Chapter 3
Physiological Roles of D-Serine in the Central Nervous System

Jean-Pierre Mothet, Grégoire Mondielli, and Magalie Martineau

Abstract The N-methyl D-aspartic acid receptors (NMDARs) are key glutamate receptors that transduce glutamatergic signals throughout the developing and adult central nervous system (CNS). Despite diversity in their subunit composition, their subcellular localization, and their biophysical and pharmacological properties, activation of NMDARs always requires in addition to glutamate the binding of a co-agonist that has long been thought to be glycine. However, research over the last two decades has challenged this long-cherished model by showing that the D-isomer of serine is the preferential co-agonist for a large population of NMDARs in many areas of the adult brain. Nowadays, a totally new picture of glutamatergic synapses is emerging where both glycine and D-serine are involved in a complex interplay to regulate NMDAR functions in the CNS following time and space constraints. In this review, we focus on the particular contribution of D-serine relatively to glycine in orchestrating synapse formation, dynamics, and neuronal network activity in a time- and synapse-specific manner and its role in cognitive functions. We will discuss also how astroglia and neurons use different pathways to regulate levels of extracellular D-serine and how alterations in synaptic availability of this D-amino acid may contribute to cognitive deficits associated to healthy aging and therefore may open new avenues for therapies.

Keywords NMDA receptors • Synaptic transmission • Synaptic plasticity • Neurons • Astrocytes

The neurotransmitter glutamate once released in the synaptic cleft binds and acts on various transporters, ionotropic and metabotropic membrane receptors located onto neuronal elements (Traynelis et al. 2010), but also onto glial cells including

J.-P. Mothet (✉) • G. Mondielli
Team 'Gliotransmission and Synaptopathies', CRN2M UMR7286, Aix-Marseille Université, CNRS, 13344 cedex 15 Marseille, France
e-mail: jean-pierre.mothet@univ-amu.fr

M. Martineau
Interdisciplinary Institute for Neuroscience, UMR 5297, University of Bordeaux and Centre National de la Recherche Scientifique, F-33000 Bordeaux, France

T. Yoshimura et al. (eds.), *D-Amino Acids*, DOI 10.1007/978-4-431-56077-7_3

astrocytes and oligodendrocytes (Parpura and Verkhratsky 2013). The ionotropic glutamate-gated receptor (iGluR) family encodes 18 gene products or subunits subdivided into three main subtypes characterized by their preferential agonists: AMPA receptors (GluARs), kainate receptors (GluKRs), and NMDARs (GluNs) (Traynelis et al. 2010; Paoletti et al. 2013). AMPARs and KARs are cation-permeable ion channels which upon binding of glutamate mediate fast excitatory synaptic transmission, while NMDARs are calcium permeable and drive the transduction of specific temporal patterns of synaptic activity into long-term structural and functional changes. N-Methyl D-aspartic receptors (NMDARs) are ionotropic glutamate-gated receptors that are central to many physiological processes including learning and memory and are involved in neurotoxicity and psychiatric disorders (Traynelis et al. 2010; Paoletti et al. 2013).

3.1 The NMDA Receptors

3.1.1 Structure and Subunit Diversity of NMDARs in the CNS

NMDA receptors are heterotetramers associating two obligatory GluN1 subunits with two identical or different GluN2 or a mixture of GluN2 and GluN3 subunits (Traynelis et al. 2010; Paoletti et al. 2013) (Fig. 3.1). The GluN1 subunit exists as eight distinct splice variants of a single encoding gene (*GRIN1*). Four distinct GluN2 subunits (GluN2A-D) and two GluN3 subunits (GluN3A, B) have been identified which are encoded by four (*GRIN2A-D*) and two (*GRIN3A*, *B*) different genes, respectively (Traynelis et al. 2010; Paoletti et al. 2013). The large repertoire of diheteromeric and triheteromeric NMDARs display distinct trafficking, biophysical, and pharmacological properties and are differentially populating synapses depending on neuronal subpopulations and on development (Traynelis et al. 2010; Paoletti et al. 2013).

Similar to all iGluR subunits, every GluN subunit has four domains (Fig. 3.1). The extracellular region comprises the amino-terminal domain (NTD) involved in subunit association and the agonist-binding domain (ABD) formed by two segments S1 and S2 which bind the co-agonists glycine and D-serine on GluN1 and GluN3 subunits or glutamate on GluN2 subunits. The transmembrane domain (TMD) is formed by three helices (M1, M3, M4) and a hairpin (M2) forming the channel pore and conferring the ion selectivity. The cytoplasmic carboxy-terminal domain (CTD) is involved in protein-protein interactions which determine trafficking (membrane targeting, lateral diffusion, stabilization, recycling), posttranslational regulation, and coupling to specific intracellular signaling cascades. All domains except the CTD are the targets of multiple endogenous or synthetic allosteric modulators (Traynelis et al. 2010; Paoletti et al. 2013).

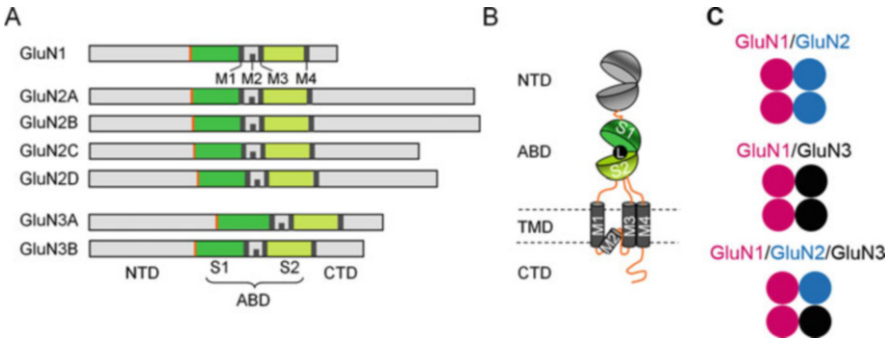

Fig. 3.1 The NMDA receptors. (**a**) Linear organization of monomeric subunits. Seven subunits (GluN) have been identified: GluN1, GluN2A–GluN2D, and GluN3A and GluN3B. M1–M4 refer to membrane segments. (**b**) Modular organization of a GluN subunit. The N-terminal domain (*NTD*); the agonist-binding domain (*ABD*) that binds to the ligand (L), i.e., glycine or D-serine in GluN1 and GluN3 and glutamate in GluN2; the transmembrane domain (*TMD*) containing the ion channel; and an intracellular C-terminal domain (*CTD*). With permission from Mothet et al. (2015) J Neurochem. (**c**) Possible subunits compositions for diheteromeric and triheteromeric receptors

3.1.2 NMDARs and the Glycine Modulatory Binding Site

NMDARs possess unique biophysical and pharmacological properties among the iGluR family (Traynelis et al. 2010; Paoletti et al. 2013). In contrast to GluKRs and GluARs, all NMDARs show much slower gating and deactivating kinetics, thus displaying a large single channel conductance (~40 pS) depending on the subunit composition. The NMDARs display high Ca^{2+} permeability (with subunit-specific variations) and behave as unique Hebbian-like coincidence detectors owing to their voltage-dependent Mg^{2+} block and the presence of many modulatory sites on the different domains of each subunit. But a major property of NMDAR activation of interest for us here is the need of a concomitant binding of glutamate on GluN2 subunits and of a co-agonist D-serine or glycine on GluN1 (Martineau et al. 2006; Traynelis et al. 2010; Paoletti et al. 2013).

The absolute necessity of a co-agonist together with glutamate for the activation of the NMDARs stemmed from the original observations that NMDA-evoked inward currents recorded in native NMDARs (Johnson and Ascher 1987) and from NMDARs expressed in oocytes (Kleckner and Dingledine 1988) require the addition of glycine at high nanomolar range. In their seminal work, Kleckner and Dingledine already showed that several glycine analogs such as D-serine or D-alanine could substitute in activating NMDARs (Kleckner and Dingledine 1988). This observation is consistent with early binding experiments and electrophysiological recordings showing that glycine and D-serine target NMDARs with relatively similar nanomolar affinity (Monaghan et al. 1988; Matsui et al. 1995). Cocrystal structures of the GluN1 S1-S2 ligand-binding core with glycine or D-serine reveal that agonist binding is critically dependent upon a series of hydrogen bonds to side-chain and main-chain atoms, as well as to water molecules in the binding pocket.

Because it makes three additional hydrogen bonds and displaces a water molecule, D-serine binds more tightly to the receptor in comparison to glycine (Furukawa and Gouaux 2003) and would therefore virtually make it more efficient to activate the NMDAR. Nevertheless, the affinity of GluN1 for D-serine or glycine is very similar ($EC_{50} \sim 0.1–1$ μM, (Priestley and Kemp 1994; Chen et al. 2008). The affinity of glycine or D-serine for GluN1 is in fact dictated by the identity of GluN2 subunit. Indeed, the binding sites of the co-agonist and glutamate are allosterically coupled. Accordingly, heterodimeric GluN2B-containing NMDARs display 10× higher affinity for glycine than those containing the GluN2A subunit ($EC_{50} \sim 0.1$ μM versus 1 μM). Conversely, the affinity of D-serine for GluN1 seems to be higher when the NMDARs are containing the GluN2A subunits (Priestley and Kemp 1994; Matsui et al. 1995). While the binding of D-serine at NMDARs was described more than 25 years ago, only glycine was thought to be the right co-agonist since D-serine was not expected to exist in higher living organisms. The discovery that D-amino acids including D-serine are present in organisms throughout evolution including in humans has forced neuroscientists to reconsider the mode of activation of NMDARs. In different tissues including the brain, D-amino acids have emerged as important signaling molecules (Ohide et al. 2011). The field has grown very fast since the pioneer observations made in early 1990s and results from intensive research and from many groups around the world now support that D-serine is the preferred endogenous ligand for the glycine site of NMDARs and thus that this unexpected D-amino acid is central to many brain functions.

3.2 Astrocytes and Neurons Use Different Pathways to Release D-Serine

3.2.1 D-Serine: Glial and Neuronal

In mammals, D-serine is exclusively synthesized from L-serine by serine racemase (SR) (Wolosker and Mori 2012), an enzyme widely distributed in the CNS. SR is a highly regulated enzyme reviewed in (Campanini et al. 2013; Martineau et al. 2014) that binds to several receptor-interacting proteins that modulate the production of D-serine. In addition, D-serine degradation is controlled by the flavoenzyme DAAO, a peroxisomal enzyme that eliminates the amino acid retrieved from the extracellular space (Martineau et al. 2006). The expression and activity of DAAO is widespread in the human and rodent CNS being expressed in both astrocytes and neurons (Verrall et al. 2007; Sasabe et al. 2014). Despite many progresses, our knowledge on the biochemical cascades modulating DAAO activity is still very limited. DAAO interacts with pLG72, a primate-specific protein present in mitochondria (Sacchi et al. 2008; Otte et al. 2014). The interaction with DAAO would involve interaction of the cytosolic fraction of DAAO with pLG72 potentially exposed at the surface of mitochondria as suggested by Sacchi and colleagues

(Sacchi et al. 2011). We do not know if an ortholog of pLG72 may exist in non-primate mammals. Proteomics analyses have revealed that DAAO interacts also with Bassoon and Piccolo, two proteins of the presynaptic active zone (Popiolek et al. 2011) supporting the neuronal localization for DAAO.

In which cell type D-serine is formed? Although a glial localization has been largely attributed to SR and D-serine (Schell et al. 1997; Wolosker et al. 1999; Panatier et al. 2006; Williams et al. 2006), allowing the amino acid to be considered as a gliotransmitter, several studies indicate that SR may be instead preferentially expressed in excitatory and inhibitory neurons in rodent and human brains rather than in glia (Miya et al. 2008; Ehmsen et al. 2013; Balu et al. 2014). Accordingly, neuronal-specific conditional deletion of SR induces a much larger decrease in cerebral and hippocampal D-serine contents compared to deletion limited to the astrocyte population (Benneyworth et al. 2012). However, variations in the cellular expression of SR and D-serine between species could not be totally ruled out. Accordingly, Suzuki et al. (2015), using SR-KO as negative controls, recently reported that astrocytes abundantly express SR in the human subiculum. The notion that D-serine could be formed in astroglia is further reinforced by the fact that SR interacts with DISC-1 in astrocytes (Ma et al. 2013). Furthermore, using multiplex single-cell RT-PCR, we observe that both neurons and astrocytes express SR at least at the levels of transcripts in the prefrontal cortex of adult rat (Dallerac, Turpin & Mothet, Personal communication). Thus, the cellular distribution of SR remains an open issue particularly since a recent transcriptome analysis of isolated purified brain cell groups shows that the enzyme is more expressed in glial cell lineage and notably astrocytes than in neurons in the mature cortex of mice (Zhang et al. 2014). As neurons are not capable of synthesizing much L-serine, neuronal SR activity would depend on the supply of the D-serine precursor which is primarily formed in astrocytes from glucose by 3-phosphoglycerate dehydrogenase (Yang et al. 2010; Ehmsen et al. 2013).

3.2.2 Cell-Type-Specific Mechanisms of Release: Exocytosis Versus Hetero-exchange

As shown so far, D-serine can be formed in both neurons and astrocytes (Martineau et al. 2014). Different elements of the literature support the notion that neurons and astroglia use different molecular machinery and signaling pathways to release D-serine into the extracellular space (Fig. 3.2). Early studies have shown that D-serine release is triggered by agonists of the ionotropic and metabotropic glutamate receptors (iGluR and mGluR, respectively; (Schell et al. 1995; Mothet et al. 2005; Martineau et al. 2008; Ishiwata et al. 2013). We have accumulated evidence that the release of D-serine by astrocytes is mainly dependent on an increase in cytosolic Ca^{2+}. Indeed, disrupting Ca^{2+} signaling inside astrocytes reduces D-serine release from astrocytes in culture and in hippocampal slices

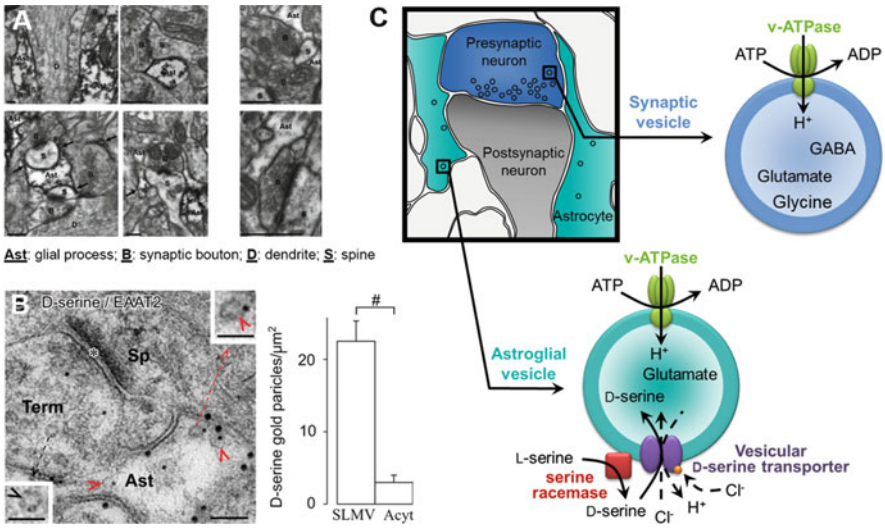

Fig. 3.2 Modes of release of D-serine. (**a**) Preembedding peroxidase immunohistochemistry showing that D-serine immunoreactivity is mainly distributed in astrocytic processes (*arrows*) at the level of asymmetric synapses in the adult rat cerebral cortex. Representative low-magnification (*top*) and high-magnification (*bottom*) electron micrographs showing that no peroxidase staining is detected with a preabsorbed anti-D-serine antibody. *Ast* astrocyte, *D* dendrite, *S* dendritic spine, *B* axonal bouton. *Scale bars*, 500 nm. Modified with permission from The Society of Neuroscience (Martineau et al. 2013). (**b**) Immunogold labeling (small gold particles) of D-serine in small synaptic-like vesicles (indicated by *red arrowheads*) in astrocytic processes (*Ast*) positive for EAAT2 (large gold particles) contacting asymmetric synapses (stars) between nerve terminals (Term) and dendritic spines (Sp). *Insets*: higher magnification showing the similarities between SLMVs (*red dotted arrows*) and SVs (*black dotted arrow*). Note that the astrocytic SLMVs are often localized in small clusters close to the plasma membrane. *Scale bars*: 100 and 50 nm in insets. Right immunogold quantification of D-serine gold particles in astrocytic processes. The *bar charts* show the mean number of D-serine gold particles/$\mu m^2 \pm$ SD in SLMVs and the cytoplasmic matrix of astrocytic processes (*Acyt*). Reprinted with permission from Oxford University Press (Bergersen et al 2012). (**c**) Graphical abstract showing that in astrocytes D-serine is mainly released by exocytosis of some vesicles containing also glutamate, while in neurons D-serine is released from the cytosol through asc-1. For explanation, see text

(Mothet et al. 2005; Henneberger et al. 2010; Shigetomi et al. 2013) but also from astrocytes in vivo (Takata et al. 2011). Calcium certainly represents the most critical signaling hub for D-serine release from astrocytes, even though we are still largely ignoring the spatial and temporal signature of these Ca^{2+} signals: activation of astrocytic transforming growth factor (TGF)-β (TGFR; (Diniz et al. 2012)), bradykinin-type2 (B2R; (Martineau et al. 2008)), adenosine-type 2 (A2R; (Scianni et al. 2013)), ephrinB3 (Zhuang et al. 2010), and muscarinic acetylcholine (Takata et al. 2011; Lopez-Hidalgo et al. 2012) receptors. The activation of these G protein-coupled receptors (GPCRs) is associated with the recruitment of Ca^{2+} from the intracellular stores mainly via inositol-1,4,5-trisphosphate receptors (IP3R) located on the endoplasmic reticulum (ER) (Zorec et al. 2012). But Ca^{2+} driving D-serine

release could also originate from the extracellular space through channel-mediated transmembrane Ca^{2+} fluxes in astrocytes. For example, astrocytic transient receptor potential A1 (TRPA1) channels contribute to basal Ca^{2+} signals which are required for D-serine release (Shigetomi et al. 2013). These studies clearly established that astrocytes express a plethora of functional receptors which activation is coupled to the release of D-serine. Then, what could be the mechanisms downstream Ca^{2+} triggering D-serine release? Although not exclusive of another release mechanism, we have found that D-serine release from astrocytes is dependent on soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, indicating that glial Ca^{2+}-regulated exocytosis is a main release mechanism used by astroglia to release D-serine. Indeed, astrocytes as most eukaryotic cells expressed synaptobrevins, also referred to as vesicle-associated proteins-VAMPs, or R (for arginine)-SNAREs) and the plasma membrane (syntaxins and synaptosome-associated protein of 23 kDa (SNAP23); Q (for glutamine)-SNAREs) (Jahn and Fasshauer 2012). The vesicle fusion is triggered by an increase in cytosolic Ca^{2+}, which presumably binds to vesicular synaptotagmins (Montana et al. 2006). Astroglial vesicles also possess proteins necessary for vesicular filling, such as the vacuolar type H^{+}-ATPase (V-ATPase) (Martineau et al. 2013) which provides the proton gradient necessary for intravesicular loading of gliotransmitters via appropriate transporter(s). Cleavage of Sb2 and cellubrevin by tetanus neurotoxin causes a strong inhibition of Ca^{2+}-dependent D-serine release (Mothet et al. 2005; Henneberger et al. 2010). Additionally, the blockade of D-serine vesicular uptake using a V-ATPase blocker inhibits D-serine release from astrocytes (Mothet et al. 2005). Indeed, based on electron microscopy (EM), D-serine accumulates in clear vesicles with a diameter of 36 nm in the perisynaptic processes of hippocampal and cortical astrocytes (Bergersen et al. 2012; Martineau et al. 2013). In the adult hippocampus, clear vesicles are organized in small groups of 2–15 vesicles preferentially located within 100 nm from the astrocytic plasma membrane (Bergersen et al. 2012) and observed at sites adjacent to neuronal elements bearing NMDARs (Bezzi et al. 2004). Thus, astrocytes possess small vesicles resembling those found at synaptic terminals, albeit at lower density. It should be noted, however, that the vesicular size in astrocytes appears to be more diverse than that described for neurons. In addition to Ca^{2+}- and SNARE-dependent exocytotic release of D-serine from astrocytes, this gliotransmitter can be released *via* alternative non-exocytotic conduits at the plasma membrane, including volume-regulated anion channels (VRAC; (Rosenberg et al. 2010)), alanine–serine–cysteine transporter (ASCT; (Ribeiro et al. 2002), but see (Maucler et al. 2013)) and likely through connexin 43 hemichannels (Stehberg et al. 2012). Although the vesicular transporter for D-serine has not been identified, the transport of D-serine inside astroglial vesicles was recently characterized (Martineau et al. 2013). While glutamate transport is observed in both synaptic and astroglial vesicles, the transport of D-serine is specific to astroglial vesicles (Martineau et al. 2013). Its apparent affinity is ~7 mM, consistent with the affinity of vesicular inhibitory amino acid transporter for γ-aminobutyric acid, another neutral amino acid (Chaudhry 2008; Martineau et al. 2013). Similar to glutamate, extravesicular chloride concentration modulates

D-serine transport into astroglial vesicles, reaching the maximum activity at 4 mM. Because D-serine transport induces vesicular acidification and critically relies on chloride, the vesicular D-serine transporter is proposed to be a D-serine/chloride co-transporter (Martineau et al. 2013) (Fig. 3.2). A spatial association of SR activity and D-serine vesicular transport was observed, resulting in a functional coupling between D-serine synthesis and uptake (Martineau et al. 2013). Finally, D-serine and glutamate vesicular loading exert a mutual stimulation which indicates a functional crosstalk between the two transporters (Martineau et al. 2013). This synergy at vesicular level can only be explained if both transporters reside on the same vesicle, indicating the co-storage and thus the co-release of both gliotransmitters. However, the immunogold colabeling of D-serine and glutamate in the adult hippocampus did not reveal a population of vesicles containing both gliotransmitters (Bergersen et al. 2012); this seemingly disparate findings could be a result of the limited sensitivity of the immuno-EM. Nonetheless, the mechanism underlying the vesicular synergy between D-serine and glutamate uptake requires additional investigation. The possible co-storage of glutamate and D-serine, at least in a subpopulation of astroglial vesicles, points to the possibility of interdependence of their dynamics and modulatory functions at synaptic and extrasynaptic sites.

Release of D-serine as a neurotransmitter operates through a different mechanism than those described before. Neuronal D-serine is mainly released in response to depolarization, induced by veratridine, a voltage-dependent Na^+ channel activator, or by increased extracellular potassium concentration, both in vitro and in vivo (Hashimoto et al. 2000; Rosenberg et al. 2010) (Fig. 3.2). Intriguingly, neuronal silencing by tetrodotoxin increased D-serine extracellular concentration in vivo (Maucler et al. 2013) although some studies show no effect (Hashimoto et al. 1995). Chelation of intracellular and extracellular Ca^{2+} does not affect depolarization-elicited D-serine release. In addition, inhibition of V-ATPase by bafilomycin A1 or cleavage of Sb2 by tetanus neurotoxin failed to inhibit D-serine release from neurons, excluding the vesicular release mechanism (Rosenberg et al. 2010) which is consistent with D-serine absence in the lumen of synaptic vesicles (Martineau et al. 2013), D-serine is released by neuronal presynaptic elements from a cytosolic pool through the Na^+-independent antiporter alanine–serine–cysteine transporter 1 (asc-1; (Rosenberg et al. 2010; Rosenberg et al. 2013); Fig. 3.2). Asc-1 is restricted to neurons and catalyzes neutral amino acid hetero-exchange (Fukasawa et al. 2000; Helboe et al. 2003; Matsuo et al. 2004), between D-serine and another neutral amino acid. Neuronal D-serine release could be triggered by neutral amino acids, such as L-serine, D-alanine, or D-isoleucine, through this hetero-exchange (Rosenberg et al. 2010, 2013). Two independent studies published in 2013 (Ishiwata et al. 2013; Maucler et al. 2013) have explored in vivo the dynamics of D-serine release in rat cortex. Using a second-by-second time scale microelectrode biosensors, Maucler et al. (2013) found that the application of the asc-1 transporter inhibitor, S-methyl-L-cysteine, depressed the release of D-serine evoked by L-serine, while L-asparagine, a substrate of ASCT2, did not induce D-serine release in vivo. Conversely, intra-medial frontal cortex infusion of S-methyl-L-cysteine, caused a concentration-dependent increase in the

microdialysate contents of D-serine (Ishiwata et al. 2013). The reasons of these opposite results are not known but could reflect differences in timescale of both analytical methods used in the two studies but clearly show that asc-1 could work in both directions. Which direction (uptake or release) in vivo prevails is not known so far.

3.3 Functions of D-Serine: From Development to Cognition

What could be the function of D-serine in the CNS? Early studies in the 1980–1990s evidenced that exogenous D-serine can potentiate NMDARs activity (Danysz et al. 1990). Two pioneer observations in the 1990s put D-serine in the spotlight by revealing that endogenous D-serine may be linked to NMDARs activation. First, its distribution in the rodent brain mirrors the expression of NMDARs, as one might expect for an NMDAR co-agonist, during development and in the adult animal (Hashimoto et al. 1993; Schell et al. 1995, 1997).

3.3.1 Role of D-Serine in the Adult Brain

Although D-serine could serve as a co-agonist of NMDAR, the experimental evidence for it awaited for the development of specific pharmacological and genetic tools allowing manipulating the levels of the endogenous amino acid.

A first approach we introduced consisted in the use of purified DAAO on top cultured neurons or brain slices to deplete endogenous D-serine while recording synaptic events (Mothet et al. 2000). We then discovered that depleting D-serine with DAAO consistently reduces synaptic events and NMDAR-mediated currents just demonstrating that D-serine rather than glycine as initially proposed would be the endogenous ligand for synaptic NMDARS in the hippocampus and juvenile cerebellum (Mothet et al. 2000). Therefore, it was predicted that the functions of D-serine and glycine could be dependent on the location of specific synapses within different brain area. But, subsequently acute depletion of D-serine with recombinant *Rhodotorula gracilis* DAAO (*Rg*DAAO) or serine deaminase (Dsda), two enzymatic D-serine scavengers, consistently affects NMDAR activity on glutamatergic neurons in widespread area of the mature brain including the Schaeffer collateral (SC)-CA1 synapse in the hippocampus (Papouin et al. 2012; Le Bail et al. 2015), medial prefrontal cortex (Fossat et al. 2012), hypothalamic supraoptic nucleus (Panatier et al. 2006), nucleus accumbens (Curcio et al. 2013), the medial nucleus of the trapezoid body (MNTB) (Reyes-Haro et al. 2010), or even the retina (Stevens et al. 2003). These observations lead us to jump on the conclusion that D-serine and not glycine is the right endogenous co-agonist for synaptic NMDARs at most central synapses. This conclusion was reinforced by the observations that a concomitant removal of endogenous glycine with *Bacillus subtilis* GO (*Bs*GO) has only

weak or no effects at the SC-CA1 glutamatergic synapse in the hippocampus (Papouin et al. 2012; Le Bail et al. 2015) or in the hypothalamus (Panatier et al. 2006). Conversely, in some case like the nucleus tractus solitarii (NTS), a structure located in the brainstem, glycine acts as the primary co-agonist for synaptic receptors (Panatier et al. 2006). In the structures where D-serine action predominates, it is thought that extrasynaptic glia-derived glycine should be unable to reach synaptic sites due to efficient capture by GlyT1 (Fossat et al. 2012; Papouin et al. 2012; Le Bail et al. 2015). Nevertheless, this dichotomic view is challenged by the fact that a substantial fraction of the synaptic NMDAR responses remained systematically resistant to the enzymatic degradation of D-serine. In addition, neuronal-derived glycine released through Asc-1 physiologically modulates NMDAR activation (Rosenberg et al. 2013) at the SC-CA1 hippocampal synapses. Introduction of animal models for D-serine represented another important opening to define the function of D-serine. A first model was developed early by Konno and collaborators which is a mutant mouse strain (ddY/DAO^{-}) lacking DAO activity (Konno and Yasumura 1983). The ddY/DAO^{-} mice have a missense mutation (G181R) that causes a complete loss of enzyme activity (Sasaki et al. 1992). As expected, these mutant mice exhibit larger NMDA/AMPA ratio due to a higher occupancy of the glycine site of NMDARs in diverse areas like the hippocampus (Maekawa et al. 2005) and the retina (Romero et al. 2014) and also show enhanced hippocampal LTP (Maekawa et al. 2005). More recently, specific DAAO inhibitors have been developed by different companies. Duplantier and colleagues (2011) show that inhibition of DAAO increases NMDA receptor-mediated synaptic currents in primary neuronal cultures from rat hippocampus and resulted in a significant increase in evoked hippocampal theta rhythm, an in vivo electrophysiological model of hippocampal activity (Strick et al. 2011). Likewise, inhibition of DAAO was reported to increase NMDAR activity and LTP formation in the mature hippocampus (Hopkins et al. 2013a, b; Le Bail et al. 2015) and to normalize the activity of impaired NMDARs in the supraoptic nucleus of lactating rats (Hopkins et al. 2013a) by increasing D-serine levels. These results predict that DAAO inhibition would have a positive effect on cognition, most probably through its ability to augment NMDAR-mediated currents. In 2009, Coyle and colleagues introduced the first genetically invalidation of SR in mice (Basu et al. 2009). Despite some subtle behavioral and structural deficits (see Sects. 3.3.2 and 3.3.3), electrophysiological phenotyping of NMDARs activity and synaptic plasticity intriguingly revealed that a 90 % decrease in forebrain D-serine content (Basu et al. 2009), had only a moderate impact on NMDARs at SC-CA1 synapses. Indeed, the amplitude of NMDA-mediated synaptic currents was normal; only the decay kinetics of evoked NMDAR-mediated currents were slower in hippocampal slices in SR-KO mice (Basu et al. 2009) due certainly to a compensatory mechanism by glycine. The most striking effect in SR-KO is the absence of LTP induced by a pairing protocol (Basu et al. 2009; Rosenberg et al. 2013; Le Bail et al. 2015). It is worth noticing that the selective depletion of both D-serine and glycine reduce NMDAR synaptic responses in the lateral nucleus of the amygdala (LNA) (Li et al. 2013). Therefore, the contribution of the two co-agonists in activating

NMDARs shows general overlap in the brain, but regional differences in the preference for one co-agonist over the other certainly occur.

Could the preference for a co-agonist be related to the molecular composition of synaptic NMDARs? We have recently discovered that in the mature hippocampus, D-serine is the main co-agonist at SC-CA1 hippocampal synapses whereas glycine is preferentially concerned at connections within the dentate gyrus (Le Bail et al. 2015). The role of D-serine prevails in brain regions where the GluN2A-containing subtype of NMDARs predominates such as hippocampal SC-CA1 and prefrontal cortex synapses (Fossat et al. 2012; Papouin et al. 2012; Le Bail et al. 2015), while glycine acts as the preferred co-agonist in regions where the contribution of the GluN2B subunit is favored, as seen in the dentate gyrus (Le Bail et al. 2015). This segregation would explain why SR-deficient mice display moderate synaptic dysfunctions at the medial perforant path-dentate gyrus synapses (Balu et al. 2013).

But in addition to be synapse specific, the function of D-serine is developmentally regulated (Le Bail et al. 2015) (Fig. 3.3). Indeed, we have found that glycine is the preferred co-agonist at immature SC-CA1 synapses and demonstrated that a switch from glycine to D-serine occurs at these synapses during postnatal development (Le Bail et al. 2015) that parallels a progressive replacement of GluN2B by GluN2A subunits in NMDAR assemblies (Rodenas-Ruano et al. 2012). Nevertheless, a strict association between the identity of the co-agonist and the NMDAR molecular composition is not a general rule since D-serine acts as the main co-agonist in the supraoptic nucleus (Panatier et al. 2006) where GluN2B-containing receptors predominate but also in the nucleus accumbens and the prefrontal cortex (Fossat et al. 2012; Curcio et al. 2013), where both GluN2 subunits are equally expressed. Additionally, one has to keep in mind that a substantial fraction of synaptic NMDARs are triheteromers containing both GluN2A and GluN2B subunits (reviewed in (Traynelis et al. 2010; Paoletti et al. 2013)) and are thus expected to be regulated by both D-serine and glycine.

Besides the impact of the co-agonist regional specificity, a segregated role for D-serine and glycine at the synaptic cleft has also been reported. Indeed, NMDARs are not only present synaptic sites but are also located at extra- and peri-synaptic sites (Petralia 2012) where they may have different roles in cell death or survival programs (Hardingham and Bading 2010) and may be involved in different forms of synaptic plasticity (Papouin et al 2012; Mothet et al 2015). Enzymatic depletion of D-serine with *Rg*DAAO consistently reduces NMDAR synaptic events at mature SC-CA1 connections (Papouin et al. 2012; Le Bail et al. 2015), while the pharmacologically isolated extrasynaptic NMDAR activation or the NMDAR-driven tonic glutamate current remain mostly unaffected by changes in D-serine levels (Papouin et al. 2012). Opposite effects were observed when endogenous glycine was depleted using *Bacillus subtilis* GO (*Bs*GO), indicating that at those hippocampal synapses, D-serine would gate preferentially the synaptic GluN2A-NMDARs while glycine would target extrasynaptic receptors (Papouin et al. 2012). The segregated role of the two co-agonists at gating synaptic and extrasynaptic NMDARs at SC-CA1 synapses delineates their respective role in synaptic plasticity, with D-serine

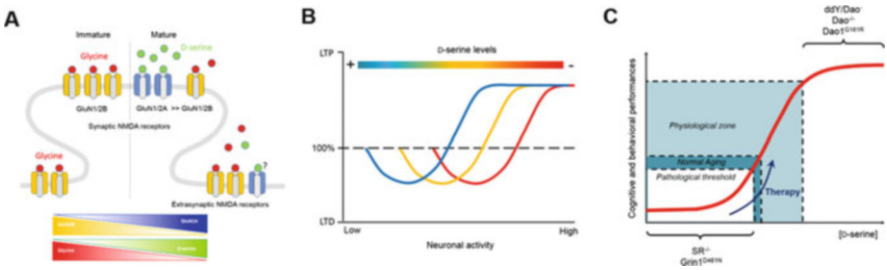

Fig. 3.3 Functions of D-serine. (**a**) Immature versus mature synapses. At immature synapse where only GluN1/2B receptors are present, glycine serves as the co-agonist. During postnatal development, the replacement of GluN2B- by GluN2A-containing NMDARs at synapses parallels a change in the identity of the co-agonist from glycine to D-serine. Conversely, glycine is the main co-agonist for extrasynaptic NMDA receptors. (**b**) D-serine and metaplasticity. D-serine levels govern the activity of NMDA receptor-dependent synaptic plasticity. Decreasing the levels of D-serine shifts the relationship to higher activity values, whereas increasing them has the opposite effect. Reprinted with permission from Cell Press (Panatier et al. 2006). (**c**) D-serine and cognition. D-serine levels drive cognitive and behavioral performances. Decreased levels as observed in normal aging and below a pathological threshold as observed in SR–KO or GrinD481N mice induce deficits. Conversely, increased levels of D-serine as observed under DAAO inactivation increases performances. Current therapy interventions aimed to normalize D-serine levels

specifically required for the expression of LTP and both D-serine and glycine acting for the induction of long-term depression (LTD) (Papouin et al. 2012). Our observation for a role of D-serine in the formation of hippocampal LTD in the mature brain expands earlier observations made by Xu and colleagues (2008) that the D-amino acid regulates LTD in the hippocampal CA1 region in a "bell-shaped" concentration-dependent manner (Zhang et al. 2008). The retina is offering another model to appreciate the relative functions of D-serine and glycine at synaptic and extrasynaptic sites. The group of Miller have nourished evidence that AMPA receptor-dependent and light-evoked NMDA receptor-mediated currents recorded at retinal ganglion cells are shaped by the release of D-serine from Muller cells (Stevens et al. 2003, 2010; Gustafson et al. 2007), while glycine would rather activate extrasynaptic NMDARs (Sullivan and Miller 2012). However, this appealing dichotomic synaptic versus extrasynaptic segregation of the functions of D-serine and glycine has limitations. Several studies demonstrate that GluN2B-NMDARs preferring glycine are also required to induce LTP (reviewed in (Paoletti et al. 2013)). Furthermore, the repetitive observation that LTP at SC-CA1 synapses is only partly affected in SR-KO mice displaying trace levels of D-serine (Basu et al. 2009; Rosenberg et al. 2013) supports the idea that glycine could compensate for any D-serine synaptic dysfunction. Indeed, a couple of very recent studies have shown that the two endogenous co-agonists are necessary for LTP since both treatments with *Rg*DAAO and *Bs*GO result in significantly reduced LTP expression in CA1 hippocampal area and dentate gyrus, as well as in the LNA (Li et al. 2013; Le Bail et al. 2015). Interestingly, a robust LTP may be induced in the LNA of

SR-KO mice by increasing the strength of presynaptic activation. This LTP is then consistently reduced under glycine depletion by *Bs*GO (Li et al. 2013). These results indicate that independent of their synaptic location in the brain, both D-serine and glycine may be recruited for activating NMDARs during enhanced synaptic activity. Accordingly, treatments with *Rg*DAAO but not *Bs*GO reduces the NMDAR component of spontaneous excitatory postsynaptic currents in the LNA while synaptic responses evoked by enhanced afferent stimulation are impacted by *Bs*GO (Li et al. 2013). Ambient D-serine could therefore drive tonic activation of NMDARs under low-synaptic activity in this structure, while the involvement of glycine should rely on increased afferent activity. In the hippocampus, although D-serine and glycine preferentially drive low-frequency-evoked NMDAR synaptic responses at mature SC-CA1 and dentate gyrus synapses, respectively, they both contribute to LTP expression driven by high-frequency stimulation at both hippocampal synapses (Le Bail et al. 2015) suggesting that both co-agonists may be necessary for memory formation. Therefore, although the identity of the NMDAR co-agonist may be synapse specific under low basal conditions, it appears that the activation by the two co-agonists is concerned when a sustained recruitment of synaptic activity is promoted.

NMDARs are also present at presynaptic sites where they regulate the release of glutamate by nerve terminals (Sjostrom et al. 2003; Corlew et al. 2008; Larsen et al. 2014). The mode of activation by either glycine or D-serine remained to be defined. Jones and colleagues, by using *Rg*DAAO and *Bs*GO to deplete D-serine and glycine, respectively, have recently reported that in the mature entorhinal cortex, these presynaptic NMDA receptors are tonically activated by D-serine but not by glycine, suggesting that D-serine is the endogenous co-agonist of presynaptic NMDARs (Lench et al. 2014).

Finally, D-serine and glycine synaptic functions closely rely on a fine coupling between astrocytes and neurons. Indeed, the profile of the tripartite synapse could also impact the identity of the predominant co-agonist acting on NMDAR. As mentioned earlier, a progressive developmental switch from glycine to D-serine occurs at SC-CA1 synapses that could reflect to some extent postnatal changes in the intimate neuron–glial coupling (Le Bail et al. 2015). At immature synapses, pharmacological blockade of astrocytic GlyT1 does not increase NMDAR activation as it does at mature connections, confirming that ambient glycine is able to saturate NMDAR binding sites early after birth. These elevated levels of glycine at birth presumably rely on a weak expression of GlyT1 (Zafra et al. 1995) related to the lack of a prominent astrocyte network that only progressively develops during the first postnatal weeks (Yang et al. 2013). The fact that inactivation of astrocyte activity with the gliotoxin fluoroacetate (FAC) does not significantly alter NMDAR activation at immature synapses further supports the view that this functional aspect of neuron–glial coupling is weakly effective early after birth (Le Bail et al. 2015). On the other hand, the postnatal formation of the tripartite synapse does not only result in a higher clearance of glycine by astrocytic GlyT1 but also in a better supply of D-serine either from astrocyte or through activation of the serine shuttle (Wolosker 2011). Further lines of experimental evidence show that D-serine derived

from glia regulates NMDARs. Rusakov and colleagues reported that in the mature hippocampus, astrocyte processes contacting SC-CA1 synapses retain the ability to control Hebbian LTP within or near their individual territories involving Ca^{2+}-and SNARE protein-dependent D-serine release (Henneberger et al. 2010) confirming earlier evidence for a vesicular release of D-serine from astrocytes (Mothet et al. 2005; Martineau et al. 2008). Because astrocytes express a plethora of receptors, they could link different neurotransmitter systems through the release of D-serine. Accordingly, potentiating effect of nicotine on hippocampal synaptic transmission and long-term synaptic plasticity recorded at SC-CA1 synapses depends on D-serine furnished by astrocytes (Lopez-Hidalgo et al. 2012). Noteworthy, α7 nAChR gene deletion leads to impaired synaptic NMDAR functions through specific loss of D-serine in the neocortex (Lin et al. 2014). Likewise, astrocyte TRPA1 channels contribute to basal Ca^{2+} levels and are required for constitutive D-serine release into the extracellular space, which in turn contributes to NMDA receptor-dependent LTP (Shigetomi et al. 2013). In layer 2/3 of the neocortex, activation of either astroglial cannabinoid or α1-adrenoreceptors receptors induces Ca^{2+}-signaling and synaptic plasticity through notably the release of D-serine (Rasooli-Nejad et al. 2014; Pankratov and Lalo 2015). Such a relationship between the degree of the astrocyte–neuron coupling and the prevalence of D-serine as a co-agonist agrees with the differential impact of the amino acid in activating NMDARs at hypothalamus synapses during lactation where D-serine efficiency is linearly related to the extent of astrocytic coverage of synapses (Panatier et al. 2006). Indeed in virgin rats, astrocytic coverage of synapses is maximal guaranteeing an optimal activity of NMDARs and enabling formation of LTP by D-serine. Conversely, lactation induces loss of synapses coverage by glia. This anatomical remodeling leads to a decrease of occupancy by D-serine of NMDARs (Panatier et al. 2006). As a consequence, the activity dependence of LTP and LTD whose induction depends on NMDAR activation is modified by this neuron–glial remodeling. The levels of D-serine shift the activity dependence of long-term changes toward higher activity (Fig. 3.3).

But, astrocyte-derived D-serine could play also a critical role in neurovascular coupling. Indeed astrocytes modulate hemodynamism of microcirculation by providing a physical linkage from synapses to arterioles and through notably the release of vasoactive gliotransmitters (Haydon and Carmignoto 2006). In two elegant studies, Anderson and colleagues have discovered that endogenous D-serine released by astrocytes contributes to the vasodilatory response of penetrating cortical arterioles produced by astrocyte activation (LeMaistre et al. 2012; Stobart et al. 2013). Still, we could also predict that the progressive setup of a close astrocyte–neuron interaction will favor the astrocyte-derived precursor L-serine to be provided to neurons to boost D-serine synthesis. According to this possibility, the FAC-induced decrease in NMDAR activation recorded at mature SC-CA1 synapses is rescued by increasing synaptic availability of L-serine (Le Bail et al. 2015).

Adult neurogenesis offers another example where the functions of D-serine have been recently explored. In the adult mammalian brain, neurogenesis occurs mainly in the subventricular zone (SVZ) and the subgranular zone (SGZ) of DG in the

hippocampus (Aimone et al. 2014). NSCs are the progenitor cells in the brain, which are capable of continuous self-renewal and differentiation into neurons, astrocytes, and oligodendrocytes (Aimone et al. 2014). The local environment may dictate the fate choice of NSCs. Nevertheless, we are still largely ignoring the identity of the extrinsic mediators that regulate proliferation, survival, migration, and differentiation of NSCs. Hu and colleagues have shown that forebrain neural stem cells (NSCs) in vitro could synthesize and release D-serine and that D-serine supports the proliferation and neuronal differentiation of NSCs (Huang et al. 2012). Furthermore, they show that D-serine promotes neurogenesis through acting on NMDARs and subsequently regulating the Ca^{2+}-related signaling pathways, including the phosphorylation of ERK1/2-CREB and GSK-3β. These in vitro observations were later expanded by Toni and colleagues (2013) who reported that D-serine increased adult hippocampal neurogenesis in vivo and in vitro and increased the density of neural stem cells and transit amplifying progenitors. Furthermore, D-serine increased the survival of newborn neurons (Sultan et al. 2013).

3.3.2 Role of D-Serine in the Developing Brain

Even that D-serine function may preferentially dominate at mature synapses, this co-agonist could already play an important role in the developing brain by controlling neuronal migration and synaptogenesis. Indeed, series of evidence indicate that D-serine and its enzymes are already present early during embryonic and postnatal development in different like the retina (Romero et al. 2014) and the vestibular nuclei (Puyal et al. 2006) where it could critically influence postnatal development. Therefore defects in neonatal D-serine could lead to severe brain defaults. Mice with a 3-phosphoglycerate dehydrogenase deficiency lack both L-serine and D-serine throughout their lives (Yang et al. 2010), but maternal D-serine supplementation can completely reverse the abnormal neurological phenotype (Fuchs et al. 2006).

During neocorticogenesis, migrating neurons can adopt different types of trajectories and a large proportion of neurons migrate radially, along radial glial cells, from the germinative zone to their final place (Yacubova and Komuro 2003). Glutamate acting on NMDARs exerts a crucial role by acting as a motility-promoting signal for immature neurons. Blocking NMDARs impairs neuronal migration during postnatal development (Yacubova and Komuro 2003). Radial migration of immature granule cells in the developing cerebellum, along the Bergmann glia, is one of the best studied models. How NMDARs of migrating immature neurons are activated remains unknown. Because D-serine levels peak at P14 in the cerebellum at the time of intense granule cell migration (Schell et al. 1997) and due to the absolute requirement of NMDARs activation for normal migration, one can imagine that D-serine is involved in this process. Snyder and colleagues (2005) have demonstrated that D-serine released by Bergmann glial cells promotes the migration of granule cells from the external to the internal granular

layer through activation of NMDARs (Kim et al. 2005). The physiological influence of D-serine on neuronal migration involves the activation of SR by GRIP.

The motility-promoting role of D-serine is probably not restricted to the postnatal development of the brain and may be involved earlier during fetal development. Indeed SR is present in the perireticular nucleus, a transient structure of the human fetal brain supposed to be engaged in the lamination of the forebrain (Martineau et al. 2006). Furthermore, D-serine and NMDARs are already present at embryonic day 14 (Paoletti et al. 2013; Martineau et al. 2006). Thus D-serine is well positioned both spatially and temporally to control NMDAR-mediated neuronal migration and synaptogenesis. Although SR knockout mice develop normally and show no obvious deficit in lamination or ectopic neurons, the mice have a significantly reduced cortical volume (Balu et al. 2012, 2013). Detailed structural analysis of the CNS of SR knockout mice reveals that SR deletion when achieved before weaning results in altered cortical dendritic morphology of pyramidal neurons in the prefrontal cortex and in the primary somatosensory cortex (DeVito et al. 2011) supporting the hypothesis that D-serine through the modulation of NMDARs could contribute to neuronal differentiation and therefore to synaptogenesis. Noteworthy, SR knockout mice have a reduction in total brain-derived neurotrophic factor (BDNF) protein levels (Balu et al. 2012), an activity-regulated neurotrophin that plays a key role in promoting synapse formation and maturation and in regulating the functional development of neuronal circuits (Park and Poo 2013). Accordingly, Gomes and colleagues (2012) have discovered that astrocytes induce the formation of glutamatergic synapses through *transforming growth factor* (TGF)-β1 pathway and the TGF-β1 synaptogenic property is dependent on D-serine signaling (Diniz et al. 2012). Indeed, the growth factor increases D-serine release from astrocytes, and blocking the synthesis of D-serine or its function prevents TGF-β1 to promote the development of functional glutamatergic synapses in the cerebral cortex. Another study demonstrates that differentiation of P19 cells induces SR expression and D-serine formation and that D-serine shapes synaptogenesis, potentially by preventing widespread untargeted synaptogenesis (Fuchs et al. 2012). These results contribute to expand evidence for a role of D-serine in synaptic shaping and wiring of neuronal circuitry during brain development.

3.3.3 Role of D-Serine in Cognition and Healthy Aging

In addition to their role in shaping neuronal circuitries and in synapse dynamics, NMDARs are essential for memory formation and for higher cognitive functions and social interactions (Paoletti et al. 2013). Genetically or pharmacologically driven hypofunction of NMDARs leads to profound deficits in cognition and social activity (Paoletti et al. 2013). For example, mice with a point mutation in Grin1 (D481N) showed abnormally persistent latent inhibition (a measure of information-processing deficit), reduced social approach behaviors, and enhanced startle reactivity (measure of sensory gatting) and impairments in nonassociative spatial object

recognition task (Labrie et al. 2008). These deficits are reversed by administration of agents that enhance NMDAR glycine site function like D-serine (Labrie et al. 2008). Various regimens of systemic administration of low-dose D-serine (50 mg/kg/day) increase performances on BALB/c mice performing object recognition, T-maze alternation, and open-field exploration tasks (Bado et al. 2011). Analysis of SR-KO mice revealed that the animals exhibited a significantly disrupted representation of the order of events in distinct experimental paradigms as shown by object recognition and odor sequence tests (DeVito et al. 2011); However, SR mutant mice showed normal detection of novel objects and in spatial displacement and showed intact relational memory in a test of transitive inference. SR mice exhibited normal sociability and preference for social novelty (DeVito et al. 2011). Analysis of mice with inactive DAAO further offers complementary evidence that D-serine is crucial for normal cognition and social behavior. First, Roder and colleagues have generated mice carrying point mutations in DAO (*Dao1*^{G181R}). These mice have no active DAAO and display elevated brain levels of D-serine. Introducing the *Dao1*^{G181R} mutations in Grin1(D481N) mice corrects all cognitive deficits and normalizes social behavior deficit normally observed in Grin1(D481N) mice (Labrie et al. 2010). Second, Dao$^{-/-}$ mice generated more recently by Peirson and colleagues (2015) demonstrate enhanced spatial recognition memory performance, improved odor recognition memory performance, and enhanced spontaneous alternation in the T-maze (Pritchett et al. 2015). Overall, these observations are fully consistent with, and extend, findings in the natural mutant ddY/Dao^{-} line introduced and largely popularized by Konno and colleagues (Almond et al. 2006; Zhang et al. 2011). Thus, an increased level of D-serine resulting from decreased catalysis (i.e., DAAO inactivation) increases the cognitive performance and behavior of mice in striking contrast to SR deficient mice (Fig. 3.3). Building on this ground, inhibitors for DAAO show remarkable benefit in improving cognitive performance in animal models of NMDAR hypofunction (Smith et al. 2009; Strick et al. 2011; Hopkins et al. 2013a, b). Nicotine has long been known to improve long-term hippocampal-dependent memory in both laboratory animals and humans (Changeux 2010). García-Colunga and colleagues (2012) have found that the facilitating effect of nicotine on long-term memory evaluated with the one-trial step-through inhibitory-avoidance task depends on glial D-serine (Lopez-Hidalgo et al. 2012). Accordingly, nicotine activates nAChRs present on glial cells stimulating the release of D-serine and then the activation of NMDARs in the hippocampus. These data further expand the earlier observation that glial cells through the release of D-serine contribute to spatial memory retrieval (Zhang et al. 2008).

Non-pathological brain aging is certainly one of the best documented situation where accumulated experimental evidence have shown that deficits in synaptic plasticity and cognitive functions notably memory and learning result from reduced activation of NMDARs. In collaboration with Jean-Marie Billard, we have discovered that those deficits are caused by a reduction in the levels of D-serine produced by SR during aging (Mothet et al. 2006; Turpin et al. 2011) thus resulting in a hypofunction of NMDARs (Fig. 3.3). More detailed analysis revealed that

oxidative stress as occurring during aging affects SR and then the production of D-serine and that treatment with the reducing agent N-acetyl cysteine prevents hippocampal synaptic plasticity deficits by protecting D-serine-dependent NMDA receptor activation (Haxaire et al. 2012). Recently the notion that D-serine contributes to age-related cognitive decline has been expanded by Saitoe and colleagues who demonstrated that age-related memory impairments in *Drosophila melanogaster* are a result of reduced D-serine production by glia and that D-serine feeding suppresses these deficits (Yamazaki et al. 2014) thus showing that D-serine metabolism and functions may be conserved throughout evolution (Schell 2004).

3.4 Conclusion

D-serine has overturned fundamental axioms of biology. Intensive research during the last two decades has shown that this D-amino acid has emerged as a very important brain messenger with implication for Human disease. Despite many progresses since the breakthrough of Nishikawa (1992) and Snyder (1995) groups, there are still some opened questions regarding the functions of D-serine in the CNS and in the peripheral nervous system. The discovery that NMDAR co-activation takes more than glycine illustrates perfectly there's no simple rule governing the regulation of NMDA receptors. There is still a great need for efforts to discriminate the relative contribution of these two NMDAR neuromodulators in the healthy and diseased nervous system.

Acknowledgments We thank our collaborators who help to expand the field. We apologize to our colleagues whose work could not be cited due to space limitations. Work in Dr Mothet's lab is supported by operating grants from the Centre National de la Recherche Scientifique, France Alzheimer, the Fondation pour la Recherche Médicale, Direction Générale de l'Armement and the Université Aix-Marseille.

References

Aimone JB, Li Y, Lee SW et al (2014) Regulation and function of adult neurogenesis: from genes to cognition. Physiol Rev 94:991–1026

Almond SL, Fradley RL, Armstrong EJ et al (2006) Behavioral and biochemical characterization of a mutant mouse strain lacking D-amino acid oxidase activity and its implications for schizophrenia. Mol Cell Neurosci 32:324–334

Bado P, Madeira C, Vargas-Lopes C et al (2011) Effects of low-dose D-serine on recognition and working memory in mice. Psychopharmacology 218:461–470

Balu DT, Basu AC, Corradi JP et al (2012) The NMDA receptor co-agonists, D-serine and glycine, regulate neuronal dendritic architecture in the somatosensory cortex. Neurobiol Dis 45:671–682

Balu DT, Li Y, Puhl MD et al (2013) Multiple risk pathways for schizophrenia converge in serine racemase knockout mice, a mouse model of NMDA receptor hypofunction. Proc Natl Acad Sci U S A 110:31

Balu DT, Takagi S, Puhl MD et al (2014) D-serine and serine racemase are localized to neurons in the adult mouse and human forebrain. Cell Mol Neurobiol 34:419–435
Basu AC, Tsai GE, Ma CL et al (2009) Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. Mol Psychiatry 14:719–727
Benneyworth MA, Li Y, Basu AC et al (2012) Cell selective conditional null mutations of serine racemase demonstrate a predominate localization in cortical glutamatergic neurons. Cell Mol Neurobiol 32:613–624
Bergersen LH, Morland C, Ormel L et al (2012) Immunogold detection of L-glutamate and D-serine in small synaptic-like microvesicles in adult hippocampal astrocytes. Cereb Cortex 22:1690–1697
Bezzi P, Gundersen V, Galbete JL et al (2004) Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. Nat Neurosci 7:613–620
Campanini B, Spyrakis F, Peracchi A et al (2013) Serine racemase: a key player in neuron activity and in neuropathologies. Front Biosci 18:1112–1128
Changeux JP (2010) Allosteric receptors: from electric organ to cognition. Annu Rev Pharmacol Toxicol 50:1–38
Chaudhry FA, Boulland JL, Jenstad M et al (2008) Pharmacology of neurotransmitter transport into secretory vesicles. Handb Exp Pharmacol 184:77–106
Chen PE, Geballe MT, Katz E et al (2008) Modulation of glycine potency in rat recombinant NMDA receptors containing chimeric NR2A/2D subunits expressed in Xenopus laevis oocytes. J Physiol 586:227–245
Corlew R, Brasier DJ, Feldman DE et al (2008) Presynaptic NMDA receptors: newly appreciated roles in cortical synaptic function and plasticity. Neuroscientist 14:609–625
Curcio L, Podda MV, Leone L et al (2013) Reduced D-serine levels in the nucleus accumbens of cocaine-treated rats hinder the induction of NMDA receptor-dependent synaptic plasticity. Brain 136:1216–1230
Danysz W, Fadda E, Wroblewski JT et al (1990) [3H]D-serine labels strychnine-insensitive glycine recognition sites of rat central nervous system. Life Sci 46:155–164
DeVito LM, Balu DT, Kanter BR et al (2011) Serine racemase deletion disrupts memory for order and alters cortical dendritic morphology. Genes Brain Behav 10:210–222
Diniz LP, Almeida JC, Tortelli V et al (2012) Astrocyte-induced synaptogenesis is mediated by transforming growth factor beta signaling through modulation of D-serine levels in cerebral cortex neurons. J Biol Chem 287:41432–41445
Ehmsen JT, Ma TM, Sason H et al (2013) D-serine in glia and neurons derives from 3-phosphoglycerate dehydrogenase. J Neurosci 33:12464–12469
Fossat P, Turpin FR, Sacchi S et al (2012) Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. Cereb Cortex 22:595–606
Fuchs SA, Dorland L, de Sain-van der Velden MG et al (2006) D-serine in the developing human central nervous system. Ann Neurol 60:476–480
Fuchs SA, Roeleveld MW, Klomp LW et al (2012) D-serine influences synaptogenesis in a p19 cell model. JIMD Rep 6:47–53
Fukasawa Y, Segawa H, Kim JY et al (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
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
Gustafson EC, Stevens ER, Wolosker H et al (2007) Endogenous D-serine contributes to NMDA-receptor-mediated light-evoked responses in the vertebrate retina. J Neurophysiol 98:122–130
Hardingham GE, Bading H (2010) Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nat Rev Neurosci 11:682–696
Hashimoto A, Nishikawa T, Oka T et al (1993) Endogenous D-serine in rat brain: N-methyl-D-aspartate receptor-related distribution and aging. J Neurochem 60:783–786

Hashimoto A, Oka T, Nishikawa T (1995) Extracellular concentration of endogenous free D-serine in the rat brain as revealed by in vivo microdialysis. Neuroscience 66:635–643

Hashimoto A, Kanda J, Oka T (2000) Effects of N-methyl-D-aspartate, kainate or veratridine on extracellular concentrations of free D-serine and L-glutamate in rat striatum: an in vivo microdialysis study. Brain Res Bull 53:347–351

Haxaire C, Turpin FR, Potier B et al (2012) Reversal of age-related oxidative stress prevents hippocampal synaptic plasticity deficits by protecting D-serine-dependent NMDA receptor activation. Aging Cell 11:336–344

Haydon PG, Carmignoto G (2006) Astrocyte control of synaptic transmission and neurovascular coupling. Physiol Rev 86:1009–1031

Helboe L, Egebjerg J, Moller M et al (2003) Distribution and pharmacology of alanine-serine-cysteine transporter 1 (asc-1) in rodent brain. Eur J Neurosci 18:2227–2238

Henneberger C, Papouin T, Oliet SH et al (2010) Long-term potentiation depends on release of D-serine from astrocytes. Nature 463:232–236

Hopkins SC, Heffernan ML, Saraswat LD et al (2013a) Structural, kinetic, and pharmacodynamic mechanisms of D-amino acid oxidase inhibition by small molecules. J Med Chem 56:3710–3724

Hopkins SC, Campbell UC, Heffernan ML et al (2013b) Effects of D-amino acid oxidase inhibition on memory performance and long-term potentiation in vivo. Pharmacol Res Perspect 1:1

Huang X, Kong H, Tang M et al (2012) D-Serine regulates proliferation and neuronal differentiation of neural stem cells from postnatal mouse forebrain. CNS Neurosci Ther 18:4–13

Ishiwata S, Umino A, Umino M et al (2013) Modulation of extracellular D-serine content by calcium permeable AMPA receptors in rat medial prefrontal cortex as revealed by in vivo microdialysis. Int J Neuropsychopharmacol 16:1395–1406

Jahn R, Fasshauer D (2012) Molecular machines governing exocytosis of synaptic vesicles. Nature 490:201–207

Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325:529–531

Kim PM, Aizawa H, Kim PS et al (2005) Serine racemase: activation by glutamate neurotransmission via glutamate receptor interacting protein and mediation of neuronal migration. Proc Natl Acad Sci U S A 102:2105–2110

Kleckner NW, Dingledine R (1988) Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. Science 241:835–837

Konno R, Yasumura Y (1983) Mouse mutant deficient in D-amino acid oxidase activity. Genetics 103:277–285

Labrie V, Lipina T, Roder JC (2008) Mice with reduced NMDA receptor glycine affinity model some of the negative and cognitive symptoms of schizophrenia. Psychopharmacology (Berlin) 200:217–230

Labrie V, Wang W, Barger SW et al (2010) Genetic loss of D-amino acid oxidase activity reverses schizophrenia-like phenotypes in mice. Genes Brain Behav 9:11–25

Larsen RS, Smith IT, Miriyala J et al (2014) Synapse-specific control of experience-dependent plasticity by presynaptic NMDA receptors. Neuron 83:879–893

Le Bail M, Martineau M, Sacchi S et al (2015) Identity of the NMDA receptor coagonist is synapse specific and developmentally regulated in the hippocampus. Proc Natl Acad Sci U S A 112:30

LeMaistre JL, Sanders SA, Stobart MJ et al (2012) Coactivation of NMDA receptors by glutamate and D-serine induces dilation of isolated middle cerebral arteries. J Cereb Blood Flow Metab 32:537–547

Lench AM, Massey PV, Pollegioni L et al (2014) Astroglial D-serine is the endogenous co-agonist at the presynaptic NMDA receptor in rat entorhinal cortex. Neuropharmacology 83:118–127

Li Y, Sacchi S, Pollegioni L et al (2013) Identity of endogenous NMDAR glycine site agonist in amygdala is determined by synaptic activity level. Nat Commun 4:1760

Lin H, Hsu FC, Baumann BH et al (2014) Cortical synaptic NMDA receptor deficits in alpha7 nicotinic acetylcholine receptor gene deletion models: implications for neuropsychiatric diseases. Neurobiol Dis 63:129–140

Lopez-Hidalgo M, Salgado-Puga K, Alvarado-Martinez R et al (2012) Nicotine uses neuron-glia communication to enhance hippocampal synaptic transmission and long-term memory. PLoS ONE 7:21

Ma TM, Abazyan S, Abazyan B et al (2013) Pathogenic disruption of DISC1-serine racemase binding elicits schizophrenia-like behavior via D-serine depletion. Mol Psychiatry 18:557–567

Maekawa M, Watanabe M, Yamaguchi S et al (2005) Spatial learning and long-term potentiation of mutant mice lacking D-amino-acid oxidase. Neurosci Res 53:34–38

Martineau M, Baux G, Mothet JP (2006) D-serine signalling in the brain: friend and foe. Trends Neurosci 29:481–491

Martineau M, Galli T, Baux G et al (2008) Confocal imaging and tracking of the exocytotic routes for D-serine-mediated gliotransmission. Glia 56:1271–1284

Martineau M, Shi T, Puyal J et al (2013) Storage and uptake of D-serine into astrocytic synaptic-like vesicles specify gliotransmission. J Neurosci 33:3413–3423

Martineau M, Parpura V, Mothet JP (2014) Cell-type specific mechanisms of D-serine uptake and release in the brain. Front 6:12. doi:10.3389/fnsyn.2014.00012

Matsui T, Sekiguchi M, Hashimoto A et al (1995) Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. J Neurochem 65:454–458

Matsuo H, Kanai Y, Tokunaga M et al (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

Maucler C, Pernot P, Vasylieva N et al (2013) In vivo D-serine hetero-exchange through alanine-serine-cysteine (ASC) transporters detected by microelectrode biosensors. ACS Chem Neurosci 4:772–781

Miya K, Inoue R, Takata Y et al (2008) Serine racemase is predominantly localized in neurons in mouse brain. J Comp Neurol 510:641–654

Monaghan DT, Olverman HJ, Nguyen L et al (1988) Two classes of N-methyl-D-aspartate recognition sites: differential distribution and differential regulation by glycine. Proc Natl Acad Sci U S A 85:9836–9840

Montana V, Malarkey EB, Verderio C et al (2006) Vesicular transmitter release from astrocytes. Glia 54:700–715

Mothet JP, Parent AT, Wolosker H et al (2000) D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A 97:4926–4931

Mothet JP, Pollegioni L, Ouanounou G et al (2005) Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. Proc Natl Acad Sci U S A 102:5606–5611

Mothet JP, Rouaud E, Sinet PM et al (2006) A critical role for the glial-derived neuromodulator D-serine in the age-related deficits of cellular mechanisms of learning and memory. Aging Cell 5:267–274

Mothet JP, Le Bail M, Billard JM (2015) Time and space profilling of NMDA receptor co-agonist functions. J Neurochem. doi:10.1111/jnc.13204

Ohide H, Miyoshi Y, Maruyama R et al (2011) D-Amino acid metabolism in mammals: biosynthesis, degradation and analytical aspects of the metabolic study. J Chromatogr B Anal Technol Biomed Life Sci 879:3162–3168

Otte DM, Rasko T, Wang M et al (2014) Identification of the mitochondrial MSRB2 as a binding partner of LG72. Cell Mol Neurobiol 34:1123–1130

Panatier A, Theodosis DT, Mothet JP et al (2006) Glia-derived D-serine controls NMDA receptor activity and synaptic memory. Cell 125:775–784

Pankratov Y, Lalo U (2015) Role for astroglial alpha1-adrenoreceptors in gliotransmission and control of synaptic plasticity in the neocortex. Front 9:230

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

Papouin T, Ladepeche L, Ruel J et al (2012) Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell 150:633–646

Park H, Poo MM (2013) Neurotrophin regulation of neural circuit development and function. Nat Rev Neurosci 14:7–23

Parpura V, Verkhratsky A (2013) Astroglial amino acid-based transmitter receptors. Amino Acids 44:1151–1158

Petralia RS (2012) Distribution of extrasynaptic NMDA receptors on neurons. ScientificWorldJournal 2012:30

Popiolek M, Ross JF, Charych E et al (2011) D-amino acid oxidase activity is inhibited by an interaction with bassoon protein at the presynaptic active zone. J Biol Chem 286:28867–28875

Priestley T, Kemp JA (1994) Kinetic study of the interactions between the glutamate and glycine recognition sites on the N-methyl-D-aspartic acid receptor complex. Mol Pharmacol 46:1191–1196

Pritchett D, Hasan S, Tam SK et al (2015) D-amino acid oxidase knockout (Dao(−/−)) mice show enhanced short-term memory performance and heightened anxiety, but no sleep or circadian rhythm disruption. Eur J Neurosci 41:1167–1179

Puyal J, Martineau M, Mothet JP et al (2006) Changes in D-serine levels and localization during postnatal development of the rat vestibular nuclei. J Comp Neurol 497:610–621

Rasooli-Nejad S, Palygin O, Lalo U et al (2014) Cannabinoid receptors contribute to astroglial Ca (2)(+)-signalling and control of synaptic plasticity in the neocortex. Philos Trans R Soc Lond B Biol Sci 369:0077

Reyes-Haro D, Muller J, Boresch M et al (2010) Neuron-astrocyte interactions in the medial nucleus of the trapezoid body. J Gen Physiol 135:583–594

Ribeiro CS, Reis M, Panizzutti R et al (2002) Glial transport of the neuromodulator D-serine. Brain Res 929:202–209

Rodenas-Ruano A, Chavez AE, Cossio MJ et al (2012) REST-dependent epigenetic remodeling promotes the developmental switch in synaptic NMDA receptors. Nat Neurosci 15:1382–1390

Romero GE, Lockridge AD, Morgans CW et al (2014) The postnatal development of D-serine in the retinas of two mouse strains, including a mutant mouse with a deficiency in D-amino acid oxidase and a serine racemase knockout mouse. ACS Chem Neurosci 5:848–854

Rosenberg D, Kartvelishvily E, Shleper M et al (2010) Neuronal release of D-serine: a physiological pathway controlling extracellular D-serine concentration. FASEB J 24:2951–2961

Rosenberg D, Artoul S, Segal AC et al (2013) Neuronal D-serine and glycine release via the Asc-1 transporter regulates NMDA receptor-dependent synaptic activity. J Neurosci 33:3533–3544

Sacchi S, Cappelletti P, Giovannardi S et al (2008) Evidence for the interaction of D-amino acid oxidase with pLG72 in a glial cell line. Mol Cell Neurosci 48:20–28

Sacchi S, Cappelletti P, Giovannardi S et al (2011) Evidence for the interaction of D-amino acid oxidase with pLG72 in a glial cell line. Mol Cell Neurosci 48(1):20–28

Sasabe J, Suzuki M, Imanishi N et al (2014) Activity of D-amino acid oxidase is widespread in the human central nervous system. Front Synaptic Neurosci 6:14

Sasaki M, Konno R, Nishio M et al (1992) A single-base-pair substitution abolishes D-amino-acid oxidase activity in the mouse. Biochim Biophys Acta 1139:315–318

Schell MJ (2004) The N-methyl D-aspartate receptor glycine site and D-serine metabolism: an evolutionary perspective. Philos Trans R Soc Lond B Biol Sci 359:943–964

Schell MJ, Molliver ME, Snyder SH (1995) D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proc Natl Acad Sci U S A 92:3948–3952

Schell MJ, Brady RO Jr, Molliver ME et al (1997) D-serine as a neuromodulator: regional and developmental localizations in rat brain glia resemble NMDA receptors. J Neurosci 17:1604–1615

Scianni M, Antonilli L, Chece G et al (2013) Fractalkine (CX3CL1) enhances hippocampal N-methyl-D-aspartate receptor (NMDAR) function via D-serine and adenosine receptor type A2 (A2AR) activity. J Neuroinflammation 10:1742–2094

Shigetomi E, Jackson-Weaver O, Huckstepp RT et al (2013) TRPA1 channels are regulators of astrocyte basal calcium levels and long-term potentiation via constitutive D-serine release. J Neurosci 33:10143–10153

Sjostrom PJ, Turrigiano GG, Nelson SB (2003) Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. Neuron 39:641–654

Smith SM, Uslaner JM, Yao L et al (2009) The behavioral and neurochemical effects of a novel D-amino acid oxidase inhibitor compound 8 [4H-thieno [3,2-b]pyrrole-5-carboxylic acid] and D-serine. J Pharmacol Exp Ther 328:921–930

Stehberg J, Moraga-Amaro R, Salazar C et al (2012) Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. FASEB J 26:3649–3657

Stevens ER, Esguerra M, Kim PM et al (2003) D-serine and serine racemase are present in the vertebrate retina and contribute to the physiological activation of NMDA receptors. Proc Natl Acad Sci U S A 100:6789–6794

Stevens ER, Gustafson EC, Sullivan SJ et al (2010) Light-evoked NMDA receptor-mediated currents are reduced by blocking D-serine synthesis in the salamander retina. Neuroreport 21:239–244

Stobart JL, Lu L, Anderson HD et al (2013) Astrocyte-induced cortical vasodilation is mediated by D-serine and endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 110:3149–3154

Strick CA, Li C, Scott L et al (2011) Modulation of NMDA receptor function by inhibition of D-amino acid oxidase in rodent brain. Neuropharmacology 61:1001–1015

Sullivan SJ, Miller RF (2012) AMPA receptor-dependent, light-evoked D-serine release acts on retinal ganglion cell NMDA receptors. J Neurophysiol 108:1044–1051

Sultan S, Gebara EG, Moullec K et al (2013) D-serine increases adult hippocampal neurogenesis. Front 7:155

Suzuki M, Sasabe J, Miyoshi Y et al (2015) Glycolytic flux controls D-serine synthesis through glyceraldehyde-3-phosphate dehydrogenase in astrocytes. Proc Natl Acad Sci U S A 112:13

Takata N, Mishima T, Hisatsune C et al (2011) Astrocyte calcium signaling transforms cholinergic modulation to cortical plasticity in vivo. J Neurosci 31:18155–18165

Traynelis SF, Wollmuth LP, McBain CJ et al (2010) Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev 62:405–496

Turpin FR, Potier B, Dulong JR et al (2011) Reduced serine racemase expression contributes to age-related deficits in hippocampal cognitive function. Neurobiol Aging 32:1495–1504

Verrall L, Walker M, Rawlings N et al (2007) D-Amino acid oxidase and serine racemase in human brain: normal distribution and altered expression in schizophrenia. Eur J Neurosci 26:1657–1669

Williams SM, Diaz CM, Macnab LT et al (2006) Immunocytochemical analysis of D-serine distribution in the mammalian brain reveals novel anatomical compartmentalizations in glia and neurons. Glia 53:401–411

Wolosker H (2011) Serine racemase and the serine shuttle between neurons and astrocytes. Biochim Biophys Acta 2011:1558–1566

Wolosker H, Mori H (2012) Serine racemase: an unconventional enzyme for an unconventional transmitter. Amino Acids 43:1895–1904

Wolosker H, Blackshaw S, Snyder SH (1999) Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. Proc Natl Acad Sci U S A 96:13409–13414

Yacubova E, Komuro H (2003) Cellular and molecular mechanisms of cerebellar granule cell migration. Cell Biochem Biophys 37:213–234

Yamazaki D, Horiuchi J, Ueno K et al (2014) Glial dysfunction causes age-related memory impairment in Drosophila. Neuron 84:753–763

Yang JH, Wada A, Yoshida K et al (2010) Brain-specific Phgdh deletion reveals a pivotal role for L-serine biosynthesis in controlling the level of D-serine, an N-methyl-D-aspartate receptor co-agonist, in adult brain. J Biol Chem 285:41380–41390

Yang Y, Higashimori H, Morel L (2013) Developmental maturation of astrocytes and pathogenesis of neurodevelopmental disorders. J Neurodev Disord 5:1866–1955

Zafra F, Gomeza J, Olivares L et al (1995) Regional distribution and developmental variation of the glycine transporters GLYT1 and GLYT2 in the rat CNS. Eur J Neurosci 7:1342–1352

Zhang Z, Gong N, Wang W et al (2008) Bell-shaped D-serine actions on hippocampal long-term depression and spatial memory retrieval. Cereb Cortex 18:2391–2401

Zhang M, Ballard ME, Basso AM et al (2011) Behavioral characterization of a mutant mouse strain lacking D-amino acid oxidase activity. Behav Brain Res 217:81–87

Zhang Y, Chen K, Sloan SA et al (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34:11929–11947

Zhuang Z, Yang B, Theus MH et al (2010) EphrinBs regulate D-serine synthesis and release in astrocytes. J Neurosci 30:16015–16024

Zorec R, Araque A, Carmignoto G et al (2012) Astroglial excitability and gliotransmission: an appraisal of Ca2+ as a signalling route. ASN Neuro 4:2