Priestley T, Tridgett R, Iversen LL, et al. 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the N-methyl-D-aspartate receptor complex. Proc Natl Acad Sci U S A. 1988;85(17):6547–50.
Kinney GG, Sur C, Burno M, Mallorga PJ, Williams JB, Figueroa DJ, et al. The glycine transporter type 1 inhibitor N-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine potentiates NMDA receptor-mediated responses in vivo and produces an antipsychotic profile in rodent behavior. J Neurosci. 2003;23(20):7586–91.
Kleckner NW, Dingledine R. Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. Science. 1988;241(4867):835–7.
Komatsu H, Furuya Y, Sawada K, Asada T. Involvement of the strychnine-sensitive glycine receptor in the anxiolytic effects of GlyT1 inhibitors on maternal separation-induced ultrasonic vocalization in rat pups. Eur J Pharmacol. 2015;746:252–7.
Krishtal OA, Osipchuk YV, Shelest TN, Smirnoff SV. Rapid extracellular pH transients related to synaptic transmission in rat hippocampal slices. Brain Res. 1987;436(2):352–6.
Krystal JH, Sanacora G, Duman RS. Rapid-acting glutamatergic antidepressants: the path to ketamine and beyond. Biol Psychiatry. 2013;73(12):1133–41.
Lane HY, Liu YC, Huang CL, Chang YC, Liau CH, Perng CH, et al. Sarcosine (N-methylglycine) treatment for acute schizophrenia: a randomized, double-blind study. Biol Psychiatry. 2008;63(1):9–12.
Lavezzari G, McCallum J, Dewey CM, Roche KW. Subunit-specific regulation of NMDA receptor endocytosis. J Neurosci. 2004;24(28):6383–91.
Le Bail M, Martineau M, Sacchi S, Yatsenko N, Radzishevsky I, Conrod S, et al. Identity of the NMDA receptor coagonist is synapse specific and developmentally regulated in the hippocampus. Proc Natl Acad Sci U S A. 2015;112(2):E204–13.
Lee CH, Lü W, Michel JC, Goehring A, Du J, Song X, et al. NMDA receptor structures reveal subunit arrangement and pore architecture. Nature. 2014;511(7508):191–7.
Leonetti M, Desvignes C, Bougault I, Souilhac J, Oury-Donat F, Steinberg R. 2-Chloro-N-[(S)-phenyl [(2S)-piperidin-2-yl] methyl]-3-trifluoromethyl benzamide, monohydrochloride, an inhibitor of the glycine transporter type 1, increases evoked-dopamine release in the rat nucleus accumbens in vivo via an enhanced glutamatergic neurotransmission. Neuroscience. 2006;137(2):555–64.

Lester RA, Tong G, Jahr CE. Interactions between the glycine and glutamate binding sites of the NMDA receptor. J Neurosci. 1993;13(3):1088–96.
Li Y, Krupa B, Kang JS, Bolshakov VY, Liu G. Glycine site of NMDA receptor serves as a spatiotemporal detector of synaptic activity patterns. J Neurophysiol. 2009;102(1):578–89.
Li Y, Sacchi S, Pollegioni L, Basu AC, Coyle JT, Bolshakov VY. Identity of endogenous NMDAR glycine site agonist in amygdala is determined by synaptic activity level. Nat Commun. 2013;4:1760.
Lidö HH, Ericson M, Marston H, Söderpalm B. A role for accumbal glycine receptors in modulation of dopamine release by the glycine transporter-1 inhibitor org25935. Front Psychiatry. 2011;2:8.
Lim R, Hoang P, Berger AJ. Blockade of glycine transporter-1 (GLYT-1) potentiates NMDA receptor-mediated synaptic transmission in hypoglossal motorneurons. J Neurophysiol. 2004;92(4):2530–7.
Liu QR, López-Corcuera B, Mandiyan S, Nelson H, Nelson N. Cloning and expression of a spinal cord- and brain-specific glycine transporter with novel structural features. J Biol Chem. 1993;268(30):22802–8.
López-Colomé AM, Gadea A. Regulation of glycine transport in cultured Müller cells by Ca^{2+}/calmodulin-dependent enzymes. Ann N Y Acad Sci. 1999;868:685–8.
Luccini E, Romei C, Raiteri L. Glycinergic nerve endings in hippocampus and spinal cord release glycine by different mechanisms in response to identical depolarizing stimuli. J Neurochem. 2008;105(6):2179–89.
Mackenzie B, Erickson JD. Sodium-coupled neutral amino acid (System N/A) transporters of the SLC38 gene family. Pflugers Arch. 2004;447(5):784–95.
Mao SC, Lin HC, Gean PW. Augmentation of fear extinction by infusion of glycine transporter blockers into the amygdala. Mol Pharmacol. 2009;76(2):369–78.
Marcaggi P, Attwell D. Role of glial amino acid transporters in synaptic transmission and brain energetics. Glia. 2004;47(3):217–25.
Martel MA, Ryan TJ, Bell KF, Fowler JH, McMahon A, Al-Mubarak B, et al. The subtype of GluN2 C-terminal domain determines the response to excitotoxic insults. Neuron. 2012;74(3):543–56.
Martina M, Gorfinkel Y, Halman S, Lowe JA, Periyalwar P, Schmidt CJ, et al. Glycine transporter type 1 blockade changes NMDA receptor-mediated responses and LTP in hippocampal CA1 pyramidal cells by altering extracellular glycine levels. J Physiol. 2004;557(Pt 2):489–500.
Martina M, B-Turcotte ME, Halman S, Tsai G, Tiberi M, Coyle JT, Bergeron R. Reduced glycine transporter type 1 expression leads to major changes in glutamatergic neurotransmission of CA1 hippocampal neurones in mice. J Physiol. 2005;563(3):777–93.
Martineau M, Shi T, Puyal J, Knolhoff AM, Dulong J, Gasnier B, et al. Storage and uptake of D-serine into astrocytic synaptic-like vesicles specify gliotransmission. J Neurosci. 2013;33(8):3413–23.
Martineau M, Parpura V, Mothet JP. Cell-type specific mechanisms of D-serine uptake and release in the brain. Front Synaptic Neurosci. 2014;6:12.
Marvin JS, Borghuis BG, Tian L, Cichon J, Harnett MT, Akerboom J, et al. An optimized fluorescent probe for visualizing glutamate neurotransmission. Nat Methods. 2013 Feb;10(2):162–70.
Mayer ML, Westbrook GL, Guthrie PB. Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. Nature. 1984;309:261.
Meunier CN, Dallérac G, Le Roux N, Sacchi S, Levasseur G, Amar M, et al. D-serine and glycine differentially control neurotransmission during visual cortex critical period. PLoS One. 2016;11(3):e0151233.
Mezler M, Hornberger W, Mueller R, Schmidt M, Amberg W, Braje W, et al. Inhibitors of GlyT1 affect glycine transport via discrete binding sites. Mol Pharmacol. 2008;74(6):1705–15.
Miguel TT, Nunes-de-Souza RL. Anxiogenic-like effects induced by NMDA receptor activation are prevented by inhibition of neuronal nitric oxide synthase in the periaqueductal gray in mice. Brain Res. 2008;1240:39–46.

Miyamoto Y, Yamada K, Noda Y, Mori H, Mishina M, Nabeshima T. Hyperfunction of dopaminergic and serotonergic neuronal systems in mice lacking the NMDA receptor epsilon1 subunit. J Neurosci. 2001;21(2):750–7.

Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology. 2012;37(1):4–15.

Möhler H, Boison D, Singer P, Feldon J, Pauly-Evers M, Yee BK. Glycine transporter 1 as a potential therapeutic target for schizophrenia-related symptoms: evidence from genetically modified mouse models and pharmacological inhibition. Biochem Pharmacol. 2011;81(9):1065–77.

Mohn AR, Gainetdinov RR, Caron MG, Koller BH. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell. 1999;98(4):427–36.

Molander A, Lidö HH, Löf E, Ericson M, Söderpalm B. The glycine reuptake inhibitor Org 25935 decreases ethanol intake and preference in male wistar rats. Alcohol Alcohol. 2007;42(1):11–8.

Morita K, Motoyama N, Kitayama T, Morioka N, Kifune K, Dohi T. Spinal antiallodynia action of glycine transporter inhibitors in neuropathic pain models in mice. J Pharmacol Exp Ther. 2008;326(2):633–45.

Mothet JP, Parent AT, Wolosker H, Brady RO Jr, Linden DJ, Ferris CD, et al. D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A. 2000;97(9):4926–31.

Mothet JP, Le Bail M, Billard JM. Time and space profiling of NMDA receptor co-agonist functions. J Neurochem. 2015;135(2):210–25.

Motoyama N, Morita K, Shiraishi S, Kitayama T, Kanematsu T, Uezono Y, et al. Relief of cancer pain by glycine transporter inhibitors. Anesth Analg. 2014;119(4):988–95.

Muller E, Bakkar W, Martina M, Sokolovski A, Wong AY, Legendre P, et al. Vesicular storage of glycine in glutamatergic terminals in mouse hippocampus. Neuroscience. 2013;242:110–27.

Nakanishi T, Kekuda R, Fei YJ, Hatanaka T, Sugawara M, Martindale RG, et al. Cloning and functional characterization of a new subtype of the amino acid transport system N. Am J Phys Cell Physiol. 2001;281(6):C1757–68.

Nakazawa K, McHugh TJ, Wilson MA, Tonegawa S. NMDA receptors, place cells and hippocampal spatial memory. Nat Rev Neurosci. 2004;5(5):361–72.

Nishikawa H, Inoue T, Izumi T, Nakagawa S, Koyama T. SSR504734, a glycine transporter-1 inhibitor, attenuates acquisition and expression of contextual conditioned fear in rats. Behav Pharmacol. 2010;21(5-6):576–9.

Nong Y, Huang YQ, Ju W, Kalia LV, Ahmadian G, Wang YT, et al. Glycine binding primes NMDA receptor internalization. Nature. 2003;422(6929):302–7.

Panatier A, Theodosis DT, Mothet JP, Touquet B, Pollegioni L, Poulain DA, et al. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. Cell. 2006;125(4):775–84.

Paoletti P, Bellone C, Zhou Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci. 2013;14(6):383–400.

Paolone G, Botreau F, Stewart J. The facilitative effects of D-cycloserine on extinction of a cocaine-induced conditioned place preference can be long lasting and resistant to reinstatement. Psychopharmacology. 2009;202(1-3):403–9.

Papouin T, Ladépêche L, Ruel J, Sacchi S, Labasque M, Hanini M, et al. Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell. 2012;150(3):633–46.

Pearlman RJ, Aubrey KR, Vandenberg RJ. Arachidonic acid and anandamide have opposite modulatory actions at the glycine transporter, GLYT1a. J Neurochem. 2003;84(3):592–601.

Pérez-Siles G, Núñez E, Morreale A, Jiménez E, Leo-Macías A, Pita G, et al. An aspartate residue in the external vestibule of GLYT2 (glycine transporter 2) controls cation access and transport coupling. Biochem J. 2012;442(2):323–34.

Peykov S, Berkel S, Degenhardt F, Rietschel M, Nöthen MM, Rappold GA. Rare SHANK2 variants in schizophrenia. Mol Psychiatry. 2015;20(12):1487–8.

Pinto MC, Simão F, da Costa FL, Rosa DV, de Paiva MJ, Resende RR, et al. Sarcosine preconditioning induces ischemic tolerance against global cerebral ischemia. Neuroscience. 2014;271:160–9.

Pinto MC, Lima IV, da Costa FL, Rosa DV, Mendes-Goulart VA, Resende RR, et al. Glycine transporters type 1 inhibitor promotes brain preconditioning against NMDA-induced excitotoxicity. Neuropharmacology. 2015;89:274–81.

Puhl MD, Berg AR, Bechtholt AJ, Coyle JT. Availability of N-methyl-d-aspartate receptor coagonists affects cocaine-induced conditioned place preference and locomotor sensitization: implications for comorbid schizophrenia and substance abuse. J Pharmacol Exp Ther. 2015;353(3):465–70.

Qian J, Noebels JL. Visualization of transmitter release with zinc fluorescence detection at the mouse hippocampal mossy fibre synapse. J Physiol. 2005;566(Pt 3):747–58.

Ramsey AJ. NR1 knockdown mice as a representative model of the glutamate hypothesis of schizophrenia. Prog Brain Res. 2009;179:51–8. doi:10.1016/S0079-6123(09)17906-2.

Rodríguez A, Ortega A, Berumen LC, García-Alcocer MG, Giménez C, Zafra F. Expression of the System N transporter (SNAT5/SN2) during development indicates its plausible role in glutamatergic neurotransmission. Neurochem Int. 2014;73:166–71.

Rose CR, Ransom BR. Mechanisms of H+ and Na+ changes induced by glutamate, kainate, and D-aspartate in rat hippocampal astrocytes. J Neurosci. 1996;16(17):5393–404.

Rosenberg D, Artoul S, Segal AC, Kolodney G, Radzishevsky I, Dikopoltsev E, et al. Neuronal D-serine and glycine release via the Asc-1 transporter regulates NMDA receptor-dependent synaptic activity. J Neurosci. 2013;33(8):3533–44.

Rousseau F, Aubrey KR, Supplisson S. The glycine transporter GlyT2 controls the dynamics of synaptic vesicle refilling in inhibitory spinal cord neurons. J Neurosci. 2008;28(39):9755–68.

Roux MJ, Supplisson S. Neuronal and glial glycine transporters have different stoichiometries. Neuron. 2000;25(2):373–83.

Rutter AR, Fradley RL, Garrett EM, Chapman KL, Lawrence JM, Rosahl TW, et al. Evidence from gene knockout studies implicates Asc-1 as the primary transporter mediating d-serine reuptake in the mouse CNS. Eur J Neurosci. 2007;25(6):1757–66.

Safory H, Neame S, Shulman Y, Zubedat S, Radzishevsky I, Rosenberg D, et al. The alanine-serine-cysteine-1 (Asc-1) transporter controls glycine levels in the brain and is required for glycinergic inhibitory transmission. EMBO Rep. 2015;16(5):590–8.

Saransaari P, Oja SS. Characteristics of hippocampal glycine release in cell-damaging conditions in the adult and developing mouse. Neurochem Res. 2001;26(7):845–52.

Sato K, Adams R, Betz H, Schloss P. Modulation of a recombinant glycine transporter (GLYT1b) by activation of protein kinase C. J Neurochem. 1995;65(5):1967–73.

Schell MJ, Molliver ME, Snyder SH. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proc Natl Acad Sci U S A. 1995;92(9):3948–52.

Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511(7510):421–7.

Schmitz Y, Castagna C, Mrejeru A, Lizardi-Ortiz JE, Klein Z, Lindsley CW, et al. Glycine transporter-1 inhibition promotes striatal axon sprouting via NMDA receptors in dopamine neurons. J Neurosci. 2013;33(42):16778–89.

Schoemaker JH, Jansen WT, Schipper J, Szegedi A. The selective glycine uptake inhibitor org 25935 as an adjunctive treatment to atypical antipsychotics in predominant persistent negative symptoms of schizophrenia: results from the GIANT trial. J Clin Psychopharmacol. 2014;34(2):190–8.

Shen HY, van Vliet EA, Bright KA, Hanthorn M, Lytle NK, Gorter J, et al. Glycine transporter 1 is a target for the treatment of epilepsy. Neuropharmacology. 2015;99:554–65.

Sheng M, Cummings J, Roldan LA, Jan YN, Jan LY. Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature. 1994;368(6467):144–7.

Shibasaki K, Hosoi N, Kaneko R, Tominaga M, Yamada K. Glycine release from astrocytes via functional reversal of GlyT1. J Neurochem. 2016; doi:10.1111/jnc.13741. [Epub ahead of print]

Singer P, Feldon J, Yee BK. The glycine transporter 1 inhibitor SSR504734 enhances working memory performance in a continuous delayed alternation task in C57BL/6 mice. Psychopharmacology. 2009a;202(1-3):371–84.

Singer P, Yee BK, Feldon J, Iwasato T, Itohara S, Grampp T, et al. Altered mnemonic functions and resistance to N-METHYL-d-aspartate receptor antagonism by forebrain conditional knockout of glycine transporter 1. Neuroscience. 2009b;161(2):635–54.

Singer P, Dubroqua S, Yee BK. Inhibition of glycine transporter 1: the yellow brick road to new schizophrenia therapy? Curr Pharm Des. 2015;21(26):3771–87.

Singh SP, Singh V. Meta-analysis of the efficacy of adjunctive NMDA receptor modulators in chronic schizophrenia. CNS Drugs. 2011;25(10):859–85.

Smith KE, Borden LA, Hartig PR, Branchek T, Weinshank RL. Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. Neuron. 1992;8(5):927–35.

Socała K, Nieoczym D, Rundfeldt C, Wlaź P. Effects of sarcosine, a glycine transporter type 1 inhibitor, in two mouse seizure models. Pharmacol Rep. 2010;62(2):392–7.

Song W, Chattipakorn SC, McMahon LL. Glycine-gated chloride channels depress synaptic transmission in rat hippocampus. J Neurophysiol. 2006;95(4):2366–79.

Spike RC, Watt C, Zafra F, Todd AJ. An ultrastructural study of the glycine transporter GLYT2 and its association with glycine in the superficial laminae of the rat spinal dorsal horn. Neuroscience. 1997;77(2):543–51.

Stevens ER, Gustafson EC, Miller RF. Glycine transport accounts for the differential role of glycine vs. D-serine at NMDA receptor coagonist sites in the salamander retina. Eur J Neurosci. 2010;31(5):808–16.

Straub H, Köhling R, Speckmann EJ. Strychnine-induced epileptiform activity in hippocampal and neocortical slice preparations: suppression by the organic calcium antagonists verapamil and flunarizine. Brain Res. 1997;773(1-2):173–80.

Strzelecki D, Podgórski M, Kałużyńska O, Gawlik-Kotelnicka O, Stefańczyk L, Kotlicka-Antczak M, et al. Supplementation of antipsychotic treatment with sarcosine – GlyT1 inhibitor – causes changes of glutamatergic (1)NMR spectroscopy parameters in the left hippocampus in patients with stable schizophrenia. Neurosci Lett. 2015;606:7–12.

Tanabe M, Takasu K, Yamaguchi S, Kodama D, Ono H. Glycine transporter inhibitors as a potential therapeutic strategy for chronic pain with memory impairment. Anesthesiology. 2008;108(5):929–37.

Tsai G, Lane HY, Yang P, Chong MY, Lange N. Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry. 2004a;55(5):452–6.

Tsai G, Ralph-Williams RJ, Martina M, Bergeron R, Berger-Sweeney J, Dunham KS, Jiang Z, Caine SB, Coyle JT. Gene knockout of glycine transporter 1: characterization of the behavioral phenotype. Proc Natl Acad Sci U S A. 2004b;101(22):8485–90.

Turecek R, Trussell LO. Presynaptic glycine receptors enhance transmitter release at a mammalian central synapse. Nature. 2001;411(6837):587–90.

Umbricht D, Alberati D, Martin-Facklam M, Borroni E, Youssef EA, Ostland M, et al. Effect of bitopertin, a glycine reuptake inhibitor, on negative symptoms of schizophrenia: a randomized, double-blind, proof-of-concept study. JAMA Psychiatry. 2014;71(6):637–46.

Vengeliene V, Leonardi-Essmann F, Sommer WH, Marston HM, Spanagel R. Glycine transporter-1 blockade leads to persistently reduced relapse-like alcohol drinking in rats. Biol Psychiatry. 2010;68(8):704–11.

Verleysdonk S, Martin H, Willker W, Leibfritz D, Hamprecht B. Rapid uptake and degradation of glycine by astroglial cells in culture: synthesis and release of serine and lactate. Glia. 1999;27(3):239–48.

Werdehausen R, Mittnacht S, Bee LA, Minett MS, Armbruster A, Bauer I, et al. The lidocaine metabolite N-ethylglycine has antinociceptive effects in experimental inflammatory and neuropathic pain. Pain. 2015;156(9):1647–59.

Whitehead KJ, Pearce SM, Walker G, Sundaram H, Hill D, Bowery NG. Positive N-methyl-D-aspartate receptor modulation by selective glycine transporter-1 inhibition in the rat dorsal spinal cord in vivo. Neuroscience. 2004;126(2):381–90.

Wiescholleck V, Manahan-Vaughan D. Long-lasting changes in hippocampal synaptic plasticity and cognition in an animal model of NMDA receptor dysfunction in psychosis. Neuropharmacology. 2013;74:48–58.

Wilcox KS, Fitzsimonds RM, Johnson B, Dichter MA. Glycine regulation of synaptic NMDA receptors in hippocampal neurons. J Neurophysiol. 1996;76(5):3415–24.

Williams K, Russell SL, Shen YM, Molinoff PB. Developmental switch in the expression of NMDA receptors occurs in vivo and in vitro. Neuron. 1993;10(2):267–78.

Xie X, Dumas T, Tang L, Brennan T, Reeder T, Thomas W, et al. Lack of the alanine-serine-cysteine transporter 1 causes tremors, seizures, and early postnatal death in mice. Brain Res. 2005;1052(2):212–21.

Yang Y, Ge W, Chen Y, Zhang Z, Shen W, Wu C, et al. Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. Proc Natl Acad Sci U S A. 2003;100(25):15194–9.

Yee BK, Balic E, Singer P, Schwerdel C, Grampp T, Gabernet L, et al. Disruption of glycine transporter 1 restricted to forebrain neurons is associated with a procognitive and antipsychotic phenotypic profile. J Neurosci. 2006;26(12):3169–81.

Zafra F, Giménez C. Characteristics and adaptive regulation of glycine transport in cultured glial cells. Biochem J. 1989;258(2):403–8.

Zafra F, Alcantara R, Gomeza J, Aragon C, Gimenez C. Arachidonic acid inhibits glycine transport in cultured glial cells. Biochem J. 1990;271(1):237–42.

Zafra F, Aragón C, Olivares L, Danbolt NC, Giménez C, Storm-Mathisen J. Glycine transporters are differentially expressed among CNS cells. J Neurosci. 1995a;15(5 Pt 2):3952–69.

Zafra F, Gomeza J, Olivares L, Aragón C, Giménez C. Regional distribution and developmental variation of the glycine transporters GLYT1 and GLYT2 in the rat CNS. Eur J Neurosci. 1995b;7(6):1342–52.

Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, et al. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature. 2016;533(7604):481–6.

Zhang LH, Gong N, Fei D, Xu L, Xu TL. Glycine uptake regulates hippocampal network activity via glycine receptor-mediated tonic inhibition. Neuropsychopharmacology. 2008;33(3):701–11.

Zhang XY, Ji F, Wang N, Chen LL, Tian T, Lu W. Glycine induces bidirectional modifications in N-methyl-D-aspartate receptor-mediated synaptic responses in hippocampal CA1 neurons. J Biol Chem. 2014;289(45):31200–11.

Zhao J, Tao H, Xian W, Cai Y, Cheng W, Yin M, et al. A highly selective inhibitor of glycine transporter-1 elevates the threshold for maximal electroshock-induced tonic seizure in mice. Biol Pharm Bull. 2016;39(2):174–80.