

# Functional crosstalk of the glycine transporter GlyT1 and NMDA receptors

Dolores Piniella <sup>a,b</sup>, Francisco Zafra <sup>a,b,\*</sup>

<sup>a</sup> Centro de Biología Molecular Severo Ochoa, Facultad de Ciencias, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid, Madrid, Spain

<sup>b</sup> IdiPAZ, Institute of Health Carlos III (ISCIII), Spain

## ARTICLE INFO

Handling Editor: Bruno Frenguelli

### Keywords:

Glutamate  
Glycine  
NMDA receptors  
Glycine transporters  
Synapse

## ABSTRACT

NMDA-type glutamate receptors (NMDARs) constitute one of the main glutamate (Glu) targets in the central nervous system and are involved in synaptic plasticity, which is the molecular substrate of learning and memory. Hypofunction of NMDARs has been associated with schizophrenia, while overstimulation causes neuronal death in neurodegenerative diseases or in stroke. The function of NMDARs requires coincidental binding of Glu along with other cellular signals such as neuronal depolarization, and the presence of other endogenous ligands that modulate their activity by allosterism. Among these allosteric modulators are zinc, protons and Gly, which is an obligatory co-agonist. These characteristics differentiate NMDARs from other receptors, and their structural bases have begun to be established in recent years. In this review we focus on the crosstalk between Glu and glycine (Gly), whose concentration in the NMDAR microenvironment is maintained by various Gly transporters that remove or release it into the medium in a regulated manner. The GlyT1 transporter is particularly involved in this task, and has become a target of great interest for the treatment of schizophrenia since its inhibition leads to an increase in synaptic Gly levels that enhances the activity of NMDARs. However, the only drug that has completed phase III clinical trials did not yield the expected results. Notwithstanding, there are additional drugs that continue to be investigated, and it is hoped that knowledge gained from the recently published 3D structure of GlyT1 may allow the rational design of more effective new drugs.

This article is part of the Special Issue on "The receptor-receptor interaction as a new target for therapy".

## 1. Introduction

In quantitative terms, glutamate (Glu) is the main neurotransmitter of the nervous system. It is estimated that 40% of neurons are glutamatergic, and more than 90% of all neurons have Glu receptors (GluRs) which control their activity (Gasiórowska et al., 2021). A large part of the motor, sensory, cognitive, behavioral and affective functions of the nervous system are regulated by brain regions and nuclei deeply interconnected by glutamatergic pathways (for a recent review see (Demchenko et al., 2022)). This includes the basic functional mechanisms of the brain such as synaptic plasticity, the molecular substrate of learning and memory. Consequently, alterations to these pathways, either due to overstimulation or to hypofunction, are associated with a wide variety of acute and chronic diseases. Epilepsy, autism spectrum diseases (ASD), depression, schizophrenia and other behavioral disorders each have important contributions from the glutamatergic system (Barker-Haliski and White, 2015; Duman et al., 2019; Javitt et al., 2011). No less important is the contribution of Glu to neuronal death associated with

stroke or trauma, chronic pain or neurodegenerative diseases such as Parkinson's, Alzheimer's, and amyotrophic lateral sclerosis (Choi, 2020; Lewerenz and Maher, 2015; Pajarillo et al., 2019). The study of glutamatergic neurotransmission is one of the most active fields in neurobiology and neuropharmacology. Over several decades, a variety of drugs have been discovered that enhance or diminish glutamatergic activity (Hansen et al., 2021). However, one must keep in mind the complexity of glutamatergic networks, in which some pathways can be activated while others are simultaneously inhibited when the brain performs a certain task. Sometimes, it is glutamate itself that controls both the activation of a pathway and the feedback mechanisms that inhibit it, through different types of receptors (Reiner and Levitz, 2018). Therefore, the pharmacological manipulation of all these processes is not always accessible to current pharmacology. However, the success of drugs such as ketamine (antagonist of one on the subunits of NMDA receptors [NMDARs]) in the treatment of depression (Yavi et al., 2022) encourages the search for new drugs that can efficiently modulate glutamatergic function.

\* Corresponding author. Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, C / Nicolás Cabrera 1, 28049, Madrid, Spain.

E-mail address: [francisco.zafra@uam.es](mailto:francisco.zafra@uam.es) (F. Zafra).

<https://doi.org/10.1016/j.neuropharm.2023.109514>

Received 29 December 2022; Received in revised form 10 March 2023; Accepted 20 March 2023

Available online 31 March 2023

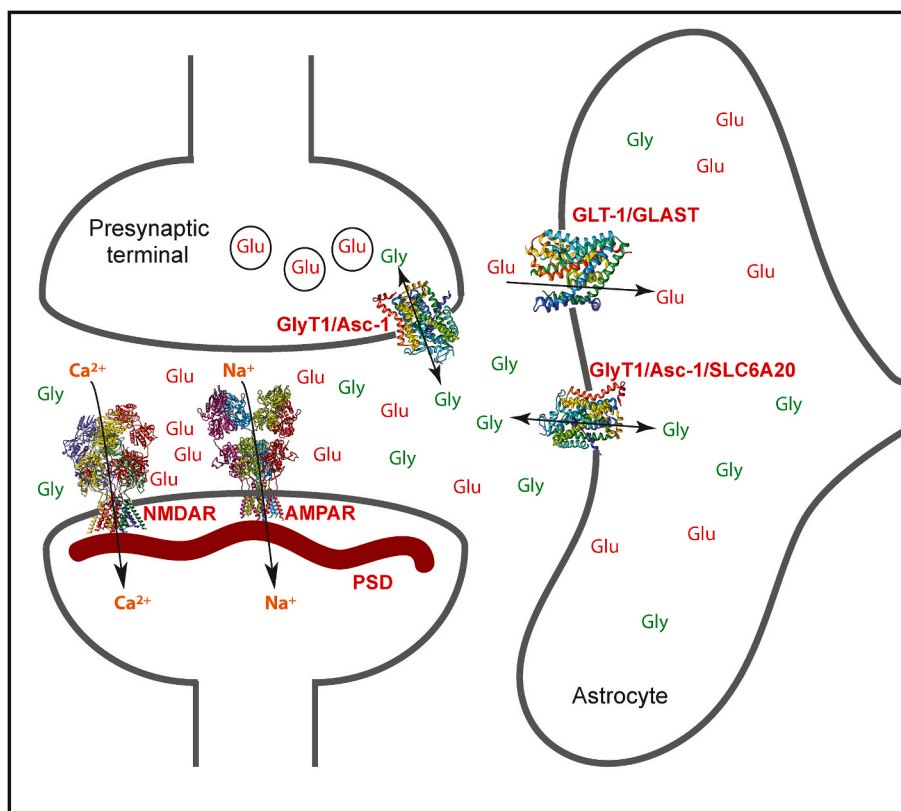
0028-3908/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Glu exerts its function by binding to its specific receptors, generally located in postsynaptic neurons, although they are frequently also located in presynaptic neurons and in adjacent glia (Bouvier et al., 2018; Verkhratsky and Chvátal, 2020) (Fig. 1). These receptors are integral membrane proteins that are classified into two categories: ionotropic (iGluR of the AMPA, Kainate, NMDA and GluR subtypes); and metabotropic (mGluR 1-5). Activation of the former is associated with changes in the ionic permeability of the membrane, while the latter are coupled to intracellular signaling pathways that regulate metabolism and cell function through second messengers (Hansen et al., 2021; Paoletti, 2011). Given the importance of these receptors in the aforementioned functions, and their involvement in pathological processes such as neuronal death due to excessive stimulation (excitotoxicity) or hypofunction (Choi, 2020; Javitt et al., 2011; Paoletti et al., 2013; Sanacora et al., 2012), the large variety of receptors offers many possibilities for pharmacological intervention.

However, Glu is not the only neurotransmitter in the brain, and it must dialogue with other transmitters and signals at different levels to carry out its various functions. In this review we will focus on the interaction between Glu and Gly (Gly), and the consequences of this dialogue on brain function and pathology. Simultaneous binding of Glu and Gly to specific cavities (site A and Gly modulatory site [GMS], respectively) that are located in several subtypes of NMDARs is necessary for their activation (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988). The relevance of the GMS has not been without controversy: After its discovery by Johnson and Ascher (1987), the significance of the finding was questioned since the affinity of NMDAR for Gly was so high that it was predicted to permanently occupy the GMS and therefore would be physiologically irrelevant (Kemp et al., 1988). Despite these early uncertainties, evidence has accumulated over the years indicating that the GMS *does* have a function, and that Gly levels, at least in some brain regions and developmental periods undergo oscillations that modulate the activity of NMDARs (Bergeron et al., 1998). The importance of the GMS has been shown *in vivo*, with mice carrying

mutations at this site displaying marked NMDAR hypofunction paralleled by deficits in long-term potentiation (LTP), spatial learning and social abilities (Ballard et al., 2002; Kew et al., 2000; Labrie et al., 2008). These and other observations support the idea that NMDAR hypofunction is a key etiological component of schizophrenia, and potentiation of NMDAR activity would be desirable as antipsychotic therapy (Javitt, 2004, 2023; Kruse and Bustillo, 2022). Similarly, there is evidence for glutamatergic dysfunction in depression (Duman et al., 2019; Sanacora et al., 2012). However, in order to increase the activity of NMDARs, direct pharmacological manipulation of the Glu binding site has proven difficult and undesirable, probably due to the similarity of the electronic structure of the Glu binding site in the various GluRs, which risks promoting neurotoxicity. In contrast, targeting the numerous NMDAR allosteric sites, which are less conserved among GluRs, offer the possibility both to enhance and to decrease their activity (Zhu and Paoletti, 2015). Regarding the GMS, both direct and indirect stimulation has been attempted as an antipsychotic and/or antidepressant strategy. Direct modulation has been tried by treatments with endogenous ligands (Gly, D-serine and D-cycloserine). These compounds have been used in preclinical and clinical trials with abundant evidence for antipsychotic, antidepressant and procognitive effects (revised by (Pei et al., 2021; Peyrovian et al., 2019). A synthetic compound, rapastinel (previously known as GLYX-13) was initially described as a partial agonist of the GMS, and had antidepressant and procognitive effects, but recent evidence indicated that the binding site is distinct from the GMS (Donello et al., 2019).

Indirect stimulation refers to manipulations that increase Gly or D-serine levels in the environment of NMDARs. Regulation of D-serine has been reviewed previously and is mediated mainly by Asc-1, a neutral amino acid transporter (Coyle et al., 2020). Regarding Gly, its levels are regulated by distinct mechanisms in the different regions of the central nervous system (CNS). In caudal areas of the CNS such as the spinal cord, the brainstem, or the cerebellum, Gly does act as a conventional neurotransmitter, being released from glycinergic neurons and acting on



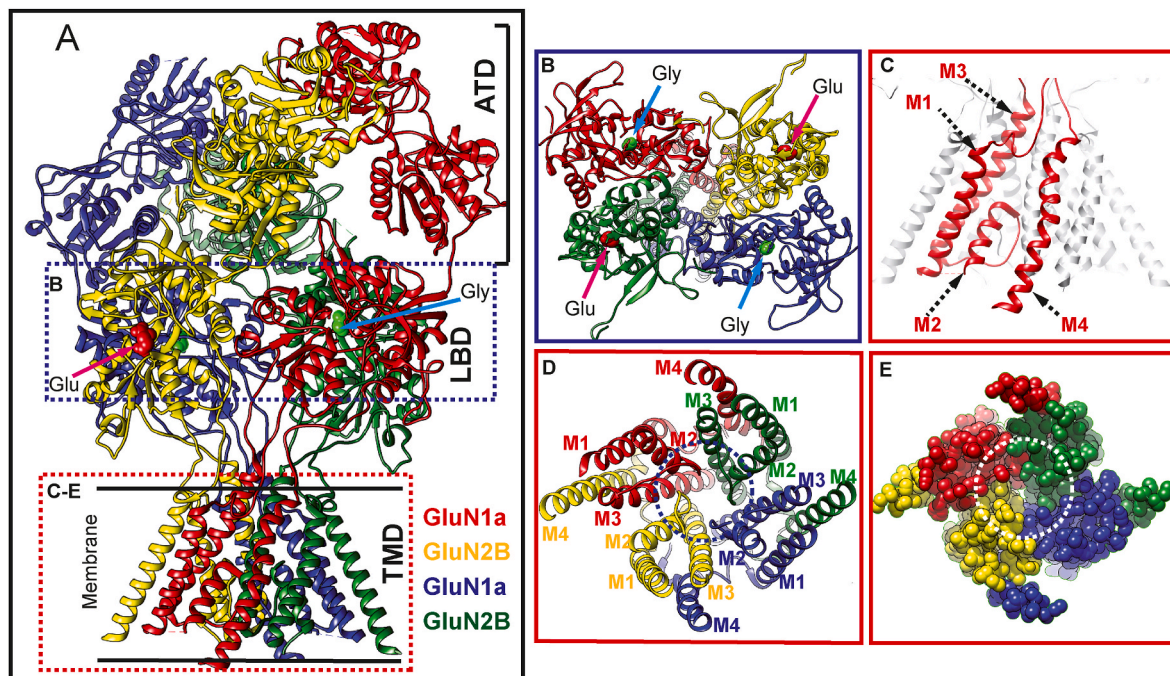
**Fig. 1. Scheme of a glutamatergic synapse and main glutamate and glycine receptors and transporters at the plasma membrane of neurons and astrocytes.** Glutamate is released upon depolarization of presynaptic neurons and binds to GluR (AMPA and NMDAR, among others), mainly located in the postsynaptic membrane, where they control the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  flux. NMDAR also need glycine (Gly) for activation. Glu is removed from the extracellular compartment by glutamate transporters (mainly glial GLT-1 and GLAST). Glycine is regulated by glial and neuronal glycine transporters, mainly GlyT1, although Asc-1 and SLC6A20 might also contribute.

a postsynaptic inhibitory Gly receptor (GlyR). The synaptic concentration of glycine is then reduced by glycine transporters located in glial cells and presynaptic neurons (Fig. 1). However, there is no evidence for glycinergic innervation in cortical areas of the forebrain, and consequently, the glycine levels in the extracellular milieu of these major glutamatergic areas depend exclusively on fluxes of this amino acid mediated by glycine transporters. In this review we will focus first on recent advances in the structure and function of the NMDAR, especially the GMS, continuing with Gly transporters, and ending with a brief review of the drugs that affect this Glu-Gly dialogue and that are in various phases of clinical trials.

## 2. Structure of NMDA receptors and role of the gly binding site

All iGluRs share a series of molecular characteristics, although the NMDAR presents clearly differential features. The structures of all of them are extensively covered in excellent reviews, so we will deal only with aspects related to the crosstalk between Glu and Gly in the NMDAR (Greger and Mayer, 2019; Hansen et al., 2021; Stroebel and Paoletti, 2021). A typical iGluR consists of the oligomeric assembly of four subunits, all of which are integral membrane proteins. Each subunit has the following domain organization: 1. Two bulky extracellular domains, one that contains the ligand binding site (LBS) and another that contains the binding site for allosteric modulators; 2. A transmembrane domain that creates a pore for ion permeation; 3. A cytoplasmic domain of variable length that regulate trafficking and interactions with other regulatory cellular elements. The channel opens or closes in a similar way to the iris of the eye, depending on the strain it receives from the LBS, regulating the permeability of  $\text{Na}^+$  or  $\text{Ca}^{2+}$  ions. Specifically, NMDARs are obligate heterooligomers and generally are made up of two types of subunits: two of the GluN1 class and two of the GluN2 class (Fig. 2). Alternative NMDARs are made of two GluN1 and two GluN3 subunits, and significant portion of native NMDARs seems to be triheteromers containing

two GluN1 and two different GluN2 (or a combination of GluN2 and GluN3) (Stroebel et al., 2018). This composition of subunits helps to generate structural diversity, since there are several isoforms for each subunit that combine to produce oligomers with different properties. Thus, for the NMDAR, there are eight GluN1 isoforms, generated by alternative splicing that assemble with one of the four types of GluN2 subunit (-A, -B, -C, -D) or one of the two GluN3 subunits (-A, -B) (Paoletti, 2011). The assembly of functional receptors is based on the level of expression of each subunit, which depends on epigenetic, transcriptional, and post-transcriptional mechanisms. In this manner, a large variety of receptors (up to 756 distinct combinations) with different properties can be obtained (Iacobucci and Popescu, 2017). For instance, the associations of GluN1 with GluN2A or GluN2B are the most abundant in the adult brain (Paoletti et al., 2013), whereas the combination GluN1/GluN2C is expressed postnatally in discrete regions, such as cerebellar granule cells, spinal cord, and cortical interneurons. GluN1/GluN2D is expressed preferentially during the early brain development but becomes restricted to selected neurons such as interneurons in the hippocampus, cortex, thalamus, basal ganglia, and cerebellum in adult brains (see (Chou et al., 2022b) and references therein). Different subunit combinations confer distinct electrophysiological properties, affecting genuine characteristics of NMDARs such as  $\text{Ca}^{2+}$  permeability or sensitivity to ion channel blockade by  $\text{Mg}^{2+}$ . Combinations containing GluN2A or GluN2B show a greater blockade by  $\text{Mg}^{2+}$ , greater  $\text{Ca}^{2+}$  permeability, single-channel conductance, and open probability than combinations containing GluN2C or GluN2D (Cull-Candy and Leszkiewicz, 2004; Dravid et al., 2008; Siegler Retchless et al., 2012). NMDAR subunits have two ligands: GluN2 subunits bind Glu, while GluN1 and GluN3 bind Gly. Both neurotransmitters are obligatory co-agonists whose simultaneous binding is necessary for ion channel opening (although Gly can be substituted for D-serine or other less abundant D-amino acids (Oliet and Mothet, 2009; Seckler and Lewis, 2020)). Indeed, subunit composition also impacts the ability to use Gly or



**Fig. 2.** 3D structure of heterotetrameric GluN1a-GluN2B NMDAR bound to glutamate and glycine. (A) Domain organization of GluN1a (red and blue subunits) and GluN2B (yellow, and green), showing the Amino Terminal Domain (ATD), the Ligand Binding Domain (LBD) and the Transmembrane Domain (TMD). Agonists, glycine (Gly) and glutamate (Glu), bound to the LBD are shown as green and red spheres, respectively. (B) Top view of the LBD, showing the interphase between subunits. (C) Side view of the transmembrane helices (TMD). Features of one of the GluN1a subunits are highlighted. M3 helices line the extracellular part of the permeation pathway, whereas the M2 loops line the intracellular part. (D) Ribbon representation of a view of the channel from the intracellular side. (E) Sphere representation of the ionic channel from the intracellular side. Data were obtained from the Protein Data Bank (PDB) ID: 7SAA. Primary publication (Chou et al., 2022a). The diagram was generated using Chimera 1.16 software.



D-serine as a ligand. Whereas Gly appears to be the preferred co-agonist in receptors containing GluN2B subunits, D-serine is preferred by those containing GluN2A. This may explain both regional and developmental differences that have been described for the preference of one or the other endogenous co-agonist (Fossat et al., 2012; Le Bail et al., 2015; Papouin et al., 2012) ... ..

Nevertheless, contrary to this double-ligand requirement, receptors formed by GluN1/GluN3 can be activated solely in the presence of Gly (or D-serine), becoming excitatory receptors for Gly (Chatterton et al., 2002). GluN1/GluN3 receptors have been associated with functions related to brain maturation that occurs with the arrival of sensory stimuli (Larsen et al., 2014; Murillo et al., 2021). In fact, their expression peaks at the end of the first postnatal week in rodents, and it is thought that they delay the maturation of synapses until the arrival of sensory stimuli (revised by (Pérez-Otaño et al., 2016)). However, in some brain regions GluN1/GluN3 receptor expression remains in adulthood, most notably in nuclei of the amygdala, medial habenula, association cortices and high-order thalamic nuclei (Murillo et al., 2021). Indeed, recent observations revealed that expression of GluN3A subunits controls the excitability of mouse adult cortical and amygdalar circuits via an unusual signaling mechanism involving the formation of excitatory Gly GluN1/GluN3 receptors and their tonic activation by extracellular Gly (see section 3, Gly transporters) (Bossi et al., 2022).

The discovery of the specific characteristics of each iGluR began from the cloning of the different subunits in the early 90s, but functional details of these molecular machines were not known until structural data became available beginning in the 2000s. The first studies that provided information on the NMDAR 3D structure focused on isolated receptor domains, given the difficulties in crystallizing the complete structure (Furukawa, 2012; Furukawa et al., 2005; Furukawa and Gouaux, 2003; Jespersen et al., 2014; Karakas et al., 2009, 2011; Vance et al., 2011; Yao et al., 2008, 2013). The atomic structure of a complete NMDAR was finally solved in 2014 after numerous technical improvements, in two studies that showed their domain-layered architecture (Karakas and Furukawa, 2014; Lee et al., 2014). Indeed, all ionotropic receptors share a similar layered organization in the extracellular region, reflecting a common evolutionary origin, despite the early separation of NMDARs in the phylogenetic tree (Ramos-Vicente et al., 2018, p.; Stroebel and Paoletti, 2021). The extracellular portion, starting at the furthest point from the membrane, is placed the amino terminal domain (ATD), which contains sites for several allosteric effectors, such as zinc and polyamines, and participates in the control of ion channel open probability and deactivation kinetics (Gielen et al., 2009; Hansen et al., 2010; Yuan et al., 2009) (Fig. 2). Next comes the LBD, a globular region folded with a clamshell structure that closes in the presence of the ligand. As already mentioned, the LBD of NMDARs participates in binding of the two co-agonists. Glutamate fits into a pocket located in GluN2 that shuts in the manner of a Venus flytrap when the neurotransmitter binds. Similarly, the GluN1 subunit houses the Gly binding site (GBS), which also causes a conformational change that traps this amino acid in the corresponding cleft (Fig. 2A and B). The binding sites of both ligands dialogue by negative allosteric mechanisms, such that Gly binding is transmitted to the Glu binding site as a conformational change that decreases its affinity, and vice versa (Mayer et al., 1989; Regalado et al., 2001). These movements are transmitted across the interface between the LBDs of GluN1 and GluN2 (Durham et al., 2020). The essential role of GMS in the function of NMDARs was demonstrated in transgenic mice carrying mutations at this site, in which NMDAR hypofunction was observed. These mice have been proposed as models of schizophrenia since their behavioral alterations are similar to those observed in patients with this disease (Ballard et al., 2002; Labrie et al., 2008). Likewise, a *de novo* mutation in the glycine binding site has been described in humans, with consequences not only for glycine binding, but also for desensitization kinetics, calcium permeability and trafficking to the cell surface of some oligomers (those containing GluN3) (Skrenkova et al., 2020).

Subsequently, connected by a short linker to the LBD, is located the membrane insertion domain, also called the transmembrane domain (TMD). The peptide chain of each subunit crosses the membrane three times with helices M1, M3 and M4, to which must be added a re-entrant loop, M2 (Fig. 2C). Part of these helices create a transmembrane pore in the center of the tetrameric assembly. The pore is lined by hydrophilic residues on M3 in the part closest to the cell exterior, and by M2 in the innermost part of the membrane (Traynelis et al., 2010) (Fig. 2C–E). This hydrophilic channel can open or close depending on the state of occupation of the LBD and contains the necessary elements to select Na<sup>+</sup> or Ca<sup>2+</sup> ions. This region of NMDARs also controls the Mg<sup>2+</sup> block of the receptor when the cell is at rest. This block occurs from the cytosolic face and is only removed when the neuron depolarizes because of activation of non-NMDA receptors located in the vicinity of the NMDARs. Thus, Ca<sup>2+</sup> conductance regulated by NMDARs occurs in a late phase of neuronal activation. In other words, this property converts NMDARs into Hebbian-type coincidence detectors, which is necessary for the development of synaptic plasticity (Mayer et al., 1984; Nowak et al., 1984).

Lastly in the layered structure of NMDARs is the C-terminal domain (CTD), which is longer than that of other iGluRs and contains sequences that may anchor them to postsynaptic densities and other postsynaptic proteins involved in regulating processes such as intracellular trafficking or lateral mobility in the membrane (Groc and Choquet, 2020; O'Brien et al., 1998; Prybylowski and Wenthold, 2004).

Although the best-known effects of NMDAR activation are those mediated by changes in ionic permeability, it should be noted that there are several ion-independent actions of these receptors. Examples of these “metabotropic” actions of NMDARs were anticipated by (Vissel et al., 2001), who demonstrated that Glu binding to the NMDAR enhanced tyrosine dephosphorylation of the GluN2A subunit, independent of ion flux. More recently, it has been shown that the binding of ligands promotes conformational movements of the GluN1 CTD in the absence of ionic flow (Dore et al., 2015). These metabotropic actions are essential for control of plastic processes such as long-term depression (LTD), which is in turn dependent on signaling pathways and morphological changes in dendritic spines (Nabavi et al., 2013; Stein et al., 2015, 2020, 2021; Stein and Zito, 2019).

Furthermore, these actions are not only initiated by glutamate. Gly binding to GluN1 primes NMDARs for AP2-clathrin-mediated and dynamin-dependent endocytosis (Nong et al., 2003), although this is critically dependent on the absence of the N1 alternatively spliced cassette in the ATD of GluN1 (Li et al., 2021). Gly was also shown to decrease the lateral mobility of GluN2A-, but not GluN2B-containing NMDARs, independent of NMDAR currents (Ferreira et al., 2017; Papouin et al., 2012). Similarly, Gly acting on receptors containing GluN2A induced a potentiation of AMPAR currents through the activation of ERK1/2 signaling (Li et al., 2016).

The NMDAR structure has been refined over the last few years, and 3D structures have been solved for the receptors bound to Glu and Gly as well as to various agonist or antagonist drugs, providing well founded hypotheses about the mechanism of activation, inhibition, desensitization and allostery (Krieger et al., 2019; Zhu and Gouaux, 2017). Perhaps the greatest recent advances have come from single-particle cryo-electron microscopy, which although providing lower structural resolution than X-ray crystallography, yields a dynamic view of the receptor properties (Tajima et al., 2016; Zhu et al., 2016). Using this technique, the binding mode of some pharmacologically relevant compounds such as phencyclidine (PCP, a drug of abuse), ketamine (an antidepressant) or nemantine (used in Alzheimer's treatment) has recently been resolved (Chou et al., 2022a). All of them are channel blockers, and this structural knowledge could provide the basis for the assisted development of therapeutic drugs with fewer side effects. Structural details also help to explain the functional properties of NMDARs, such as the effect of the presence of exon 5A in GluN1 (Regan et al., 2018) or the effect of GluN2C or GluN2D subunits in association with GluN1, which contrasts

what was previously described for the most abundant GluN1/GluN2A or GluN1/GluN2B pairings (Chou et al., 2022b). It has been possible to observe details on conformational changes and inter-subunit and inter-domain reorientation leading to agonist-gating and subunit-dependent competitive inhibition (Chou et al., 2020), the conformational effects of anti-NMDAR blocking antibodies (Tajima et al., 2022) or the conformational effects of zinc and protons, two physiological allosteric modulators of NMDARs (Jalali-Yazdi et al., 2018). However, there is still room for improvement, through the application of other technologies that do not need to work at cryotemperatures and in the presence of detergents, which might lead to non-physiological dissociations or deformations in the molecules. Methods such as luminescence resonance energy transfer (LRET) and single-molecule fluorescence resonance energy transfer (smFRET) (MacLean et al., 2019; Wang and Furukawa, 2019) have the advantage of operating at room temperature, and, therefore, provide data of higher physiological relevance. Nevertheless, many questions remain open in the structural field, such as the importance of unconventional assemblies of NMDAR subunits such as triheteromer assemblies (Stroebel et al., 2018).

### 3. Gly transporters in the nervous system

As already mentioned, Gly plays the dual role of inhibitor and activator in neurotransmission. In the caudal regions of the CNS (spinal cord, brain stem, and cerebellum) and in the retina, it acts primarily as an inhibitor. Accumulating in the synaptic vesicles of glycinergic neurons, it is released by their depolarization and then binds to the postsynaptic GlyR, historically known for its sensitivity to strychnine (Dutertre et al., 2012). This activity is essential for the control of motor and sensory neurons in these areas and, therefore, in processes such as locomotion, breathing or the transmission of pain signals. As explained in the previous section, Gly acts as a co-agonist of the NMDAR in all regions of the CNS and, therefore, in excitatory neurotransmission (Harvey and Yee, 2013). However, glycinergic and glutamatergic neurons only coexist in caudal regions, and synapses containing terminals of both neurotransmitters are rare. Therefore, the dialogue between Gly and Glu cannot follow classical neurotransmission mechanisms. In these caudal areas, Gly can reach glutamatergic synapses by spillover from nearby glycinergic synapses (Ahmadi et al., 2003), but the generic mechanism for regulating Gly levels in glutamatergic synapses of regions without this type of terminal are the Gly transporters. These proteins generally function by removing Gly from the extracellular milieu to concentrate it within neurons and glia, using the energy of transmembrane ionic gradients. However, increasing evidence support that under some circumstances, Gly can be released from astrocytic stores through reversal of the transporters. For instance, as mentioned in section 2, stimulation of the thalamic afferents of the lateral amygdala promoted the GlyT1-dependent release of Gly, which resulted in the stimulation of both conventional NMDARs and excitatory Gly GluN1/GluN3A-containing receptors (Bossi et al., 2022; Li et al., 2013). Keep in mind that the reversal of Gly transport requires reversion of the ionic gradients, and this could be achieved by depolarization of glial AMPA receptor and/or by signaling mechanisms, such as activation of astrocytic dopamine receptors (Adermark et al., 2022; Nimitvilai-Roberts et al., 2021; Shibasaki et al., 2017). However, it remains to be shown whether this reversal occurs *in vivo* under physiological stimuli. Regardless of how frequently the transporter-mediated efflux process contributes to synaptic physiology, we find it biophysically more plausible that the increase in extracellular Gly concentration might occur by regulated cessation of the forward activity of Gly transporters. The contribution of the glutamatergic terminals themselves is also yet to be established, since one Gly transporter is concentrated within them (see next section), placed in a privileged location to regulate synaptic Gly (Musante et al., 2011). In addition, several additional transporters might contribute to the equilibrium between uptake and efflux of Gly. Indeed,

at least four Gly transporters have been described: the sodium-dependent Gly transporter-1 (GlyT1, SLC6A9) and -2 (GlyT2, SLC6A5), the alanine-serine-cysteine transporter-1 (Asc-1, SLC7A10) and the Gly/proline transporter SLC6A20.

#### 3.1. Localization and function of Gly transporters

##### 3.1.1. Localization of GlyT1 transporters

Among the four Gly transporters, GlyT1 and GlyT2 seem to play a major role in controlling Gly levels. They are encoded by two genes, now called *SLC6A9* and *SLC6A5*, identified in the early 90s using homology cloning techniques (Borowsky et al., 1993; Guastella et al., 1992; Liu et al., 1993; Smith et al., 1992). They share approximately 50% sequence identity and are also homologous to other neurotransmitter transporters, like those of GABA, dopamine, serotonin, or norepinephrine, defining the SLC6 gene family. Nevertheless, GlyT2 has a distinctive feature in its cytoplasmic N-terminal domain, which is larger than in any other related transporter (Liu et al., 1993). GlyT1 and GlyT2 accumulate Gly within the cell using the Na<sup>+</sup> electrochemical gradient as an energy source, and they also require the presence of Cl<sup>-</sup>, which is co-transported with Na<sup>+</sup> and Gly. Both transporters are abundantly expressed in glycinergic regions of the spinal cord, brain stem, and to a lesser extent in the cerebellum, but GlyT1 and GlyT2 are not redundant, neither in their cellular location nor in their function. GlyT2 is mainly expressed in glycinergic neurons of these caudal areas (although sparse expression in the hippocampus has been reported) (Danglot et al., 2004). However, GlyT1 is preferentially located in glial cells in these same caudal regions. GlyT1 is also present at lower concentrations in the rest of the brain, both in glia and in terminals of glutamatergic neurons, defined by the vGLUT1 marker (vesicular Glu transporter-1) (Cubelos et al., 2005, 2014). The use of radiotracers for *in vivo* positron emission tomography has confirmed GlyT1 in glutamatergic pathways of both rodents and primates, even though at a spatial resolution lower than immunohistochemistry (Fuchigami et al., 2011; Herdon et al., 2010; Hoffmann et al., 2021; Passchier et al., 2010; Zeng et al., 2008). Unfortunately, the only antibody available for detecting neuronal forms of GlyT1 by immunohistochemistry in rats (Cubelos et al., 2005) does not cross-react with mouse or human GlyT1. In the retina, the distribution of these transporters does not follow this scheme; there, glycinergic amacrine neurons do not express GlyT2, but rather GlyT1, and there is no evidence of a glial localization of GlyT1 (Eulenburg et al., 2018; Pow and Hendrickson, 1999; Zafra et al., 1995).

More recent evidence indicated that GlyT1 and GlyT2 are not the only transporters that contribute to Gly fluxes in the brain under physiological conditions. The Asc-1 transporter (encoded by gene *SLC7A10*) is capable of transporting Gly as well as the D- and L-series of the neutral amino acids like serine, alanine, or cysteine. This transporter is a heteromeric protein, with a heavy subunit (termed 4F2hc/SLC3A2) involved in trafficking to the cell surface of the heterodimer, and a catalytic one, which translocates the substrate in a sodium-independent manner (Fukasawa et al., 2000). This sodium independence allows a bidirectional flux of substrates through the plasma membrane, depending on their concentration gradient. Moreover, Asc-1 can perform hetero-exchange among the different substrates, which might contribute to equilibration of substrates on both sides of the membrane, according to cellular needs. It has been shown that Asc-1 mediates the efflux of both Gly and D-serine from brain slices and primary neuronal cultures, contributing to modulate the activity of the GMS on NMDARs (Rosenberg et al., 2013). Initial immunohistochemical staining localized Asc-1 to neuronal structures including dendrites and nerve terminals (Helboe et al., 2003; Matsuo et al., 2004), but later it was preferentially associated with glia, with enrichment in caudal areas (Ehmsen et al., 2016). This glial isoform might mediate efflux of Gly from astrocytes for its subsequent capture by GlyT2 in glycinergic neurons of the brainstem and spinal cord (Ehmsen et al., 2016). Nevertheless, it remains to be determined if this applies also to forebrain regions, where it has been

shown that neuronal forms of Asc-1 might contribute to NMDAR function by releasing D-serine and perhaps also by setting the extracellular glycine levels. Thus, the balance between D-serine and glycine, adjusted by this transporter, may be a determinant in the different responses observed in paradigms such as LTP or LTD (Safory et al., 2015; Sason et al., 2017).

Finally, a fourth transporter, SLC6A20, is reportedly involved in maintenance of Gly homeostasis, (Bae et al., 2021). Initially this was classified as an orphan transporter (rB21 or XT3) (Nash et al., 1998; Smith et al., 1995), but later it was characterized as a Na<sup>+</sup>-dependent imino acid transporter, transporting proline and hydroxyproline (Kowalczyk et al., 2005; Takanaga et al., 2005). Its involvement in Gly transport was rather surprising, since it showed no such activity in *Xenopus* oocytes injected with the SLC6A20 mRNA (Kowalczyk et al., 2005; Takanaga et al., 2005). However, HEK293 cells expressing SLC6A20 transported Gly and proline with similar efficiency, and a lack of this gene in the brain increased Gly levels in the extracellular milieu, while its overexpression decreased them, the latter correlating with a decrease in NMDAR activity (Bae et al., 2021). SLC6A20 has expression that extends throughout the brain, more abundant in astrocytes and microglia, and with modest expression in glutamatergic neurons. Additional work is undoubtedly required to uncover the mechanisms by which this protein participates in regulation of brain Gly levels in the environment of NMDARs. The brain expresses a series of additional transporters for neutral amino acids that could potentially transport Gly, but their role in the regulation of NMDARs is unknown (López-Corcuera et al., 2017). Whereas the involvement of SLC6A20, Asc-1 or other transporters in Gly flux has relatively little experimental support, there is much more evidence for GlyT1 and GlyT2 deriving from genetic and pharmacological experiments.

### 3.1.2. Glycine transporters in inhibitory neurotransmission

Deletion of the SLC6A9 (GlyT1) gene in mice resulted in the death of the pups on the first day of life because of an inability to breathe properly. Respiratory activity was depressed due to an increased accumulation of extracellular glycine and, consequently, a sustained activation of inhibitory GlyRs and over-inhibition of the respiratory network (Gomez et al., 2003a). However, GlyT2 KO mice were able to live longer, until the second postnatal week, although with notable deficits in their neural function (tremor, muscular spasticity, and impaired motor coordination) (Gomez et al., 2003b). These dysfunctions were due to a reduction in the amount of Gly available for release in glycinergic neurons, implying that the main function of GlyT2 is to maintain adequate levels of Gly for synaptic vesicle refilling. Similar dysfunctions are observed in patients suffering from hyperekplexia, a rare human disease caused by mutations either in GlyR or in GlyT2 (Harvey and Yee, 2013; López-Corcuera et al., 2019). This accumulative role of GlyT2 is consistent with its abnormal stoichiometry, in which the cellular import of one molecule of Gly is coupled to that of 3 Na<sup>+</sup> (Roux and Supplisson, 2000), allowing the formation of a much steeper gradient of the amino acid than that obtained with GlyT1, where Gly is coupled to 2 Na<sup>+</sup>. Studies on the structure/function relationship have identified Gly binding sites and coupled ions, although the location of the permeation pathway for the third sodium is still debated (Benito-Muñoz et al., 2018; Le Guellec et al., 2022; Subramanian et al., 2016).

### 3.1.3. GlyT1 in glutamatergic function. Genetic and pharmacological evidence

The role of glycine in NMDAR function was more difficult to demonstrate, as some of the data obtained with genetically modified mice were contradictory. In addition, interpretation of these results was further complicated by the possible alternative use of D-serine by the GMS of NMDARs. However, over time, genetic and pharmacological evidence has accumulated in favor of the existence of a dialogue between NMDARs and glycine, controlled by transporters, especially GlyT1.

Regarding the genetic data, the link between GlyT1 and glutamatergic function has been demonstrated in heterozygous KO mice and in conditional KO mice. In hippocampal slices from heterozygous KO mice, the normal NMDAR stimulatory response resulting from addition of exogenous Gly was lost. This is compatible with saturation of the GMS resulting from a 50% decrease in GlyT1, and consequently the ability to remove Gly from the extracellular medium. These mice also had enhanced memory retention and were protected against amphetamine disruption of sensory gating (Tsai et al., 2004b). On the other hand, mice with conditional forebrain global GlyT1 deletion and forebrain neuronal specific deletion also showed signs of enhanced cognitive function (Yee et al., 2006). Both mice showed complex behavioral patterns including increased memory for object recognition, as well as increased resistance to the effects of the NMDAR inhibitor PCP (which causes hyperlocomotion) (reviewed by (Möhler et al., 2011)). Conditional GlyT1 KOs have also been generated in neurons that did not show effects on NMDA receptors, although the reasons for these discrepancies are not clear (Eulenburg et al., 2010). Furthermore, in mice with selective deletion of GlyT1 in glia, glycinergic symptoms predominated during postnatal development, with most dying due to overstimulation of GlyR, much like in homozygous KO. The few mice from this line that survived to adulthood did not show motor symptoms, suggesting the existence of a compensatory mechanisms (Eulenburg et al., 2010).

Further evidence supporting the involvement of GlyT1 in glutamatergic function has been obtained after treatment with specific inhibitors. The possible involvement of GlyT1 in widely prevalent diseases such as schizophrenia, ASD, obsessive-compulsive disorder, or even in Alzheimer's cognitive impairment, triggered an interest by important pharmaceutical companies in the search for new drugs. The latest advances are collected in the exhaustive review by Cioffi and Guzzo (2016) and summarized in Table 1, so here we will only comment on the proof-of-concept and their use in clinical trials, as well as some recent developments. In theory, an increase in extracellular Gly concentration would potentiate NMDAR activity only in the synapses in which these receptors are active, as Glu release converges there with the pharmacological increase in Gly levels. However, one concern about the use of these inhibitors is the potential affectation of GlyR-regulated sensory and motor pathways. In addition, GlyT1 is located in other cell types outside of the CNS such as erythrocytes, where it participates in supplying Gly for heme synthesis, which could be reduced by these compounds (Halloy et al., 2021; Matte et al., 2019).

The first group of compounds to be developed was based on the structure of sarcosine (N-methyl-Gly), which specifically inhibited GlyT1, but not GlyT2. Indeed, when co-administered with antipsychotic drugs, sarcosine reduced negative symptoms and cognitive impairment in schizophrenic patients as well as drug-naïve acute schizophrenic patients (Chang et al., 2020; Lane et al., 2006, 2010; Tsai et al., 2004a). The first sarcosine-based inhibitor was (±)-N-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine (NFPS) (the R-enantiomer is named ALX5407), which showed non-competitive binding with high affinity and slow dissociation, leading to prolonged elevation in synaptic Gly concentrations in brain and modulation of NMDAR-mediated responses in several animal models, including enhanced LTP as well as to improved behavioral performance in diverse test for associative learning, spatial and object memory, and social memory (Atkinson et al., 2001; Aubrey and Vandenberg, 2001; Hashimoto et al., 2008; Karasawa et al., 2008; Kinney et al., 2003; Mallorga et al., 2003; Mao et al., 2009; Shimazaki et al., 2010). However, at high doses this compound produced ataxia and depressed respiratory function due to potentiation of GlyR activity in caudal areas of the CNS (Perry et al., 2008). Nevertheless, NFPS is still widely used in animal experiments, which recently revealed a new application of potential importance in ischemic stroke. NFPS decreased the infarct volume and decreased motor behavioral deficits when injected before or even after the ischemic episode (photothrombosis or endothelin-1 models in mice) (Cappelli et al., 2022). Paradoxically, NMDAR-mediated responses were reduced, an effect that was attributed to the long-known phenomenon of NMDAR endocytosis triggered by high Gly (Nong et al.,

**Table 1**  
GlyT1 inhibitors.

Compound	Also known as	Clinical trials (Phase/Disease/ NTC number)	Reference or patent number
<b>sarcosine based GlyT1 inhibitors</b>			
(±)-N-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl] sarcosine	NFPS		Mallorga et al. (2003)
(R)-N-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl] sarcosine	ALX-5407		Atkinson et al. (2001)
(r,s)-(+/-)-N-methyl-N-[(4-trifluoromethyl)phenoxy]-3-phenyl-propyl-glycine	Org 24461		Brown et al. (2001)
(r)(+/-)-N-methyl-N-[(4-trifluoromethyl)phenoxy]-3-phenyl-propyl-glycine	Org24598		Le Pen et al. (2003)
N-[3-phenyl-3-(4'-(4-toluoyl)phenoxy)-propyl]sarcosine	NPTS		Lowe et al. (2003)
indandione-sarcosine derivatives	series of compounds		Thomson et al. (2006)
2-arylsulfanylphenyl piperazinyl acetic acid derivatives	series of compounds		Smith et al. (2004)
2-arylsulfanylphenyl-1-oxyalkylamino derivatives	series of compounds		Smith et al. (2006)
N-{3-(4-Chlorophenyl)-3-[4-(1,3-thiazole-2-carbonyl)phenoxy]propyl}-N-methylglycine	CP-802,079		Martina et al. (2004)
N-[2-[4-(1,3-Benzodioxol-5-yl)-2-(1,1-dimethylethyl)phenoxy]ethyl]-N-methylglycine hydrochloride	LY 2365109		Perry et al. (2008)
4-[(R)-Phenyl[3-(trifluoromethyl)phenyl]methyl]-1-piperazineacetic acid	AMG 747/Tilapertin	II/SCZ/01568229, 01568216	Dunayevich et al. (2017)
2-[[[(1R,2S)-6-Methoxy-1-phenyl-1,2,3,4-tetrahydronaphthalen-2-yl]methyl-methylamino]acetic acid	Org25935/SCH 900435/ MK-8435	II/SCZ/00725075 II/AUD/00764660 II/PAD/00725725	Molander et al. (2007)
<b>Non-sarcosine GlyT1 inhibitors</b>			
<b>Methylphenidate-Derived GlyT1 inhibitors</b>			
2-chloro-N-[phenyl(piperidin-2-yl)methyl]-3-(trifluoromethyl)benzamide	SSR504734		(Depoortère et al., 2005; Singer et al., 2013)
N-[(S)-(3S)-1-Azabicyclo[2.2.2]oct-3-yl(phenyl)methyl]-2,6-dichloro-3-(trifluoromethyl)benzamide	SSR103800		Boulay et al. (2008)
Derivatives of SSR103800	series of compounds		WO 2010/065701 A1, WO 2008/037881 A2, WO 2010/092286A1, WO 2010/092287 A1, WO 2008/018639
2-methoxy-N-[(1R)-2-methyl-1-phenyl-2-(1-pyrrolidinyl)propyl]-4,6-bis(trifluoromethyl)-Benzamide	GSK1018921	I/SCZ/00929370	Ouellet et al. (2011)
N-[(S)-[1-(dimethylamino)cyclopentyl](phenyl)methyl]-2,6-dimethylbenzamide	GSK931145		WO 2006/067414 A2
3-chloro-N-[[3-(1-ethyl-1H-pyrazol-4-yl)phenyl](1-hydroxycyclohexyl)methyl]-4-(trifluoromethyl)pyridine-2-carboxamide	example of a series based on GSK931145		WO 2014/199960 A1
N-cycloalkylmethyl and Noxetanylmethyl derivatives of GSK931145	series of compounds		WO 2010/020548 A1
3-Amido-3-aryl-piperidines series ring constrained variation of GSK1018921	series of compounds		Pinard et al. (2018)
Tetrahydro-pyran derivatives	series of compounds		WO 2011/095434 A1
<b>Alkyl and Heteroaromatic Substituted Sulfonamide and Sulfone GlyT-1 Inhibitors</b>			
(S)-2-amino-4-chloro-N-(1-(4-phenyl-1-(propylsulfonyl)piperidin-4-yl)ethyl)benzamide	ACPPB		Lindsley et al. (2006)
2,4-dichloro-N-[[1-(cyclopropylmethyl)-4-[(cyclopropylmethyl)sulfonyl]cyclohexyl)methyl]-benzamide	DCCyB		Blackaby et al. (2010)
Alkylsulfonyl-2,3-dihydrospiro[indene-1,4'-piperidine] analogs	series of compounds		WO 2010/102003 A2
Sulfonyl-azetidin-3-yl-methylamine amide analogs	series of compounds		WO 2010/114907 A1
N-[(1-(4-(sulfonyl)piperazin-1-yl)cycloalkyl)methyl]benzamide	series of compounds		Cioffi et al. (2016)
1-Methyl-1 H-imidazole-4-sulfonic acid {2-[3-(2-amino-1-benzyl-ethyl)-4-fluoro-phenoxy]-ethyl}-amide	example of Phenylalkylamine derivatives		WO 2012/020130 A1,
N-[2-(3-benzyl-2-(methylaminomethyl)indan-5-yl)oxyethyl]-1-cyclopropyl-methanesulfonamide	example of tetraline and indane derivatives		WO 2012/020133
Aminochromane,aminothiochromane and amino-1,2,3,4-tetrahydroquinoline derivatives	series of compounds		WO 2015/055770 A1,
N-(Azetidin-3-yl)-N-(2-(7-(1-(4-chlorophenyl)cyclobutyl)-4,5,6,7-tetrahydro-2H-pyrazolo[3,4-c]pyridin-2-yl)ethyl)propane-1-sulfonamide	example of heterocyclic compounds		WO 2010/092181 A1,
N-[2-(3-benzyl-1-oxo-isoindolin-5-yl)oxyethyl]-1-methyl-1H-imidazole-4-sulfonamide	example of Isoindoline derivatives		WO 2013/120835
2-chloro-N-{trans-1-[(1-methyl-1 H-imidazole-4-yl)sulfonyl]-4-phenylpyrrolidin-3-yl}-3-(trifluoromethyl)benzamide	example of Pyrrolidine derivatives		WO/2014/140310

**Heteroaryl Amide GlyT-1 Inhibitors**

(continued on next page)



Table 1 (continued)

Compound	Also known as	Clinical trials (Phase/Disease/NTC number)	Reference or patent number
1-Methyl-1H-imidazole-4-carboxylic acid (3-chloro-4-fluorobenzyl)-(3-methyl-3-aza-bicyclo[3.1.0]hex-6-ylmethyl)-amide	example of cyclohexyl N-methyl imidazo amides		Lowe et al. (2009)
N-[(3-chloro-4-fluorophenyl)methyl]-1-methyl-N-[[[(1R,5S)-3-methyl-3-azabicyclo[3.1.0]hexan-6-yl)methyl]imidazole-4-carboxamide	PF-03463275	II/SCZ/00567203	Roberts et al. (2010)
N-[[[6-Fluoro-4'-(trifluoromethoxy)biphenyl-3-yl)methyl]-L-methyl-N-(propan-2-yl)-1H-imidazole-4-carboxamide	example of cyclic heteroaryl amides		WO 2011/007899
1-methyl-N-(tetrahydro-2H-pyran-4-yl)-N-[3-(trifluoromethoxy)benzyl]-1H-imidazole-4-carboxamide	example of heterocyclic pyranamides		WO2010107115
<b>Benzoylpiperazines</b>			
2-{4-[5-Methanesulfonyl-2-(2,2,2-trifluoro-ethoxy)-benzoyl]-piperazin-1-yl}-thiazole-5-carbonitrile	example of benzoylpiperazin series		Pinard et al. (2008)
{4-[3-Fluoro-5-(trifluoromethyl)pyridin-2-yl]piperazin-1-yl}{5-(methylsulfonyl)-2-[(1S)-2,2,2-trifluoro-1-methylethoxy]phenyl}methanone	Bitopertin	II/SCZ/00616798, 01116830 III/SCZ/01192867, 01192906, 01192880, 01235520, 01235559, 01235585 II/OCD/01674361	(Alberati et al., 2012; Martin-Facklam et al., 2013; Umbricht et al., 2014)
<b>Benzoylisoindolines</b>			
N-[2-(3-benzyl-1-oxo-isoindolin-5-yl)oxyethyl]-1-methyl-imidazole-4-sulfonamide	example of Benzoylpiperazines		Pinard et al. (2010)
[5-Methanesulfonyl-2-(2,2,2-trifluoro-1-methyl-ethoxy)-phenyl]-[1-(5-trifluoromethyl-1,2,4-oxadiazol-3-yl)-3-azabicyclo[3.1.0]hex-3-yl]-methanone	Icleptine/BI 425809	III/SCZ/038599739, 05211947, 04846868, 0486881, 04860830 II/AD/02788513	WO 2013/017657 A1
<b>Bis-Amide GlyT1 Inhibitors</b>			
N-[(Benzhydryl-methyl-carbamoyl)-methyl]-3-phenyl-propionamide	example of di-aromatic substituted amides		WO 2008/022938 (Jolidon et al., 2008)
2-[3-(4-Bromophenyl)-2,2-dioxido-2-thia-1,3-diazaspiro[4.5]dec-1-yl]-N-[3-(trifluoromethyl)phenyl]acetamide	partially constrained bisamide		WO 2010/010133
cyclohexyl-1-(4-methoxyphenyl) imidazolidine-2-one	partially constrained bisamide		WO 2012/081665 A1
N-(2-methylphenyl)-2-[3-(2-methoxy-5-nitrophenyl)-thioureido]-2-phenyl acetamide	example of arylglycine derivatives		WO 2004/022528
<b>Miscellaneous</b>			
3-biphenyl-4-yl-4-phenyl-4H-1,2,4-triazoles	series of compounds		Sugane et al. (2012)
Atropisomeric 4-Phenyl-4H-1,2,4-triazoles	series of compounds		Sugane et al. (2013)
N-[[1-(propylsulfonyl)-4-pyridin-2-yl]piperidin-4-yl)methyl]-benzamides	series of compounds		Zhao et al. (2009)
4-[3-isopropyl-5-(6-phenyl-3-pyridyl)-4H-1,2,4-triazol-4-yl]-2,1,3-benzoxadiazole	ASP2535		Harada et al. (2012)
N-(2-hydroxy-2-aryl-cyclohexyl) substituted spiropiperidines	derived from RO454338		Ceccarelli et al. (2006)
1,3-diaminopropan-2-ol sulfonamides	derived from SB733993		Rahman et al. (2007)
4-Amino-1,5-substituted 1,5-dihydroimidazol-2-ones	series of compounds		WO 2007/101802 A1
4-benzylaminoquinolines	series of compounds		2009/024611 A2
Benzoxazine derivatives	series of compounds		WO 2011/023753 A1
Piperazine derivatives	series of compounds		WO 2015/055698 A1
N-(2-azepan-1-yl)-2-phenylethyl-benzenesulfonamides	series of compounds		Varnes et al. (2010)
1,4-disubstituted piperidine	series of compounds		WO 2005/058882 A1

Abbreviations: AD, Alzheimer's Disease; AUD, Alcohol Use Dependence; OCD, Obsessive Compulsive Disorder; PAD, Panic Disorder; SCZ, Schizophrenia.

2003), levels presumably elicited during NFPS treatment. For this reason, it would be interesting to investigate whether other GlyT1 inhibitors with a better pharmacodynamic profile have a similar neuroprotective effect in stroke.

Other 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-arylsulfanyphenyl-1-oxyalkylamino derivatives), Merck (indandione sarcosine derivatives), and Amgen (benzhydryl piperazine analogue AMG 747), among others (Table 1). Several of these compounds were better tolerated than NFPS and demonstrated an increase in cerebrospinal fluid Gly concentration and efficacy in preclinical animal models of psychotic illness. However, even the most promising of them, Org25935, did not show efficacy in clinical trials (Schoemaker et al., 2014). Several companies developed

second generation GlyT1 inhibitors that were not analogous to sarcosine. Chemically, they can be divided into several categories: methylphenidate-derived, alkyl- and heteroaromatic-substituted sulfonamides and sulfones, heteroaryl amides, benzoylpiperazines, benzoylisoindolines (derived from benzoylpiperazines), and several others (Cioffi and Guzzo, 2016) (Table 1). In general, all these compounds showed better selectivity for GlyT1 versus GlyT2, better water solubility, and shorter residence times on the transporter, all of which should improve their therapeutic capabilities. Attempts are still being made to find new series heads and to improve the methodology to characterize them (Ackermann et al., 2019, 2021). A new series of pyrrolo[3,4-c]pyrazole-based GlyT1 inhibitors has recently been developed (Santora et al., 2018), and these compounds were later modified through a strategy to conformationally restrain them, leading to a series of



azetidine-based inhibitors (Hudson et al., 2020).

Summarizing a large number of preclinical and small clinical studies on diverse GlyT1 inhibitors, there is experimental support for their utility to improve the cognitive dysfunctions of schizophrenia (Chaki et al., 2015; Deiana et al., 2022; Depoortere et al., 2005; Fone et al., 2020; Harada et al., 2012). In fact, Bitopertin, from the group of benzoylpiperazines, advanced through clinical trials and improved negative symptoms of schizophrenia in a phase-IIb study in stable medicated patients (Pinard et al., 2018; Umbricht et al., 2014). However, due to a failure to improve Positive And Negative Syndrome Scale scores in phase-II/III trials, and failure to ameliorate negative symptoms in a phase-III trial (Bugarski-Kirola et al., 2014, 2016, 2017), trials were suspended by Hoffman-LaRoche. And although schizophrenia is a heterogeneous disease, no subgroups of patients with a greater response to Bitopertin were found. However, additional trials are underway with other compounds. BI 425809 (Iclepertin) was tested in preclinical models of schizophrenia (Rosenbrock et al., 2022) as well various phase I clinical trials (Moschetti et al., 2018a, 2018b; Rosenbrock et al., 2018). In phase II studies, when assayed at doses of 10 mg and 25 mg for 12 weeks, it improved cognition in schizophrenic patients (Fleischhacker et al., 2021). Other phase II and phase III trials are currently on course for Iclepertin (NCT038599739, NCT05211947, NCT04846868, NCT0486881, NCT04860830). Also, a phase II study (NCT01911676) looked at PF-03463275, a compound that was effective in facilitating NMDAR function in the ketamine assay in primate (Roberts et al., 2010), but not in humans, although it enhanced neuroplasticity in schizophrenia patients (D'Souza et al., 2018).

Although these drugs were designed to treat schizophrenia, they may have applications in other diseases. For example, the GlyT1 inhibitor VU0410120 improved sociability in BALB/c mice, a model of ASD (Burket et al., 2015). Similarly, sarcosine decreased aggressive behavior in an animal model of CHARGE syndrome, a disease of the autism spectrum (Liu and Liu, 2020). A beneficial effect has also been described for ALX5407 on dyskinesia caused by prolonged DOPA treatment in an animal model of Parkinson's disease (Frouni et al., 2021). Bitopertin and other inhibitors of GlyT1 and GlyT2 might be repurposed for the treatment of erythropoietic protoporphyria, a rare disease in which patients experience severe light sensitivity produced by excess heme production (Halloy et al., 2021). A phase II clinical trial is currently recruiting participants (NCT05308472), and the rationale for this application lies in the fact that Gly is a heme precursor and is transported to erythrocytes through GlyT1. Bitopertin also improves anemia in a mouse model of  $\beta$ -thalassemia (Matte et al., 2019).

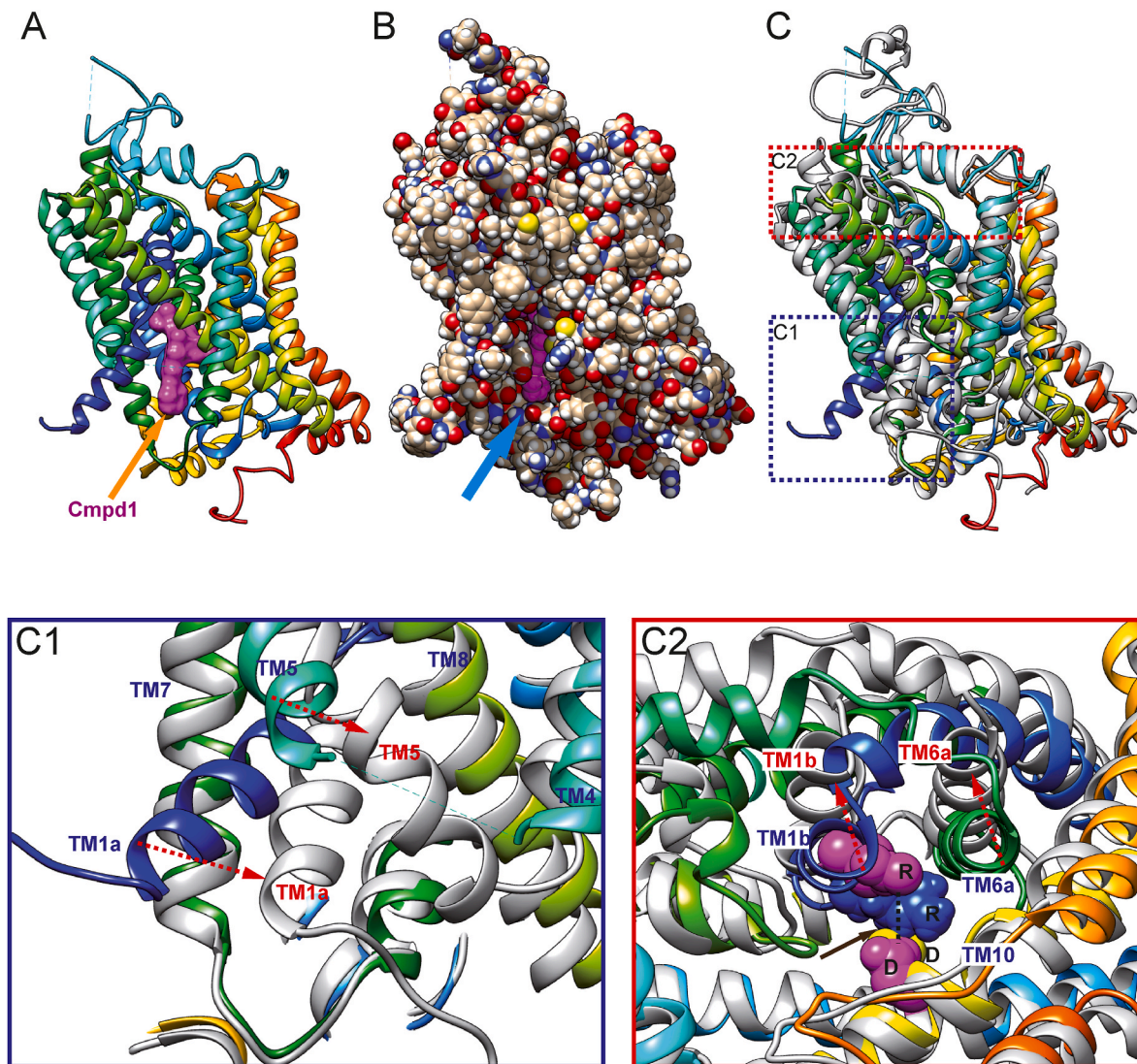
Several possible explanations have been proposed for the failure of Bitopertin in large scale phase III trials, from too low a dose (due to fear of side effects affecting heme synthesis) to a greater response in the placebo group. We propose that additional factors should be carefully evaluated during future drug development. For instance, these compounds appear to have a narrow therapeutic window, with inverted U-shaped dose-response curves. High concentrations can affect the glycinergic system producing unacceptable side effects. In addition, at high doses, the mechanism of NMDAR endocytosis might be activated, something that would be counterproductive in diseases associated with NMDAR hypofunction. Nevertheless, this mechanism may make sense in a pathological situation such as cerebral ischemia, which might improve by decreasing NMDAR levels in the cell surface (Cappelli et al., 2022). No less important may be the contribution of other transporters to the regulation of Gly levels in the glutamatergic synapse, such that GlyT1 blockade could trigger compensatory responses in Asc-1 or *SCL6A20*. Equally, compensatory responses might occur for other endogenous ligands like D-amino acids. Therefore, combination therapies with modulators of these coexisting systems might be considered in order to further increase the levels of GMS ligand in the environment of NMDARs.

#### 4. Structure of gly transporters

Parallel to these advances in the functional knowledge of Gly transporters, progress was also made in the understanding of the structure/function relationship, which may provide the basis for the future development of new and improved drugs. In this respect, a breakthrough came in 2005, when Gouaux's group solved the 3D structure of a bacterial ortholog of SLC6 transporters, LeuT (Yamashita et al., 2005). Since then, additional 3D images of LeuT and other SLC6 transporters bound to specific ligands have illustrated the different intermediate conformations of the translocation cycle. The 12 transmembrane helices of LeuT are organized in two halves of five transmembrane segments (TM1-5 and TM6-10), which are placed in the plane of the membrane with an inverted pseudo-twofold symmetry. The TM1 and TM6 helices are aligned in the middle of these two halves, oriented in an antiparallel manner, each one displaying a partially unwound segment halfway across the membrane bilayer, splitting TM1 and TM6 helices into TM1a-TM1b and TM6a-TM6b, respectively. This uncoiled segment contains part of the binding site for the substrate and cotransported  $\text{Na}^+$ , and allows formation of a hinge in both helices (Yamashita et al., 2005) (see review by (Penmatsa and Gouaux, 2014)).

In general, the 3D images as well as biophysical data obtained by FRET, electron paramagnetic resonance spectroscopy, and hydrogen/deuterium exchange mass spectrometry support the highly dynamic nature of the transporter, which suggests a mechanism of alternate access ((Billesbølle et al., 2016, 2015; Claxton et al., 2010; Kazmier et al., 2014; Merkle et al., 2018; Zhao et al., 2011, 2010)). There are three basic conformations that ensure that the permeation pathway is never completely open, while the substrate-binding site is alternatively exposed to either side of the membrane. In the first conformation, called outward open, the substrate and the ions find a hydrophilic pathway from the extracellular medium to the unwound region of TM1 and TM6, where they bind. Subsequently, the extracellular gate closes, leaving the ligands locked in the center of the structure (occluded conformation), and finally the intracellular gate opens (inward-open conformation), releasing substrates and ions into the cytoplasm. Finally, the empty transporter returns to the initial conformation with the help of a conserved Leu in the unwound region of TM1 (Malinauskaitė et al., 2016).

Recent advances in crystallization and cryo-EM techniques have made it possible to resolve the 3D structure of many eukaryotic transporters, including the human serotonin transporter in the three basic conformations of alternating access (Coleman et al., 2016, 2019; Coleman and Gouaux, 2018), the *Drosophila* dopamine transporter (drDAT) in the outward-open conformation (Penmatsa et al., 2013; Wang et al., 2015), the GABA transporter, GAT1 (inward-open) (Motiwala et al., 2022) and GlyT1 (inward-open) (Shahsavari et al., 2021). Except for GAT1, crystallization of eukaryotic transporters requires a series of modifications to thermally stabilize the structure, such as the introduction of missense mutations or deletions to certain areas of the protein, as well as the use of high-affinity ligands (inhibitors) and/or nanobodies that lock it in a single conformation. For GlyT1, crystallization was performed in the presence of Cmpd1, a structural analogue of Bitopertin. In addition, a synbody was used targeting the extracellular zone, and part of the EL2 extracellular loop was deleted ( $\Delta 240$ –256, numeration based on the variant P48067-1, or GlyT1C), removing three of the four glycosylation sites shown previously to be necessary for trafficking to the cell surface (Olivares et al., 1995). Finally, several point mutations (L153A, S297A, I368A and C633A) were introduced, and the amino and carboxyl termini were truncated ( $\Delta 1$ –90 and  $\Delta 685$ –706). While the structure of the outward open conformation is yet unknown, it can be bioinformatically modeled using dDAT (PDB:4M48) as template (Shahsavari et al., 2021) or SERT (PDB:6VRH) (see Fig. 3). Overlap of both structures permits a prediction of the major movement that likely occurs during the transition from outward-open (modeled) to the inward-open (experimental) state. Major movements occur on



**Fig. 3.** 3D structure of the glycine transporter GlyT1. A) Ribbon diagram showing a side view of GlyT1. Rainbow coloring from blue to red indicates the N- to C-terminal positions of the residues. The structure corresponds to the inward open conformation, stabilized by the inhibitor Cmdp1 (purple). B) Atoms are represented as spheres with conventional color coding. Note in A) and B) the intracellular part of the permeation pathway (arrows) that opens due to the bending away of helix TM1a and partial unwinding of TM5. C. Side view of the inward open conformation (rainbow ribbon) superimposed on a model of GlyT1 in the outward open conformation (gray ribbon). The model was built with the SWISS-MODEL tools using the human serotonin transporter (PDB:6VRH) as a template. C1. Zoom of the side view showing the predicted displacement (red arrows) of TM1a and TM5 during the transition from inward open (blue tones) to outward open (gray) conformation. C2. Top view of the extracellular gate in the superimposed conformations. Note that in the inward open conformation the extracellular gate is closed due to the tight interaction of R125 in TM1b (Blue spheres) with D528 in TM10 (yellow, partially occluded). These two residues become separated in the outward open conformation (purple spheres, gray ribbon) due to displacement of TM1b (red arrows). Data were obtained from the Protein Data Bank (PDB) ID:6ZBV. Primary publication (Shahsavari et al., 2022a). The diagram was generated using Chimera 1.16 software.

TM1a, which bends away from the GlyT1 core, and TM5, which partially unwinds, opening a hydrophilic pathway to the cytoplasm (Fig. 3C, C1). In the extracellular face, after binding of ions and Gly, D528 (TM10) and R125 (TM1b) approach establishing an ionic pair and closing the gate. There are other movements affecting the packaging of helices, especially affecting TM1b, TM6a, or the extracellular end of TM7 (Fig. 3C2).

## 5. Conclusions

NMDARs control a wide variety of brain functions and, consequently, they are also implicated in a multitude of pathologies, either due to excess or lack of activity. Numerous recent advances in understanding the structure and organization of these receptors have made it possible to clarify the mechanisms of activation, desensitization, and allostery of these proteins. It is not only Glu signals that converge on this receptor,

but also numerous allosteric effectors, among which Gly stands out. The concentration of Gly in the NMDAR environment has been shown to play a critical role in receptor activity, at least in certain brain regions and periods of development. This concentration is regulated by various transporters that may operate both to remove Gly and to release it from glial or neuronal cells. Given the functional effect of this Gly, the pharmaceutical industry has targeted specific transporters (especially GlyT1) for the treatment of schizophrenia and other diseases caused by alterations in neurotransmission mediated by NMDARs. Structural knowledge of these transporters will support the rational design of new drugs that improve upon the ones currently available.

## Declarations of competing interest

None



## Data availability

No data was used for the research described in the article.

## Acknowledgements

The work was supported by grants from the Spanish Ministry Science and Innovation (RTI 2018-098712-B-I00 to FZ, and by the Recovery, Transformation and Resilience Plan of Madrid Autonomous University, CA1/RSUE/2021-00753 to DP. The institutional support of Fundación Ramon Areces to CBMSO is also acknowledged. The professional editing service NB Revisions was used for technical preparation of the text prior to submission.

## References

- Ackermann, T.M., Bhokare, K., Höfner, G., Wanner, K.T., 2019. MS binding assays for GlyT1 based on Org24598 as nonlabelled reporter ligand. *Neuropharmacology* 161, 107561. <https://doi.org/10.1016/j.neuropharm.2019.03.004>.
- Ackermann, T.M., Höfner, G., Wanner, K.T., 2021. Screening for new inhibitors of Glycine transporter 1 and 2 by means of MS binding assays. *ChemMedChem* 16, 3094–3104. <https://doi.org/10.1002/cmdc.202100408>.
- Adermark, L., Lagström, O., Loftén, A., Licheri, V., Havenäng, A., Loi, E.A., Stomberg, R., Söderpalm, B., Domi, A., Ericson, M., 2022. Astrocytes modulate extracellular neurotransmitter levels and excitatory neurotransmission in dorsolateral striatum via dopamine D2 receptor signaling. *Neuropsychopharmacology* 47, 1493–1502. <https://doi.org/10.1038/s41386-021-01232-x>.
- Ahmadi, S., Muth-Selbach, U., Lauterbach, A., Lipfert, P., Neuhuber, W.L., Zeilhofer, H. U., 2003. Facilitation of spinal NMDA receptor currents by spillover of synaptically released glycine. *Science* 300, 2094–2097. <https://doi.org/10.1126/science.1083970>.
- Alberati, D., Moreau, J.-L., Lengyel, J., Hauser, N., Mory, R., Borroni, E., Pinard, E., Knöflach, F., Schlöterbeck, G., Hainzl, D., Wettstein, J.G., 2012. Glycine reuptake inhibitor RG1678: a pharmacologic characterization of an investigational agent for the treatment of schizophrenia. *Neuropharmacology* 62, 1152–1161. <https://doi.org/10.1016/j.neuropharm.2011.11.008>.
- Atkinson, B.N., Bell, S.C., De Vivo, M., Kowalski, L.R., Lechner, S.M., Ognjanov, V.I., Tham, C.S., Tsai, C., Jia, J., Ashton, D., Klitenick, M.A., 2001. Alx 5407: a potent, selective inhibitor of the hGlyT1 glycine transporter. *Mol. Pharmacol.* 60, 1414–1420. <https://doi.org/10.1124/mol.60.6.1414>.
- Aubrey, K.R., Vandenberg, R.J., 2001. N-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy) propyl]sarcosine (NFPS) is a selective persistent inhibitor of glycine transport. *Br. J. Pharmacol.* 134, 1429–1436. <https://doi.org/10.1038/sj.bjp.0704381>.
- Bae, M., Roh, J.D., Kim, Y., Kim, S.S., Han, H.M., Yang, E., Kang, H., Lee, S., Kim, J.Y., Kang, R., Jung, H., Yoo, T., Kim, Hyosang, Kim, Doyoun, Oh, H., Han, S., Kim, Dayeon, Han, J., Bae, Y.C., Kim, Hyun, Ahn, S., Chan, A.M., Lee, D., Kim, J.W., Kim, E., 2021. SLC6A20 transporter: a novel regulator of brain glycine homeostasis and NMDAR function. *EMBO Mol. Med.* 13, e12632 <https://doi.org/10.15252/emmm.202012632>.
- Ballard, T.M., Pauly-Evers, M., Higgins, G.A., Ouagazzal, A.-M., Mutel, V., Borroni, E., Kemp, J.A., Bluethmann, H., Kew, J.N.C., 2002. 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.* 22, 6713–6723. <https://doi.org/10.1523/JNEUROSCI.22-15-06713.2002>.
- Barker-Haliski, M., White, H.S., 2015. Glutamatergic mechanisms associated with seizures and epilepsy. *Cold Spring Harb Perspect Med* 5, a022863. <https://doi.org/10.1101/cshperspect.a022863>.
- Benito-Muñoz, C., Perona, A., Abia, D., Dos Santos, H.G., Núñez, E., Aragón, C., López-Corcuera, B., 2018. Modification of a putative third sodium site in the Glycine transporter GlyT2 influences the chloride dependence of substrate transport. *Front. Mol. Neurosci.* 11, 347. <https://doi.org/10.3389/fnmol.2018.00347>.
- Bergeron, R., Meyer, T.M., Coyle, J.T., Greene, R.W., 1998. Modulation of N-methyl-D-aspartate receptor function by glycine transport. *Proc. Natl. Acad. Sci. U. S. A.* 95, 15730–15734. <https://doi.org/10.1073/pnas.95.26.15730>.
- Billesbølle, C.B., Krüger, M.B., Shi, L., Quick, M., Li, Z., Stolzenberg, S., Kniazeff, J., Gotfryd, K., Mortensen, J.S., Javitch, J.A., Weinstein, H., Loland, C.J., Gether, U., 2015. Substrate-induced unlocking of the inner gate determines the catalytic efficiency of a neurotransmitter:sodium symporter. *J. Biol. Chem.* 290, 26725–26738. <https://doi.org/10.1074/jbc.M115.677658>.
- Billesbølle, C.B., Mortensen, J.S., Sohail, A., Schmidt, S.G., Shi, L., Sitte, H.H., Gether, U., Loland, C.J., 2016. Transition metal ion FRET uncovers K<sup>+</sup> regulation of a neurotransmitter/sodium symporter. *Nat. Commun.* 7, 12755 <https://doi.org/10.1038/ncomms12755>.
- Blackaby, W.P., Lewis, R.T., Thomson, J.L., Jennings, A.S.R., Goodacre, S.C., Street, L.J., MacLeod, A.M., Pike, A., Wood, S., Thomas, S., Brown, T.A., Smith, A., Pillai, G., Almond, S., Guscott, M.R., Burns, H.D., Eng, W., Ryan, C., Cook, J., Hamill, T.G., 2010. Identification of an orally bioavailable, potent, and selective inhibitor of GlyT1. *ACS Med. Chem. Lett.* 1, 350–354. <https://doi.org/10.1021/ml1001085>.
- Borowsky, B., Mezey, E., Hoffman, B.J., 1993. Two glycine transporter variants with distinct localization in the CNS and peripheral tissues are encoded by a common gene. *Neuron* 10, 851–863. [https://doi.org/10.1016/0896-6273\(93\)90201-2](https://doi.org/10.1016/0896-6273(93)90201-2).
- Bossi, S., Dhanasobhon, D., Ellis-Davies, G.C.R., Frontera, J., de Brito Van Velze, M., Lourenço, J., Murillo, A., Luján, R., Casado, M., Perez-Otaño, I., Bacci, A., Popa, D., Paoletti, P., Rebola, N., 2022. GluN3A excitatory glycine receptors control adult cortical and amygdalar circuits. *Neuron* 110, 2438–2454.e8. <https://doi.org/10.1016/j.neuron.2022.05.016>.
- Boulay, D., Pichat, P., Dargazanli, G., Estenne-Bouhtou, G., Terranova, J.P., Rogacki, N., Stemmelin, J., Coste, A., Lanneau, C., Desvignes, C., Cohen, C., Alonso, R., Vigé, X., Biton, B., Steinberg, R., Sevrin, M., Oury-Donat, F., George, P., Bergis, O., Griebel, G., Avenet, P., Scatton, B., 2008. Characterization of SSR103800, a selective inhibitor of the glycine transporter-1 in models predictive of therapeutic activity in schizophrenia. *Pharmacol. Biochem. Behav.* 91, 47–58. <https://doi.org/10.1016/j.pbb.2008.06.009>.
- Bouvier, G., Larsen, R.S., Rodríguez-Moreno, A., Paulsen, O., Sjöström, P.J., 2018. Towards resolving the presynaptic NMDA receptor debate. *Curr. Opin. Neurobiol.* 51, 1–7. <https://doi.org/10.1016/j.conb.2017.12.020>.
- Brown, A., Carlyle, I., Clark, J., Hamilton, W., Gibson, S., McGarry, G., McEachen, S., Rae, D., Thorn, S., Walker, G., 2001. Discovery and SAR of org 24598-a selective glycine uptake inhibitor. *Bioorg. Med. Chem. Lett.* 11, 2007–2009. [https://doi.org/10.1016/S0960-894X\(01\)00355-9](https://doi.org/10.1016/S0960-894X(01)00355-9).
- Bugarski-Kirolo, D., Blaettler, T., Arango, C., Fleischhacker, W.W., Garibaldi, G., Wang, A., Dixon, M., Bressan, R.A., Nasrallah, H., Lawrie, S., Napieralski, J., Ochi-Lohmann, T., Reid, C., Marder, S.R., 2017. Bitopertin in negative symptoms of schizophrenia—results from the phase III FlashLyte and DayLyte studies. *Biol. Psychiatry* 82, 8–16. <https://doi.org/10.1016/j.biopsych.2016.11.014>.
- Bugarski-Kirolo, D., Iwata, N., Sameljok, S., Reid, C., Blaettler, T., Millar, L., Marques, T. R., Garibaldi, G., Kapur, S., 2016. Efficacy and safety of adjunctive bitopertin versus placebo in patients with suboptimally controlled symptoms of schizophrenia treated with antipsychotics: results from three phase 3, randomised, double-blind, parallel-group, placebo-controlled, multicentre studies in the SearchLyte clinical trial programme. *Lancet Psychiatr.* 3, 1115–1128. [https://doi.org/10.1016/S2215-0366\(16\)30344-3](https://doi.org/10.1016/S2215-0366(16)30344-3).
- Bugarski-Kirolo, D., Wang, A., Abi-Saab, D., Blättler, T., 2014. A phase II/III trial of bitopertin monotherapy compared with placebo in patients with an acute exacerbation of schizophrenia - results from the CandleLyte study. *Eur. Neuropsychopharmacol.* 24, 1024–1036. <https://doi.org/10.1016/j.euroneuro.2014.03.007>.
- Burket, J.A., Benson, A.D., Green, T.L., Rook, J.M., Lindsley, C.W., Conn, P.J., Deutsch, S. I., 2015. Effects of VU0410120, a novel GlyT1 inhibitor, on measures of sociability, cognition and stereotypic behaviors in a mouse model of autism. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 61, 10–17. <https://doi.org/10.1016/j.pnpb.2015.03.003>.
- Cappelli, J., Khacho, P., Wang, B., Sokolovski, A., Bakkar, W., Raymond, S., Ahlskog, N., Pitney, J., Wu, J., Chudalayandi, P., Wong, A.Y.C., Bergeron, R., 2022. Glycine-induced NMDA receptor internalization provides neuroprotection and preserves vasculature following ischemic stroke. *iScience* 25, 103539. <https://doi.org/10.1016/j.isci.2021.103539>.
- Ceccarelli, S.M., Pinard, E., Stalder, H., Alberati, D., 2006. Discovery of N-(2-hydroxy-2-aryl-cyclohexyl) substituted spiroperidines as GlyT1 antagonists with improved pharmacological profile. *Bioorg. Med. Chem. Lett.* 16, 354–357. <https://doi.org/10.1016/j.bmc.2005.09.067>.
- Chaki, S., Shimazaki, T., Karasawa, J.-I., Aoki, T., Kaku, A., Iijima, M., Kambe, D., Yamamoto, S., Kawakita, Y., Shibata, T., Abe, K., Okubo, T., Sekiguchi, Y., Okuyama, S., 2015. Efficacy of a glycine transporter 1 inhibitor TASP0315003 in animal models of cognitive dysfunction and negative symptoms of schizophrenia. *Psychopharmacology (Berl)* 232, 2849–2861. <https://doi.org/10.1007/s00213-015-3920-3>.
- Chang, C.-H., Lin, C.-H., Liu, C.-Y., Chen, S.-J., Lane, H.-Y., 2020. Efficacy and cognitive effect of sarcosine (N-methylglycine) in patients with schizophrenia: a systematic review and meta-analysis of double-blind randomised controlled trials. *J. Psychopharmacol.* 34, 495–505. <https://doi.org/10.1177/0269881120908016>.
- Chatterton, J.E., Awobuluyi, M., Premkumar, L.S., Takahashi, H., Talantova, M., Shin, Y., Cui, J., Tu, S., Sevarino, K.A., Nakanishi, N., Tong, G., Lipton, S.A., Zhang, D., 2002. Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature* 415, 793–798. <https://doi.org/10.1038/nature715>.
- Choi, D.W., 2020. Excitotoxicity: still hammering the ischemic brain in 2020. *Front. Neurosci.* 14, 579953 <https://doi.org/10.3389/fnins.2020.579953>.
- Chou, T.-H., Epstein, M., Michalski, K., Fine, E., Biggin, P.C., Furukawa, H., 2022a. Structural insights into binding of therapeutic channel blockers in NMDA receptors. *Nat. Struct. Mol. Biol.* 29, 507–518. <https://doi.org/10.1038/s41594-022-00772-0>.
- Chou, T.-H., Kang, H., Simorowski, N., Traynelis, S.F., Furukawa, H., 2022b. Structural insights into assembly and function of GluN1-2C, GluN1-2A-2C, and GluN1-2D NMDARs. *Mol. Cell* 82, 4548–4563.e4. <https://doi.org/10.1016/j.molcel.2022.10.008>.
- Chou, T.-H., Tajima, N., Romero-Hernandez, A., Furukawa, H., 2020. Structural basis of functional transitions in mammalian NMDA receptors. *Cell* 182, 357–371.e13. <https://doi.org/10.1016/j.cell.2020.05.052>.
- Cioffi, C.L., Liu, S., Wolf, M.A., Guzzo, P.R., Sadalapure, K., Parthasarathy, V., Loong, D. T.J., Maeng, J.-H., Carulli, E., Fang, X., Karunakaran, K., Matta, L., Choo, S.H., Panduga, S., Buckle, R.N., Davis, R.N., Sakwa, S.A., Gupta, P., Sargent, B.J., Moore, N.A., Luche, M.M., Carr, G.J., Khmelinsky, Y.L., Ismail, J., Chung, M., Bai, M., Leong, W.Y., Sachdev, N., Swaminathan, S., Mhyre, A.J., 2016. Synthesis and biological evaluation of N-((1-(4-(Sulfonyl)piperazin-1-yl)cycloalkyl)methyl) benzamide inhibitors of Glycine transporter-1. *J. Med. Chem.* 59, 8473–8494. <https://doi.org/10.1021/acs.jmedchem.6b00914>.
- Claxton, D.P., Quick, M., Shi, L., de Carvalho, F.D., Weinstein, H., Javitch, J.A., Mchaourab, H.S., 2010. Ion/substrate-dependent conformational dynamics of a

- bacterial homolog of neurotransmitter:sodium symporters. *Nat. Struct. Mol. Biol.* 17, 822–829. <https://doi.org/10.1038/nsmb.1854>.
- Coleman, J.A., Gouaux, E., 2018. Structural basis for recognition of diverse antidepressants by the human serotonin transporter. *Nat. Struct. Mol. Biol.* 25, 170–175. <https://doi.org/10.1038/s41594-018-0026-8>.
- Coleman, J.A., Green, E.M., Gouaux, E., 2016. X-ray structures and mechanism of the human serotonin transporter. *Nature* 532, 334–339. <https://doi.org/10.1038/nature17629>.
- Coleman, J.A., Yang, D., Zhao, Z., Wen, P.-C., Yoshioka, C., Tajkhorshid, E., Gouaux, E., 2019. Serotonin transporter-ibogaine complexes illuminate mechanisms of inhibition and transport. *Nature* 569, 141–145. <https://doi.org/10.1038/s41586-019-1135-1>.
- Coyle, J.T., Balu, D., Wolosker, H., 2020. D-serine, the shape-shifting NMDA receptor Co-agonist. *Neurochem. Res.* 45, 1344–1353. <https://doi.org/10.1007/s11064-020-03014-1>.
- Cubelos, B., Giménez, C., Zafra, F., 2005. Localization of the GLYT1 glycine transporter at glutamatergic synapses in the rat brain. *Cerebr. Cortex* 15, 448–459. <https://doi.org/10.1093/cercor/bhh147>.
- Cubelos, B., Leite, C., Giménez, C., Zafra, F., 2014. Localization of the glycine transporter GLYT1 in glutamatergic synaptic vesicles. *Neurochem. Int.* 73, 204–210. <https://doi.org/10.1016/j.neuint.2013.09.002>.
- Cull-Candy, S.G., Leszkiewicz, D.N., 2004. Role of distinct NMDA receptor subtypes at central synapses. *Sci STKE* 2004 re16. <https://doi.org/10.1126/stke.2552004re16>.
- Danglot, L., Rostaing, P., Triller, A., Bessis, A., 2004. Morphologically identified glycinergic synapses in the hippocampus. *Mol. Cell. Neurosci.* 27, 394–403. <https://doi.org/10.1016/j.mcn.2004.05.007>.
- Deiana, S., Hauber, W., Munster, A., Sommer, S., Ferger, B., Marti, A., Schmid, B., Dörner-Ciossek, C., Rosenbrock, H., 2022. Pro-cognitive effects of the GLYT1 inhibitor Bitopertin in rodents. *Eur. J. Pharmacol.* 935, 175306. <https://doi.org/10.1016/j.ejphar.2022.175306>.
- Demchenko, I., Tassone, V.K., Kennedy, S.H., Dunlop, K., Bhat, V., 2022. Intrinsic connectivity networks of glutamate-mediated antidepressant response: a neuroimaging review. *Front. Psychiatry* 13, 864902. <https://doi.org/10.3389/fpsyt.2022.864902>.
- Depoortère, R., Dargazanli, G., Estenne-Bouhtou, G., Coste, A., Lanneau, C., Desvignes, C., Poncelet, H., Heaulme, M., Santucci, V., Decobert, M., Cudennec, A., Voltz, C., Boulay, D., Terranova, J.P., Stemmelin, J., Roger, P., Marabout, B., Sevrin, M., Vigé, X., Biton, B., Steinberg, R., Françon, D., Alonso, R., Avenet, P., Oury-Donat, F., Perrault, G., Griebel, G., George, P., Soubrié, P., Scatton, B., 2005. Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. *Neuropsychopharmacology* 30, 1963–1985. <https://doi.org/10.1038/sj.npp.1300772>.
- Donello, J.E., Banerjee, P., Li, Y.-X., Guo, Y.-X., Yoshitake, T., Zhang, X.-L., Miry, O., Kehr, J., Stanton, P.K., Gross, A.L., Burgdorf, J.S., Kroes, R.A., Moskal, J.R., 2019. Positive N-Methyl-D-Aspartate receptor modulation by rapastinel promotes rapid and sustained antidepressant-like effects. *Int. J. Neuropsychopharmacol.* 22, 247–259. <https://doi.org/10.1093/ijnp/pyy101>.
- Dore, K., Aow, J., Malinow, R., 2015. Agonist binding to the NMDA receptor drives movement of its cytoplasmic domain without ion flow. *Proc. Natl. Acad. Sci. U. S. A.* 112, 14705–14710. <https://doi.org/10.1073/pnas.1520023112>.
- Dravid, S.M., Prakash, A., Traynelis, S.F., 2008. Activation of recombinant NR1/NR2C NMDA receptors. *J. Physiol.* 586, 4425–4439. <https://doi.org/10.1113/jphysiol.2008.158634>.
- D'Souza, D.C., Carson, R.E., Driesen, N., Johannesen, J., Ranganathan, M., Krystal, J.H., 2018. Dose-related target occupancy and effects on circuitry, behavior, and neuroplasticity of the Glycine transporter-1 inhibitor PF-03463275 in healthy and schizophrenia subjects. *Biol. Psychiatry* 84, 413–421. <https://doi.org/10.1016/j.biopsych.2017.12.019>.
- Duman, R.S., Sanacora, G., Krystal, J.H., 2019. Altered connectivity in depression: GABA and glutamate neurotransmitter deficits and reversal by novel treatments. *Neuron* 102, 75–90. <https://doi.org/10.1016/j.neuron.2019.03.013>.
- Dunayevich, E., Buchanan, R.W., Chen, C.-Y., Yang, J., Dietrich, J.M., Sun, H., Marder, S., 2017. Efficacy and safety of the glycine transporter type-1 inhibitor AMG 747 for the treatment of negative symptoms associated with schizophrenia. *Schizophr. Res.* 182, 90–97. <https://doi.org/10.1016/j.schres.2016.10.027>.
- Durham, R.J., Paudyal, N., Carrillo, E., Bhatia, N.K., Maclean, D.M., Berka, V., Dolino, D.M., Gorf, A.A., Jayaraman, V., 2020. Conformational spread and dynamics in allosteric of NMDA receptors. *Proc. Natl. Acad. Sci. U. S. A.* 117, 3839–3847. <https://doi.org/10.1073/pnas.1910950117>.
- Dutertre, S., Becker, C.-M., Betz, H., 2012. Inhibitory glycine receptors: an update. *J. Biol. Chem.* 287, 40216–40223. <https://doi.org/10.1074/jbc.R112.408229>.
- Ehmsen, J.T., Liu, Y., Wang, Y., Paladugu, N., Johnson, A.E., Rothstein, J.D., du Lac, S., Mattson, M.P., Höke, A., 2016. The astrocytic transporter SLC7A10 (Asc-1) mediates glycinergic inhibition of spinal cord motor neurons. *Sci. Rep.* 6, 35592. <https://doi.org/10.1038/srep35592>.
- Eulenburg, V., Knop, G., Sedmak, T., Schuster, S., Hauf, K., Schneider, J., Feigenspan, A., Joachimsthaler, A., Brandstätter, J.H., 2018. GlyT1 determines the glycinergic phenotype of amacrine cells in the mouse retina. *Brain Struct. Funct.* 223, 3251–3266. <https://doi.org/10.1007/s00429-018-1684-3>.
- Eulenburg, V., Retziounskaja, M., Papadopoulos, T., Gomez, J., Betz, H., 2010. Glial glycine transporter 1 function is essential for early postnatal survival but dispensable in adult mice. *Glia* 58, 1066–1073. <https://doi.org/10.1002/glia.20987>.
- Ferreira, J.S., Papouin, T., Ladépêche, L., Yao, A., Langlais, V.C., Bouchet, D., Dulong, J., Mothet, J.-P., Sacchi, S., Pollegioni, L., Paoletti, P., Oliet, S.H.R., Groc, L., 2017. Co-agonists differentially tune GluN2B-NMDA receptor trafficking at hippocampal synapses. *Elife* 6, e25492. <https://doi.org/10.7554/eLife.25492>.
- Fleischhacker, W.W., Podhorna, J., Gröschl, M., Hake, S., Zhao, Y., Huang, S., Keefe, R.S.E., Desch, M., Brenner, R., Walling, D.P., Mantero-Atienza, E., Nakagome, K., Pollentier, S., 2021. Efficacy and safety of the novel glycine transporter inhibitor BI 425809 once daily in patients with schizophrenia: a double-blind, randomised, placebo-controlled phase 2 study. *Lancet Psychiatry* 8, 191–201. [https://doi.org/10.1016/S2215-0366\(20\)30513-7](https://doi.org/10.1016/S2215-0366(20)30513-7).
- Fone, K.C.F., Watson, D.J.G., Billiras, R.I., Sicard, D.I., Dekeyne, A., Rivet, J.-M., Gobert, A., Millan, M.J., 2020. Comparative pro-cognitive and neurochemical profiles of Glycine modulatory site agonists and Glycine reuptake inhibitors in the rat: potential relevance to cognitive dysfunction and its management. *Mol. Neurobiol.* 57, 2144–2166. <https://doi.org/10.1007/s12035-020-01875-9>.
- Fossat, P., Turpin, F.R., Sacchi, S., Dulong, J., Shi, T., Rivet, J.-M., Sweedler, J.V., Pollegioni, L., Millan, M.J., Oliet, S.H.R., Mothet, J.-P., 2012. Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. *Cerebr. Cortex* 22, 595–606. <https://doi.org/10.1093/cercor/bhr130>.
- Frouni, I., Belliveau, S., Maddaford, S., Nuara, S.G., Gourdon, J.C., Huot, P., 2021. Effect of the glycine transporter 1 inhibitor ALX-5407 on dyskinesia, psychosis-like behaviours and parkinsonism in the MPTP-lesioned marmoset. *Eur. J. Pharmacol.* 910, 174452. <https://doi.org/10.1016/j.ejphar.2021.174452>.
- Fuchigami, T., Haratake, M., Magata, Y., Haradahira, T., Nakayama, M., 2011. Synthesis and characterization of [<sup>125</sup>I]-2-iodo N-[(S)-((S)-1-methylpiperidin-2-yl)(phenyl)methyl]-3-trifluoromethyl-benzamide as novel imaging probe for glycine transporter 1. *Bioorg. Med. Chem.* 19, 6245–6253. <https://doi.org/10.1016/j.bmc.2011.09.010>.
- Fukasawa, Y., Segawa, H., Kim, J.Y., Chairoungdua, A., Kim, D.K., Matsuo, H., Cha, S.H., Endou, H., Kanai, Y., 2000. 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.* 275, 9690–9698. <https://doi.org/10.1074/jbc.275.13.9690>.
- Furukawa, H., 2012. Structure and function of glutamate receptor amino terminal domains. *J. Physiol.* 590, 63–72. <https://doi.org/10.1113/jphysiol.2011.213850>.
- Furukawa, H., Gouaux, E., 2003. Mechanisms of activation, inhibition and specificity: crystal structures of the NMDA receptor NR1 ligand-binding core. *EMBO J.* 22, 2873–2885. <https://doi.org/10.1093/emboj/cdg303>.
- Furukawa, H., Singh, S.K., Mancusso, R., Gouaux, E., 2005. Subunit arrangement and function in NMDA receptors. *Nature* 438, 185–192. <https://doi.org/10.1038/nature04089>.
- Gasiorowska, A., Wydrych, M., Drapich, P., Zadrozny, M., Steczkowska, M., Niewiadomska, W., Niewiadomska, G., 2021. The biology and pathobiology of glutamatergic, cholinergic, and dopaminergic signaling in the aging brain. *Front. Aging Neurosci.* 13, 654931. <https://doi.org/10.3389/fnagi.2021.654931>.
- Gielen, M., Sieglar Retchless, B., Mony, L., Johnson, J.W., Paoletti, P., 2009. Mechanism of differential control of NMDA receptor activity by NR2 subunits. *Nature* 459, 703–707. <https://doi.org/10.1038/nature07993>.
- Gomez, J., Hülsmann, S., Ohno, K., Eulenburg, V., Szöke, K., Richter, D., Betz, H., 2003a. Inactivation of the glycine transporter 1 gene discloses vital role of glial glycine uptake in glycinergic inhibition. *Neuron* 40, 785–796. [https://doi.org/10.1016/s0896-6273\(03\)00672-x](https://doi.org/10.1016/s0896-6273(03)00672-x).
- Gomez, J., Ohno, K., Hülsmann, S., Armsen, W., Eulenburg, V., Richter, D.W., Laube, B., Betz, H., 2003b. Deletion of the mouse glycine transporter 2 results in a hyperekplexia phenotype and postnatal lethality. *Neuron* 40, 797–806. [https://doi.org/10.1016/s0896-6273\(03\)00673-1](https://doi.org/10.1016/s0896-6273(03)00673-1).
- Greger, I.H., Mayer, M.L., 2019. Structural biology of glutamate receptor ion channels: towards an understanding of mechanism. *Curr. Opin. Struct. Biol.* 57, 185–195. <https://doi.org/10.1016/j.sbi.2019.05.004>.
- Groc, L., Choquet, D., 2020. Linking glutamate receptor movements and synapse function. *Science* 368, eaay4631. <https://doi.org/10.1126/science.aay4631>.
- Guastella, J., Brecha, N., Weigmann, C., Lester, H.A., Davidson, N., 1992. Cloning, expression, and localization of a rat brain high-affinity glycine transporter. *Proc. Natl. Acad. Sci. U. S. A.* 89, 7189–7193. <https://doi.org/10.1073/pnas.89.15.7189>.
- Hall, F., Iyer, P.S., Ghidini, A., Lysenko, V., Barman-Aksozen, J., Grubenmann, C.-P., Jucker, J., Wildner-Verhey van Wijk, N., Ruepp, M.-D., Minder, E.L., Minder, A.-E., Schneider-Yin, X., Theocharides, A.P.A., Schümperli, D., Hall, J., 2021. Repurposing of glycine transport inhibitors for the treatment of erythropoietic protoporphyria. *Cell Chemical Biology* 28, 1221–1234.e6. <https://doi.org/10.1016/j.chembiol.2021.02.021>.
- Hansen, K.B., Furukawa, H., Traynelis, S.F., 2010. Control of assembly and function of glutamate receptors by the amino-terminal domain. *Mol. Pharmacol.* 78, 535–549. <https://doi.org/10.1124/mol.110.067157>.
- Hansen, K.B., Wollmuth, L.P., Bowie, D., Furukawa, H., Menniti, F.S., Sobolevsky, A.I., Swanson, G.T., Swanger, S.A., Greger, I.H., Nakagawa, T., McBain, C.J., Jayaraman, V., Low, C.-M., Dell'Acqua, M.L., Diamond, J.S., Camp, C.R., Perszyk, R.E., Yuan, H., Traynelis, S.F., 2021. Structure, function, and pharmacology of glutamate receptor ion channels. *Pharmacol. Rev.* 73, 298–487. <https://doi.org/10.1124/pharmrev.120.000131>.
- Harada, K., Nakato, K., Yurimizu, J., Yamazaki, M., Morita, M., Takahashi, S., Aota, M., Saita, K., Doihara, H., Sato, Y., Yamaji, T., Ni, K., Matsuo, N., 2012. 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-benzoxadiazole), improves cognition in animal models of cognitive impairment in schizophrenia and Alzheimer's disease. *Eur. J. Pharmacol.* 685, 59–69. <https://doi.org/10.1016/j.ejphar.2012.04.013>.
- Harvey, R.J., Yee, B.K., 2013. Glycine transporters as novel therapeutic targets in schizophrenia, alcohol dependence and pain. *Nat. Rev. Drug Discov.* 12, 866–885. <https://doi.org/10.1038/nrd3893>.



- Hashimoto, K., Fujita, Y., Ishima, T., Chaki, S., Iyo, M., 2008. Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of the glycine transporter-1 inhibitor NFPS and D-serine. *Eur. Neuropsychopharmacol.* 18, 414–421. <https://doi.org/10.1016/j.euroneuro.2007.07.009>.
- Helboe, L., Egebjerg, J., Møller, M., Thomsen, C., 2003. Distribution and pharmacology of alanine-serine-cysteine transporter 1 (asc-1) in rodent brain. *Eur. J. Neurosci.* 18, 2227–2238. <https://doi.org/10.1046/j.1460-9568.2003.02966.x>.
- Herdon, H.J., Roberts, J.C., Coulton, S., Porter, R.A., 2010. Pharmacological characterisation of the GlyT-1 glycine transporter using two novel radioligands. *Neuropharmacology* 59, 558–565. <https://doi.org/10.1016/j.neuropharm.2010.07.023>.
- Hoffmann, C., Evčimán, S., Neumaier, F., Zlatopolskiy, B.D., Humpert, S., Bier, D., Holschbach, M., Schulze, A., Endepols, H., Neumaier, B., 2021. [18F]ALX5406: a brain-penetrating prodrug for GlyT1-specific PET imaging. *ACS Chem. Neurosci.* 12, 3335–3346. <https://doi.org/10.1021/acscchemneuro.1c00284>.
- Hudson, A.R., Santora, V.J., Petroski, R.E., Almos, T.A., Anderson, G., Barido, R., Basinger, J., Bellows, C.L., Bookser, B.C., Broadbent, N.J., Cabebe, C., Chai, C.-K., Chen, M., Chow, S., Chung, D.M., Heger, L., Danks, A.M., Freestone, G.C., Gitnick, D., Gupta, V., Hoffmaster, C., Kaplan, A.P., Kennedy, M.R., Lee, D., Limberis, J., Ly, K., Mak, C.C., Masatsugu, B., Morse, A.C., Na, J., Neul, D., Nikpur, J., Renick, J., Sebring, K., Sevidal, S., Tabatabaei, A., Wen, J., Xia, S., Yan, Y., Yoder, Z.W., Zook, D., Peters, M., Breitenbucher, J.G., 2020. Azetidine-based selective glycine transporter-1 (GlyT1) inhibitors with memory enhancing properties. *Bioorg. Med. Chem. Lett.* 30, 127214. <https://doi.org/10.1016/j.bmcl.2020.127214>.
- Iacobucci, G.J., Popescu, G.K., 2017. NMDA receptors: linking physiological output to biophysical operation. *Nat. Rev. Neurosci.* 18, 236–249. <https://doi.org/10.1038/nrn.2017.24>.
- Jalali-Yazdi, F., Chowdhury, S., Yoshioka, C., Gouaux, E., 2018. Mechanisms for zinc and proton inhibition of the GluN1/GluN2A NMDA receptor. *Cell* 175, 1520–1532.e15. <https://doi.org/10.1016/j.cell.2018.10.043>.
- Javitt, D.C., 2023. Cognitive impairment associated with schizophrenia: from pathophysiology to treatment. *Annu. Rev. Pharmacol. Toxicol.* 63, 119–141. <https://doi.org/10.1146/annurev-pharmtox-051921-093250>.
- Javitt, D.C., 2004. Glutamate as a therapeutic target in psychiatric disorders. *Mol. Psychiatr.* 9, 984–997. <https://doi.org/10.1038/sj.mp.4001551>, 979.
- Javitt, D.C., Schoepp, D., Kalivas, P.W., Volkow, N.D., Zarate, C., Merchant, K., Bear, M. F., Umbricht, D., Hajos, M., Potter, W.Z., Lee, C.-M., 2011. Translating glutamate: from pathophysiology to treatment. *Sci. Transl. Med.* 3 <https://doi.org/10.1126/scitranslmed.3002804>.
- Jespersen, A., Tajima, N., Fernandez-Cuervo, G., Garnier-Amblard, E.C., Furukawa, H., 2014. Structural insights into competitive antagonism in NMDA receptors. *Neuron* 81, 366–378. <https://doi.org/10.1016/j.neuron.2013.11.033>.
- Johnson, J.W., Ascher, P., 1987. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* 325, 529–531. <https://doi.org/10.1038/325529a0>.
- Jolidon, S., Alberati, D., Dowle, A., Fischer, H., Hainzl, D., Narquizaian, R., Norcross, R., Pinard, E., 2008. Design, synthesis and structure-activity relationship of simple bis-amides as potent inhibitors of GlyT1. *Bioorg. Med. Chem. Lett.* 18, 5533–5536. <https://doi.org/10.1016/j.bmcl.2008.09.005>.
- Karakas, E., Furukawa, H., 2014. Crystal structure of a heterotetrameric NMDA receptor ion channel. *Science* 344, 992–997. <https://doi.org/10.1126/science.1251915>.
- Karakas, E., Simorowski, N., Furukawa, H., 2011. Subunit arrangement and phenylethanolamine binding in GluN1/GluN2B NMDA receptors. *Nature* 475, 249–253. <https://doi.org/10.1038/nature10180>.
- Karakas, E., Simorowski, N., Furukawa, H., 2009. Structure of the zinc-bound amino-terminal domain of the NMDA receptor NR2B subunit. *EMBO J.* 28, 3910–3920. <https://doi.org/10.1038/emboj.2009.338>.
- Karasawa, J.-I., Hashimoto, K., Chaki, S., 2008. D-Serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. *Behav. Brain Res.* 186, 78–83. <https://doi.org/10.1016/j.bbr.2007.07.033>.
- Kazmier, K., Sharma, S., Quick, M., Islam, S.M., Roux, B., Weinstein, H., Javitt, J.A., Mchaourab, H.S., 2014. Conformational dynamics of ligand-dependent alternating access in LeuT. *Nat. Struct. Mol. Biol.* 21, 472–479. <https://doi.org/10.1038/nsmb.2816>.
- Kemp, J.A., Foster, A.C., Leeson, P.D., Priestley, T., Tridgett, R., Iversen, L.L., Woodruff, G.N., 1988. 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.* 85, 6547–6550. <https://doi.org/10.1073/pnas.85.17.6547>.
- Kew, J.N.C., Koester, A., Moreau, J.-L., Jenck, F., Ouagazzal, A.-M., Mutel, V., Richards, J.G., Trube, G., Fischer, G., Montkowski, A., Hundt, W., Reinscheid, R.K., Pauly-Evers, M., Kemp, J.A., Blüthmann, H., 2000. Functional consequences of reduction in NMDA receptor Glycine affinity in mice carrying targeted point mutations in the Glycine binding site. *J. Neurosci.* 20, 4037–4049. <https://doi.org/10.1523/JNEUROSCI.20-11-04037.2000>.
- Kinney, G.G., Sur, C., Burno, M., Mallorga, P.J., Williams, J.B., Figueroa, D.J., Wittmann, M., Lemaire, W., Conn, P.J., 2003. 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.* 23, 7586–7591. <https://doi.org/10.1523/JNEUROSCI.23-20-07586.2003>.
- Kleckner, N.W., Dingleline, R., 1988. Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes. *Science* 241, 835–837. <https://doi.org/10.1126/science.2841759>.
- Kowalczyk, S., Bröer, A., Munzinger, M., Tietze, N., Klingel, K., Bröer, S., 2005. Molecular cloning of the mouse IMINO system: an Na<sup>+</sup>- and Cl<sup>-</sup>-dependent proline transporter. *Biochem. J.* 386, 417–422. <https://doi.org/10.1042/BJ20050100>.
- Krieger, J., Lee, J.Y., Greger, I.H., Bahar, I., 2019. Activation and desensitization of ionotropic glutamate receptors by selectively triggering pre-existing motions. *Neurosci. Lett.* 700, 22–29. <https://doi.org/10.1016/j.neulet.2018.02.050>.
- Kruse, A.O., Bustillo, J.R., 2022. Glutamatergic dysfunction in schizophrenia. *Transl. Psychiatry* 12, 500. <https://doi.org/10.1038/s41398-022-02253-w>.
- Labrie, V., Lipina, T., Roder, J.C., 2008. Mice with reduced NMDA receptor glycine affinity model some of the negative and cognitive symptoms of schizophrenia. *Psychopharmacology* 200, 217–230. <https://doi.org/10.1007/s00213-008-1196-6>.
- Lane, H.-Y., Huang, C.-L., Wu, P.-L., Liu, Y.-C., Chang, Y.-C., Lin, P.-Y., Chen, P.-W., Tsai, G., 2006. Glycine transporter 1 inhibitor, N-methylglycine (sarcosine), added to clozapine for the treatment of schizophrenia. *Biol. Psychiatr.* 60, 645–649. <https://doi.org/10.1016/j.biopsych.2006.04.005>.
- Lane, H.-Y., Lin, C.-H., Huang, Y.-J., Liao, C.-H., Chang, Y.-C., Tsai, G.E., 2010. A randomized, double-blind, placebo-controlled comparison study of sarcosine (N-methylglycine) and D-serine add-on treatment for schizophrenia. *Int. J. Neuropsychopharmacol.* 13, 451–460. <https://doi.org/10.1017/S1461145709990939>.
- Larsen, R.S., Smith, I.T., Miriyala, J., Han, J.E., Corlew, R.J., Smith, S.L., Philpot, B.D., 2014. Synapse-specific control of experience-dependent plasticity by presynaptic NMDA receptors. *Neuron* 83, 879–893. <https://doi.org/10.1016/j.neuron.2014.07.039>.
- Le Bail, M., Martineau, M., Sacchi, S., Yatsenko, N., Radziszewsky, I., Conrod, S., Ait Ouarek, K., Wolosker, H., Pollegioni, L., Billard, J.-M., Mothet, J.-P., 2015. Identity of the NMDA receptor coagonist is synapse specific and developmentally regulated in the hippocampus. *Proc. Natl. Acad. Sci. U.S.A.* 112, E204–E213. <https://doi.org/10.1073/pnas.1416668112>.
- Le Guellec, B., Rousseau, F., Bied, M., Supplisson, S., 2022. Flux coupling, not specificity, shapes the transport and phylogeny of SLC6 glycine transporters. *Proc. Natl. Acad. Sci. U.S.A.* 119, e2205874119. <https://doi.org/10.1073/pnas.2205874119>.
- Le Pen, G., Kew, J., Alberati, D., Borroni, E., Heitz, M.P., Moreau, J.-L., 2003. Prepulse inhibition deficits of the startle reflex in neonatal ventral hippocampal-lesioned rats: reversal by glycine and a glycine transporter inhibitor. *Biol. Psychiatr.* 54, 1162–1170. [https://doi.org/10.1016/s0006-3223\(03\)00374-3](https://doi.org/10.1016/s0006-3223(03)00374-3).
- Lee, C.-H., Lü, W., Michel, J.C., Goehring, A., Du, J., Song, X., Gouaux, E., 2014. NMDA receptor structures reveal subunit arrangement and pore architecture. *Nature* 511, 191–197. <https://doi.org/10.1038/nature13548>.
- Lewerenz, J., Maher, P., 2015. Chronic glutamate toxicity in neurodegenerative diseases—what is the evidence? *Front. Neurosci.* 9 <https://doi.org/10.3389/fnins.2015.00469>.
- Li, H., Rajani, V., Han, L., Chung, D., Cooke, J.E., Sengar, A.S., Salter, M.W., 2021. Alternative splicing of GluN1 gates glycine site-dependent nonionotropic signaling by NMDAR receptors. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2026411118. <https://doi.org/10.1073/pnas.2026411118>.
- Li, L.-J., Hu, R., Lujan, B., Chen, J., Zhang, J.-J., Nakano, Y., Cui, T.-Y., Liao, M.-X., Chen, J.-C., Man, H.-Y., Feng, H., Wan, Q., 2016. Glycine potentiates AMPA receptor function through metabotropic activation of GluN2A-containing NMDA receptors. *Front. Mol. Neurosci.* 9, 102. <https://doi.org/10.3389/fnmol.2016.00102>.
- Li, Y., Sacchi, S., Pollegioni, L., Basu, A.C., Coyle, J.T., Bolshakov, V.Y., 2013. Identity of endogenous NMDAR glycine site agonist in amygdala is determined by synaptic activity level. *Nat. Commun.* 4, 1760. <https://doi.org/10.1038/ncomms2779>.
- Lindsley, C.W., Zhao, Z., Leister, W.H., O'Brien, J., Lemaire, W., Williams, D.L.J., Chen, T.-B., Chang, R.S.L., Burno, M., Jacobson, M.A., Sur, C., Kinney, G.G., Pettibone, D.J., Tiller, P.R., Smith, S., Tsou, N.N., Duggan, M.E., Conn, P.J., Hartman, G.D., 2006. Design, synthesis, and in vivo efficacy of glycine transporter-1 (GlyT1) inhibitors derived from a series of [4-phenyl-1-(propylsulfonyl)piperidin-4-yl]methyl benzamides. *ChemMedChem* 1, 807–811. <https://doi.org/10.1002/cmdc.200600097>.
- Liu, H., Liu, Z.-Z., 2020. Aggressive-like behavior and increased glycine transporters in a zebrafish model of CHARGE syndrome. *Behav. Brain Res.* 378, 112293. <https://doi.org/10.1016/j.bbr.2019.112293>.
- Liu, Q.R., López-Corcuera, B., Mandiyan, S., Nelson, H., Nelson, N., 1993. Cloning and expression of a spinal cord- and brain-specific glycine transporter with novel structural features. *J. Biol. Chem.* 268, 22802–22808.
- López-Corcuera, B., Arribas-González, E., Aragón, C., 2019. Hyperekplexia-associated mutations in the neuronal glycine transporter 2. *Neurochem. Int.* 123, 95–100. <https://doi.org/10.1016/j.neuint.2018.05.014>.
- López-Corcuera, B., Benito-Muñoz, C., Aragón, C., 2017. Glycine transporters in glia cells: structural studies. *Adv. Neurobiol.* 16, 13–32. [https://doi.org/10.1007/978-3-319-55769-4\\_2](https://doi.org/10.1007/978-3-319-55769-4_2).
- Lowe 3rd, J.A., Drozda, S.E., Fisher, K., Strick, C., Lebel, L., Schmidt, C., Hiller, D., Zandi, K.S., 2003. [3H]-(R)-NPTS, a radioligand for the type 1 glycine transporter. *Bioorg. Med. Chem. Lett.* 13, 1291–1292. [https://doi.org/10.1016/s0960-894x\(03\)00126-4](https://doi.org/10.1016/s0960-894x(03)00126-4).
- Lowe 3rd, J.A., Hou, X., Schmidt, C., David Tingley 3rd, F., McHardy, S., Kalman, M., Deninno, S., Sanner, M., Ward, K., Lebel, L., Tunucci, D., Valentine, J., Bronk, B.S., Schaeffer, E., 2009. The discovery of a structurally novel class of inhibitors of the type 1 glycine transporter. *Bioorg. Med. Chem. Lett.* 19, 2974–2976. <https://doi.org/10.1016/j.bmcl.2009.04.035>.
- MacLean, D.M., Durham, R.J., Jayaraman, V., 2019. Mapping the conformational landscape of glutamate receptors using single molecule FRET. *Trends Neurosci.* 42, 128–139. <https://doi.org/10.1016/j.tins.2018.10.003>.
- Malinauskaitė, L., Said, S., Sahin, C., Grouleff, J., Shahsavari, A., Bjerregaard, H., Noer, P., Severinsen, K., Boesen, T., Schiøtt, B., Sinning, S., Nissen, P., 2016.

- A conserved leucine occupies the empty substrate site of LeuT in the Na<sup>+</sup>(-)-free return state. *Nat. Commun.* 7, 11673 <https://doi.org/10.1038/ncomms11673>.
- Mallorga, P.J., Williams, J.B., Jacobson, M., Marques, R., Chaudhary, A., Conn, P.J., Pettibone, D.J., Sur, C., 2003. Pharmacology and expression analysis of glycine transporter GlyT1 with [3H]-(N-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]) sarcosine. *Neuropharmacology* 45, 585–593. [https://doi.org/10.1016/s0028-3908\(03\)00227-2](https://doi.org/10.1016/s0028-3908(03)00227-2).
- Mao, S.-C., Lin, H.-C., Gean, P.-W., 2009. Augmentation of fear extinction by infusion of glycine transporter blockers into the amygdala. *Mol. Pharmacol.* 76, 369–378. <https://doi.org/10.1124/mol.108.053728>.
- Martina, M., Gorfinkel, Y., Halman, S., Lowe, J.A., Periyalar, P., Schmidt, C.J., Bergeron, R., 2004. Glycine transporter type 1 blockade changes NMDA receptor-mediated responses and LTP in hippocampal CA1 pyramidal cells by altering extracellular glycine levels. *J. Physiol.* 557, 489–500. <https://doi.org/10.1113/jphysiol.2004.063321>.
- Martin-Facklam, M., Pizzagalli, F., Zhou, Y., Ostrowski, S., Raymont, V., Brasić, J.R., Parkar, N., Umbricht, D., Dannals, R.F., Goldwater, R., Wong, D.F., 2013. Glycine transporter type 1 occupancy by bitopertin: a positron emission tomography study in healthy volunteers. *Neuropsychopharmacology* 38, 504–512. <https://doi.org/10.1038/npp.2012.212>.
- Matsuoka, H., Kanai, Y., Tokunaga, M., Nakata, T., Chairoungdua, A., Ishimine, H., Tsukada, S., Oigawa, H., Nawashiro, H., Kobayashi, Y., Fukuda, J., Endou, H., 2004. High affinity D- and L-serine transporter Asc-1: cloning and dendritic localization in the rat cerebral and cerebellar cortices. *Neurosci. Lett.* 358, 123–126. <https://doi.org/10.1016/j.neulet.2004.01.014>.
- Matte, A., Federici, E., Winter, M., Koerner, A., Harmeier, A., Mazer, N., Tomka, T., Di Paolo, M.L., Defalco, L., Andolfo, I., Beneduce, E., Iolascon, A., Macias-García, A., Chen, J.-J., Janin, A., Lebouef, C., Turrini, F., Brugnara, C., De Franceschi, L., 2019. Bitopertin, a selective oral GLYT1 inhibitor, improves anemia in a mouse model of  $\beta$ -thalassemia. *JCI Insight* 4, e130111. <https://doi.org/10.1172/jci.insight.130111>.
- Mayer, M.L., Vyklícký, L., Clements, J., 1989. Regulation of NMDA receptor desensitization in mouse hippocampal neurons by glycine. *Nature* 338, 425–427. <https://doi.org/10.1038/338425a0>.
- Mayer, M.L., Westbrook, G.L., Guthrie, P.B., 1984. Voltage-dependent block by Mg<sup>2+</sup> of NMDA responses in spinal cord neurones. *Nature* 309, 261–263. <https://doi.org/10.1038/309261a0>.
- Merkle, P.S., Gotfryd, K., Cuendet, M.A., Leth-Espensen, K.Z., Gether, U., Loland, C.J., Rand, K.D., 2018. Substrate-modulated unwinding of transmembrane helices in the NSS transporter LeuT. *Sci. Adv.* 4, eaar6179. <https://doi.org/10.1126/sciadv.aar6179>.
- Möhler, H., Boisson, D., Singer, P., Feldon, J., Pauly-Evers, M., Yee, B.K., 2011. Glycine transporter 1 as a potential therapeutic target for schizophrenia-related symptoms: evidence from genetically modified mouse models and pharmacological inhibition. *Biochem. Pharmacol.* 81, 1065–1077. <https://doi.org/10.1016/j.bcp.2011.02.003>.
- Molander, A., Lidö, H.H., Löf, E., Ericson, M., Söderpalm, B., 2007. The glycine reuptake inhibitor OR 25935 decreases ethanol intake and preference in male wistar rats. *Alcohol Alcohol* 42, 11–18. <https://doi.org/10.1093/alcalag/agl085>.
- Moschetti, V., Desch, M., Goetz, S., Liesenfeld, K.-H., Rosenbrock, H., Kammerer, K.-P., Wunderlich, G., Wind, S., 2018a. Safety, tolerability and pharmacokinetics of oral BI 425809, a Glycine transporter 1 inhibitor, in healthy male volunteers: a partially randomised, single-blind, placebo-controlled, first-in-human study. *Eur. J. Drug Metab. Pharmacokinet.* 43, 239–249. <https://doi.org/10.1007/s13318-017-0440-z>.
- Moschetti, V., Schlecker, C., Wind, S., Goetz, S., Schmitt, H., Schultz, A., Liesenfeld, K.-H., Wunderlich, G., Desch, M., 2018b. Multiple rising doses of oral BI 425809, a GlyT1 inhibitor, in young and elderly healthy volunteers: a randomised, double-blind, phase I study investigating safety and pharmacokinetics. *Clin. Drug Invest.* 38, 737–750. <https://doi.org/10.1007/s40261-018-0660-2>.
- Motiwala, Z., Aduri, N.G., Shaye, H., Han, G.W., Lam, J.H., Katritch, V., Cherezov, V., Gati, C., 2022. Structural basis of GABA reuptake inhibition. *Nature* 606, 820–826. <https://doi.org/10.1038/s41586-022-04814-x>.
- Murillo, A., Navarro, A.I., Puellas, E., Zhang, Y., Petros, T.J., Pérez-Otaño, I., 2021. Temporal dynamics and neuronal specificity of Grin3a expression in the mouse forebrain. *Cerebr. Cortex* 31, 1914–1926. <https://doi.org/10.1093/cercor/bhaa330>.
- Musante, V., Summa, M., Cunha, R.A., Raiteri, M., Pittaluga, A., 2011. Pre-synaptic glycine GlyT1 transporter–NMDA receptor interaction: relevance to NMDA autoreceptor activation in the presence of Mg<sup>2+</sup> ions. *J. Neurochem.* 117, 516–527. <https://doi.org/10.1111/j.1471-4159.2011.07223.x>.
- Nabavi, S., Kessels, H.W., Alfonso, S., Aow, J., Fox, R., Malinow, R., 2013. Metabotropic NMDA receptor function is required for NMDA receptor-dependent long-term depression. *Proc. Natl. Acad. Sci. U. S. A.* 110, 4027–4032. <https://doi.org/10.1073/pnas.1219454110>.
- Nash, S.R., Giros, B., Kingsmore, S.F., Kim, K.M., el-Mestikawy, S., Dong, Q., Fumagalli, F., Seldin, M.F., Caron, M.G., 1998. Cloning, gene structure and genomic localization of an orphan transporter from mouse kidney with six alternatively-spliced isoforms. *Recept. Channel* 6, 113–128.
- Nimitvilai-Roberts, S., Gioia, D., Zamudio, P.A., Woodward, J.J., 2021. Ethanol inhibition of lateral orbitofrontal cortex neuron excitability is mediated via dopamine D1/D5 receptor-induced release of astrocytic glycine. *Neuropharmacology* 192, 108600. <https://doi.org/10.1016/j.neuropharm.2021.108600>.
- Nong, Y., Huang, Y.-Q., Ju, W., Kalia, L.V., Ahmadian, G., Wang, Y.T., Salter, M.W., 2003. Glycine binding primes NMDA receptor internalization. *Nature* 422, 302–307. <https://doi.org/10.1038/nature01497>.
- Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., Prochiantz, A., 1984. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307, 462–465. <https://doi.org/10.1038/307462a0>.
- O'Brien, R.J., Lau, L.F., Haganir, R.L., 1998. Molecular mechanisms of glutamate receptor clustering at excitatory synapses. *Curr. Opin. Neurobiol.* 8, 364–369. [https://doi.org/10.1016/s0959-4388\(98\)80062-7](https://doi.org/10.1016/s0959-4388(98)80062-7).
- Oliet, S.H.R., Mothet, J.-P., 2009. Regulation of N-methyl-D-aspartate receptors by astrocytic D-serine. *Neuroscience* 158, 275–283. <https://doi.org/10.1016/j.neuroscience.2008.01.071>.
- Olivares, L., Aragón, C., Giménez, C., Zafrá, F., 1995. The role of N-glycosylation in the targeting and activity of the GLYT1 glycine transporter. *J. Biol. Chem.* 270, 9437–9442. <https://doi.org/10.1074/jbc.270.16.9437>.
- Ouellet, D., Sutherland, S., Wang, T., Griffini, P., Murthy, V., 2011. First-time-in-human study with GSK1018921, a selective GlyT1 inhibitor: relationship between exposure and dizziness. *Clin. Pharmacol. Ther.* 90, 597–604. <https://doi.org/10.1038/clpt.2011.154>.
- Pajarillo, E., Rívor, A., Lee, J., Aschner, M., Lee, E., 2019. The role of astrocytic glutamate transporters GLT-1 and GLAST in neurological disorders: potential targets for neurotherapeutics. *Neuropharmacology* 161, 107559. <https://doi.org/10.1016/j.neuropharm.2019.03.002>.
- Paoletti, P., 2011. Molecular basis of NMDA receptor functional diversity. *Eur. J. Neurosci.* 33, 1351–1365. <https://doi.org/10.1111/j.1460-9568.2011.07628.x>.
- Paoletti, P., Bellone, C., Zhou, Q., 2013. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nat. Rev. Neurosci.* 14, 383–400. <https://doi.org/10.1038/nrn3504>.
- Papouin, T., Ladépêche, L., Ruel, J., Sacchi, S., Labasque, M., Hanini, M., Groc, L., Pollegioni, L., Mothet, J.-P., Oliet, S.H.R., 2012. Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. *Cell* 150, 633–646. <https://doi.org/10.1016/j.cell.2012.06.029>.
- Passchier, J., Gentile, G., Porter, R., Herdon, H., Salinas, C., Jakobsen, S., Audrain, H., Laruelle, M., Gunn, R.N., 2010. Identification and evaluation of [11C]GSK931145 as a novel ligand for imaging the type 1 glycine transporter with positron emission tomography. *Synapse* 64, 542–549. <https://doi.org/10.1002/syn.20760>.
- Pei, J.-C., Luo, D.-Z., Gau, S.-S., Chang, C.-Y., Lai, W.-S., 2021. Directly and indirectly targeting the Glycine modulatory site to modulate NMDA receptor function to address unmet medical needs of patients with schizophrenia. *Front. Psychiatr.* 12, 742058. <https://doi.org/10.3389/fpsy.2021.742058>.
- Penmatsa, A., Gouaux, E., 2014. How LeuT shapes our understanding of the mechanisms of sodium-coupled neurotransmitter transporters. *J. Physiol.* 592, 863–869. <https://doi.org/10.1113/jphysiol.2013.259051>.
- Penmatsa, A., Wang, K.H., Gouaux, E., 2013. X-ray structure of dopamine transporter elucidates antidepressant mechanism. *Nature* 503, 85–90. <https://doi.org/10.1038/nature12533>.
- Pérez-Otaño, I., Larsen, R.S., Wesseling, J.F., 2016. Emerging roles of GluN3-containing NMDA receptors in the CNS. *Nat. Rev. Neurosci.* 17, 623–635. <https://doi.org/10.1038/nrn.2016.92>.
- Perry, K.W., Falcone, J.F., Fell, M.J., Ryder, J.W., Yu, H., Love, P.L., Katner, J., Gordon, K.D., Wade, M.R., Man, T., Nomikos, G.G., Phebus, L.A., Cauvin, A.J., Johnson, K.W., Jones, C.K., Hoffmann, B.J., Sandusky, G.E., Walter, M.W., Porter, W. J., Yang, L., Merchant, K.M., Shannon, H.E., Svensson, K.A., 2008. Neurochemical and behavioral profiling of the selective GlyT1 inhibitors ALX5407 and LY2365109 indicate a preferential action in caudal vs. cortical brain areas. *Neuropharmacology* 55, 743–754. <https://doi.org/10.1016/j.neuropharm.2008.06.016>.
- Peyrovian, B., Rosenblat, J.D., Pan, Z., Iacobucci, M., Brietzke, E., McIntyre, R.S., 2019. The glycine site of NMDA receptors: a target for cognitive enhancement in psychiatric disorders. *Prog. Neuro Psychopharmacol. Biol. Psychiatr.* 92, 387–404. <https://doi.org/10.1016/j.pnpbp.2019.02.001>.
- Pinard, E., Alberati, D., Bender, M., Borroni, E., Brom, V., Burner, S., Fischer, H., Hainzl, D., Halm, R., Hauser, N., Jolidon, S., Lengyel, J., Marty, H.-P., Meyer, T., Moreau, J.-L., Mory, R., Narquizian, R., Norcross, R.D., Schmid, P., Wermuth, R., Zimmerli, D., 2010. Discovery of benzoylisoindolines as a novel class of potent, selective and orally active GlyT1 inhibitors. *Bioorg. Med. Chem. Lett* 20, 6960–6965. <https://doi.org/10.1016/j.bmcl.2010.09.124>.
- Pinard, E., Alberati, D., Borroni, E., Fischer, H., Hainzl, D., Jolidon, S., Moreau, J.-L., Narquizian, R., Nettekoven, M., Norcross, R.D., Stalder, H., Thomas, A.W., 2008. Discovery of benzoylpiperazines as a novel class of potent and selective GlyT1 inhibitors. *Bioorg. Med. Chem. Lett* 18, 5134–5139. <https://doi.org/10.1016/j.bmcl.2008.07.086>.
- Pinard, E., Borroni, E., Koerner, A., Umbricht, D., Alberati, D., 2018. Glycine transporter type I (GlyT1) inhibitor, bitopertin: a journey from lab to patient. *Chimia* 72, 477–484. <https://doi.org/10.2533/chimia.2018.477>.
- Pow, D.V., Hendrickson, A.E., 1999. Distribution of the glycine transporter glyt-1 in mammalian and nonmammalian retinae. *Vis. Neurosci.* 16, 231–239. <https://doi.org/10.1017/s0952523899162047>.
- Prybylowski, K., Wenthold, R.J., 2004. N-Methyl-D-aspartate receptors: subunit assembly and trafficking to the synapse. *J. Biol. Chem.* 279, 9673–9676. <https://doi.org/10.1074/jbc.R300029200>.
- Rahman, S.S., Coulton, S., Herdon, H.J., Joiner, G.F., Jin, J., Porter, R.A., 2007. 1,3-diaminopropan-2-ol sulfonamides as potent and selective inhibitors of the glycine transporter type 1. *Bioorg. Med. Chem. Lett* 17, 1741–1745. <https://doi.org/10.1016/j.bmcl.2006.12.063>.
- Ramos-Vicente, D., Ji, J., Gratacòs-Batlle, E., Gou, G., Reig-Viader, R., Luís, J., Burguera, D., Navas-Pérez, E., García-Fernández, J., Fuentes-Prior, P., Escrivá, H., Roher, N., Soto, D., Bayés, A., 2018. Metazoan evolution of glutamate receptors reveals unreported phylogenetic groups and divergent lineage-specific events. *Elife* 7, e35774. <https://doi.org/10.7554/eLife.35774>.
- Regalado, M.P., Villarroel, A., Lerma, J., 2001. Interunit cooperativity in the NMDA receptor. *Neuron* 32, 1085–1096. [https://doi.org/10.1016/S0896-6273\(01\)00539-6](https://doi.org/10.1016/S0896-6273(01)00539-6).

- Regan, M.C., Grant, T., McDaniel, M.J., Karakas, E., Zhang, J., Traynelis, S.F., Grigorieff, N., Furukawa, H., 2018. Structural mechanism of functional modulation by gene splicing in NMDA receptors. *Neuron* 98, 521–529.e3. <https://doi.org/10.1016/j.neuron.2018.03.034>.
- Reiner, A., Levitz, J., 2018. Glutamatergic signaling in the central nervous system: ionotropic and metabotropic receptors in concert. *Neuron* 98, 1080–1098. <https://doi.org/10.1016/j.neuron.2018.05.018>.
- Roberts, B.M., Shaffer, C.L., Seymour, P.A., Schmidt, C.J., Williams, G.V., Castner, S.A., 2010. Glycine transporter inhibition reverses ketamine-induced working memory deficits. *Neuroreport* 21, 390–394. <https://doi.org/10.1097/WNR.0b013e3283381a4e>.
- Rosenberg, D., Artoul, S., Segal, A.C., Kolodney, G., Radzishvsky, I., Dikopoltsev, E., Foltyn, V.N., Inoue, R., Mori, H., Billard, J.-M., Wolosker, H., 2013. Neuronal D-serine and glycine release via the Asc-1 transporter regulates NMDA receptor-dependent synaptic activity. *J. Neurosci.* 33, 3533–3544. <https://doi.org/10.1523/JNEUROSCI.3836-12.2013>.
- Rosenbrock, H., Desch, M., Kleiner, O., Dörner-Ciossek, C., Schmid, B., Keller, S., Schlecker, C., Moschetti, V., Goetz, S., Liesenfeld, K.-H., Fillon, G., Giovannini, R., Ramael, S., Wunderlich, G., Wind, S., 2018. Evaluation of pharmacokinetics and pharmacodynamics of BI 425809, a novel GlyT1 inhibitor: translational studies. *Clin. Transl. Sci* 11, 616–623. <https://doi.org/10.1111/cts.12578>.
- Rosenbrock, H., Dörner-Ciossek, C., Giovannini, R., Schmid, B., Schuelert, N., 2022. Effects of the Glycine transporter-1 inhibitor Iclepertin (BI 425809) on sensory processing, neural network function, and cognition in animal models related to schizophrenia. *J. Pharmacol. Exp. Therapeut.* 382, 223–232. <https://doi.org/10.1124/jpet.121.001071>.
- Roux, M.J., Supplisson, S., 2000. Neuronal and glial glycine transporters have different stoichiometries. *Neuron* 25, 373–383. [https://doi.org/10.1016/s0896-6273\(00\)80901-0](https://doi.org/10.1016/s0896-6273(00)80901-0).
- Safory, H., Neame, S., Shulman, Y., Zubedat, S., Radzishvsky, I., Rosenberg, D., Sason, H., Engelender, S., Avital, A., Hülsmann, S., Schiller, J., Wolosker, H., 2015. The alanine-serine-cysteine-1 (Asc-1) transporter controls glycine levels in the brain and is required for glycinergic inhibitory transmission. *EMBO Rep.* 16, 590–598. <https://doi.org/10.15252/embr.201439561>.
- Sanacora, G., Treccani, G., Popoli, M., 2012. Towards a glutamate hypothesis of depression. *Neuropharmacology* 62, 63–77. <https://doi.org/10.1016/j.neuropharm.2011.07.036>.
- Santora, V.J., Almos, T.A., Barido, R., Basinger, J., Bellows, C.L., Bookser, B.C., Breitenbucher, J.G., Broadbent, N.J., Cabebe, C., Chai, C.-K., Chen, M., Chow, S., Chung, D.M., Crickard, L., Danks, A.M., Freestone, G.C., Gitnick, D., Gupta, V., Hoffmaster, C., Hudson, A.R., Kaplan, A.P., Kennedy, M.R., Lee, D., Limberis, J., Ly, K., Mak, C.C., Masatsugu, B., Morse, A.C., Na, J., Neul, D., Nikpur, J., Peters, M., Petroski, R.E., Renick, J., Sebring, K., Sevidal, S., Tabatabaei, A., Wen, J., Yan, Y., Yoder, Z.W., Zook, D., 2018. Design and synthesis of novel and selective Glycine transporter-1 (GlyT1) inhibitors with memory enhancing properties. *J. Med. Chem.* 61, 6018–6033. <https://doi.org/10.1021/acs.jmedchem.8b00372>.
- Sason, H., Billard, J.M., Smith, G.P., Safory, H., Neame, S., Kaplan, E., Rosenberg, D., Zubedat, S., Foltyn, V.N., Christoffersen, C.T., Bundgaard, C., Thomsen, C., Avital, A., Christensen, K.V., Wolosker, H., 2017. Asc-1 transporter regulation of synaptic activity via the tonic release of D-serine in the forebrain. *Cerebr. Cortex* 27, 1573–1587. <https://doi.org/10.1093/cercor/bhv350>.
- Schoemaker, J.H., Jansen, W.T., Schipper, J., Szegedi, A., 2014. The selective glycine uptake inhibitor 025935 as an adjunctive treatment to atypical antipsychotics in predominant persistent negative symptoms of schizophrenia: results from the GIANT trial. *J. Clin. Psychopharmacol.* 34, 190–198. <https://doi.org/10.1097/JCP.0000000000000073>.
- Seckler, J.M., Lewis, S.J., 2020. Advances in D-amino acids in neurological research. *Int. J. Mol. Sci.* 21, 7325. <https://doi.org/10.3390/ijms21197325>.
- Shahsavari, A., Stohler, P., Bourenkov, G., Zimmermann, I., Siegrist, M., Guba, W., Pinard, E., Sinning, S., Seeger, M.A., Schneider, T.R., Dawson, R.J.P., Nissen, P., 2021. Structural insights into the inhibition of glycine reuptake. *Nature* 591, 677–681. <https://doi.org/10.1038/s41586-021-03274-z>.
- Shibasaki, K., Hosoi, N., Kaneko, R., Tominaga, M., Yamada, K., 2017. Glycine release from astrocytes via functional reversal of GlyT1. *J. Neurochem.* 140, 395–403. <https://doi.org/10.1111/jnc.13741>.
- Shimazaki, T., Kaku, A., Chaki, S., 2010. D-Serine and a glycine transporter-1 inhibitor enhance social memory in rats. *Psychopharmacology (Berl)* 209, 263–270. <https://doi.org/10.1007/s00213-010-1794-y>.
- Siegler Retchless, B., Gao, W., Johnson, J.W., 2012. A single GluN2 subunit residue controls NMDA receptor channel properties via intersubunit interaction. *Nat. Neurosci.* 15 (406–413), S1–S2. <https://doi.org/10.1038/nn.3025>.
- Singer, P., Zhang, W., Yee, B.K., 2013. SSR504734 enhances basal expression of prepulse inhibition but exacerbates the disruption of prepulse inhibition by apomorphine. *Psychopharmacology (Berl)* 230, 309–317. <https://doi.org/10.1007/s00213-013-3160-3>.
- Skrenkova, K., Song, J.-M., Kortus, S., Kolcheva, M., Netolicky, J., Hemelkova, K., Kaniakova, M., Krausova, B.H., Kucera, T., Korabecny, J., Suh, Y.H., Horak, M., 2020. The pathogenic S688Y mutation in the ligand-binding domain of the GluN1 subunit regulates the properties of NMDA receptors. *Sci. Rep.* 10, 18576. <https://doi.org/10.1038/s41598-020-75646-w>.
- Smith, G., Mikkelsen, G., Eskildsen, J., Bundgaard, C., 2006. The synthesis and SAR of 2-arylsulfanylphenyl-1-oxyalkylamino acids as GlyT-1 inhibitors. *Bioorg. Med. Chem. Lett* 16, 3981–3984. <https://doi.org/10.1016/j.bmcl.2006.05.017>.
- Smith, G., Ruhland, T., Mikkelsen, G., Andersen, K., Christoffersen, C.T., Alifrangis, L.H., Mørk, A., Wren, S.P., Harris, N., Wyman, B.M., Brandt, G., 2004. The synthesis and SAR of 2-arylsulfanylphenyl piperazinyl acetic acids as glyT-1 inhibitors. *Bioorg. Med. Chem. Lett* 14, 4027–4030. <https://doi.org/10.1016/j.bmcl.2004.05.043>.
- Smith, K.E., Borden, L.A., Hartig, P.R., Branchek, T., Weinshank, R.L., 1992. Cloning and expression of a glycine transporter leader colocalization with NMDA receptors. *Neuron* 8, 927–935. [https://doi.org/10.1016/0896-6273\(92\)90207-t](https://doi.org/10.1016/0896-6273(92)90207-t).
- Smith, K.E., Fried, S.G., Durkin, M.M., Gustafson, E.L., Borden, L.A., Branchek, T.A., Weinshank, R.L., 1995. Molecular cloning of an orphan transporter. A new member of the neurotransmitter transporter family. *FEBS Lett.* 357, 86–92. [https://doi.org/10.1016/0014-5793\(94\)01328-x](https://doi.org/10.1016/0014-5793(94)01328-x).
- Stein, I.S., Gray, J.A., Zito, K., 2015. Non-ionotropic NMDA receptor signaling drives activity-induced dendritic spine shrinkage. *J. Neurosci.* 35, 12303–12308. <https://doi.org/10.1523/JNEUROSCI.4289-14.2015>.
- Stein, I.S., Park, D.K., Claiborne, N., Zito, K., 2021. Non-ionotropic NMDA receptor signaling gates bidirectional structural plasticity of dendritic spines. *Cell Rep.* 34, 108664. <https://doi.org/10.1016/j.celrep.2020.108664>.
- Stein, I.S., Park, D.K., Flores, J.C., Jahncke, J.N., Zito, K., 2020. Molecular mechanisms of non-ionotropic NMDA receptor signaling in dendritic spine shrinkage. *J. Neurosci.* 40, 3741–3750. <https://doi.org/10.1523/JNEUROSCI.0046-20.2020>.
- Stein, I.S., Zito, K., 2019. Dendritic spine elimination: molecular mechanisms and implications. *Neuroscientist* 25, 27–47. <https://doi.org/10.1177/1073858418769644>.
- Stroebel, D., Casado, M., Paoletti, P., 2018. Triheteromeric NMDA receptors: from structure to synaptic physiology. *Curr Opin Physiol* 2, 1–12. <https://doi.org/10.1016/j.cophys.2017.12.004>.
- Stroebel, D., Paoletti, P., 2021. Architecture and function of NMDA receptors: an evolutionary perspective. *J. Physiol.* 599, 2615–2638. <https://doi.org/10.1113/JP279028>.
- Subramanian, N., Scopelliti, A.J., Carland, J.E., Ryan, R.M., O'Mara, M.L., Vandenberg, R.J., 2016. Identification of a 3rd Na<sup>+</sup> binding site of the Glycine transporter, GlyT2. *PLoS One* 11, e0157583. <https://doi.org/10.1371/journal.pone.0157583>.
- Sugane, T., Tobe, T., Hamaguchi, W., Shimada, I., Maeno, K., Miyata, J., Suzuki, T., Kimizuka, T., Morita, T., Sakamoto, S., Tsukamoto, S., 2012. Synthesis and biological evaluation of (4H-1,2,4-triazol-4-yl)isoquinoline derivatives as selective glycine transporter 1 inhibitors. *Bioorg. Med. Chem.* 20, 34–41. <https://doi.org/10.1016/j.bmc.2011.11.038>.
- Sugane, T., Tobe, T., Hamaguchi, W., Shimada, I., Maeno, K., Miyata, J., Suzuki, T., Kimizuka, T., Sakamoto, S., Tsukamoto, S., 2013. Atropisomeric 4-phenyl-4H-1,2,4-triazoles as selective glycine transporter 1 inhibitors. *J. Med. Chem.* 56, 5744–5756. <https://doi.org/10.1021/jm400383w>.
- Tajima, N., Karakas, E., Grant, T., Simorowski, N., Diaz-Avalos, R., Grigorieff, N., Furukawa, H., 2016. Activation of NMDA receptors and the mechanism of inhibition by ifenprodil. *Nature* 534, 63–68. <https://doi.org/10.1038/nature17679>.
- Tajima, N., Simorowski, N., Yovanno, R.A., Regan, M.C., Michalski, K., Gómez, R., Lau, A.Y., Furukawa, H., 2022. Development and characterization of functional antibodies targeting NMDA receptors. *Nat. Commun.* 13, 923. <https://doi.org/10.1038/s41467-022-28559-3>.
- Takanaga, H., Mackenzie, B., Suzuki, Y., Hediger, M.A., 2005. Identification of mammalian proline transporter SIT1 (SLC6A20) with characteristics of classical system imino. *J. Biol. Chem.* 280, 8974–8984. <https://doi.org/10.1074/jbc.M413027200>.
- Thomson, C.G., Duncan, K., Fletcher, S.R., Huscroft, I.T., Pillai, G., Raubo, P., Smith, A.J., Stead, D., 2006. Sarcosine based indandione hGlyT1 inhibitors. *Bioorg. Med. Chem. Lett* 16, 1388–1391. <https://doi.org/10.1016/j.bmcl.2005.11.041>.
- Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., Dingledine, R., 2010. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev.* 62, 405–496. <https://doi.org/10.1124/pr.109.002451>.
- Tsai, G., Lane, H.-Y., Yang, P., Chong, M.-Y., Lange, N., 2004a. Glycine transporter 1 inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. *Biol. Psychiatr.* 55, 452–456. <https://doi.org/10.1016/j.biopsych.2003.09.012>.
- Tsai, G., Ralph-Williams, R.J., Martina, M., Bergeron, R., Berger-Sweeney, J., Dunham, K. S., Jiang, Z., Caine, S.B., Coyle, J.T., 2004b. Gene knockout of glycine transporter 1: characterization of the behavioral phenotype. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8485–8490. <https://doi.org/10.1073/pnas.0402662101>.
- Umbricht, D., Alberati, D., Martin-Facklam, M., Borroni, E., Youssef, E.A., Ostland, M., Wallace, T.L., Knoflach, F., Dorflinger, E., Wettstein, J.G., Bausch, A., Garibaldi, G., Santarelli, L., 2014. Effect of bitopertin, a glycine reuptake inhibitor, on negative symptoms of schizophrenia: a randomized, double-blind, proof-of-concept study. *JAMA Psychiatry* 71, 637–646. <https://doi.org/10.1001/jamapsychiatry.2014.163>.
- Vance, K.M., Simorowski, N., Traynelis, S.F., Furukawa, H., 2011. Ligand-specific deactivation time course of GluN1/GluN2D NMDA receptors. *Nat. Commun.* 2, 294. <https://doi.org/10.1038/ncomms1295>.
- Varnes, J.G., Forst, J.M., Hoerter, T.N., Holmquist, C.R., Wilkins, D.E., Tian, G., Jonak, G., Wang, X., Potts, W.M., Wood, M.W., Alhambra, C., Brugel, T.A., Albert, J. S., 2010. Identification of N-(2-(azepan-1-yl)-2-phenylethyl)-benzenesulfonamides as novel inhibitors of GlyT1. *Bioorg. Med. Chem. Lett* 20, 4878–4881. <https://doi.org/10.1016/j.bmcl.2010.06.085>.
- Verkhatsky, A., Chvátal, A., 2020. NMDA receptors in astrocytes. *Neurochem. Res.* 45, 122–133. <https://doi.org/10.1007/s11064-019-02750-3>.
- Vissel, B., Krupp, J.J., Heinemann, S.F., Westbrook, G.L., 2001. A use-dependent tyrosine dephosphorylation of NMDA receptors is independent of ion flux. *Nat. Neurosci.* 4, 587–596. <https://doi.org/10.1038/88404>.



- Wang, J.X., Furukawa, H., 2019. Dissecting diverse functions of NMDA receptors by structural biology. *Curr. Opin. Struct. Biol.* 54, 34–42. <https://doi.org/10.1016/j.sbi.2018.12.009>.
- Wang, K.H., Penmatsa, A., Gouaux, E., 2015. Neurotransmitter and psychostimulant recognition by the dopamine transporter. *Nature* 521, 322–327. <https://doi.org/10.1038/nature14431>.
- Yamashita, A., Singh, S.K., Kawate, T., Jin, Y., Gouaux, E., 2005. Crystal structure of a bacterial homologue of Na<sup>+</sup>/Cl<sup>−</sup>-dependent neurotransmitter transporters. *Nature* 437, 215–223. <https://doi.org/10.1038/nature03978>.
- Yao, Y., Belcher, J., Berger, A.J., Mayer, M.L., Lau, A.Y., 2013. Conformational analysis of NMDA receptor GluN1, GluN2, and GluN3 ligand-binding domains reveals subtype-specific characteristics. *Structure* 21, 1788–1799. <https://doi.org/10.1016/j.str.2013.07.011>.
- Yao, Y., Harrison, C.B., Freddolino, P.L., Schulten, K., Mayer, M.L., 2008. Molecular mechanism of ligand recognition by NR3 subtype glutamate receptors. *EMBO J.* 27, 2158–2170. <https://doi.org/10.1038/emboj.2008.140>.
- Yavi, M., Lee, H., Henter, I.D., Park, L.T., Zarate, C.A., 2022. Ketamine treatment for depression: a review. *Discov. Ment. Health* 2, 9. <https://doi.org/10.1007/s44192-022-00012-3>.
- Yee, B.K., Balic, E., Singer, P., Schwerdel, C., Grampp, T., Gabernet, L., Knuesel, I., Benke, D., Feldon, J., Mohler, H., Boison, D., 2006. Disruption of glycine transporter 1 restricted to forebrain neurons is associated with a procognitive and antipsychotic phenotypic profile. *J. Neurosci.* 26, 3169–3181. <https://doi.org/10.1523/JNEUROSCI.5120-05.2006>.
- Yuan, H., Hansen, K.B., Vance, K.M., Ogden, K.K., Traynelis, S.F., 2009. Control of NMDA receptor function by the NR2 subunit amino-terminal domain. *J. Neurosci.* 29, 12045–12058. <https://doi.org/10.1523/JNEUROSCI.1365-09.2009>.
- Zafrá, F., Aragón, C., Olivares, L., Danbolt, N.C., Giménez, C., Storm-Mathisen, J., 1995. Glycine transporters are differentially expressed among CNS cells. *J. Neurosci.* 15, 3952–3969. <https://doi.org/10.1523/JNEUROSCI.15-05-03952.1995>.
- Zeng, Z., O'Brien, J.A., Lemaire, W., O'Malley, S.S., Miller, P.J., Zhao, Z., Wallace, M.A., Raab, C., Lindsley, C.W., Sur, C., Williams, D.L.J., 2008. A novel radioligand for glycine transporter 1: characterization and use in autoradiographic and in vivo brain occupancy studies. *Nucl. Med. Biol.* 35, 315–325. <https://doi.org/10.1016/j.nucmedbio.2007.12.002>.
- Zhao, Y., Terry, D., Shi, L., Weinstein, H., Blanchard, S.C., Javitch, J.A., 2010. Single-molecule dynamics of gating in a neurotransmitter transporter homologue. *Nature* 465, 188–193. <https://doi.org/10.1038/nature09057>.
- Zhao, Y., Terry, D.S., Shi, L., Quick, M., Weinstein, H., Blanchard, S.C., Javitch, J.A., 2011. Substrate-modulated gating dynamics in a Na<sup>+</sup>-coupled neurotransmitter transporter homologue. *Nature* 474, 109–113. <https://doi.org/10.1038/nature09971>.
- Zhao, Z., Leister, W.H., O'Brien, J.A., Lemaire, W., Williams, D.L.J., Jacobson, M.A., Sur, C., Kinney, G.G., Pettibone, D.J., Tiller, P.R., Smith, S., Hartman, G.D., Lindsley, C.W., Wolkenberg, S.E., 2009. Discovery of N-[[1-(propylsulfonyl)-4-pyridin-2-ylpiperidin-4-yl]methyl]benzamides as novel, selective and potent GlyT1 inhibitors. *Bioorg. Med. Chem. Lett.* 19, 1488–1491. <https://doi.org/10.1016/j.bmcl.2008.12.115>.
- Zhu, S., Gouaux, E., 2017. Structure and symmetry inform gating principles of ionotropic glutamate receptors. *Neuropharmacology* 112, 11–15. <https://doi.org/10.1016/j.neuropharm.2016.08.034>.
- Zhu, S., Paoletti, P., 2015. Allosteric modulators of NMDA receptors: multiple sites and mechanisms. *Curr. Opin. Pharmacol.* 20, 14–23. <https://doi.org/10.1016/j.coph.2014.10.009>.
- Zhu, S., Stein, R.A., Yoshioka, C., Lee, C.-H., Goehring, A., Mchaourab, H.S., Gouaux, E., 2016. Mechanism of NMDA receptor inhibition and activation. *Cell* 165, 704–714. <https://doi.org/10.1016/j.cell.2016.03.028>.