Published in final edited form as:

Trends Neurosci. 2016 November ; 39(11): 712–721. doi:10.1016/j.tins.2016.09.007.

The rise and fall of the D-serine-mediated gliotransmission hypothesis

Herman Wolosker¹, Darrick T. Balu^{2,3}, and Joseph T. Coyle^{2,4}

¹Department of Biochemistry, Rappaport Faculty of Medicine and Research Institute, Technion-Israel Institute of Technology, Haifa 31096, Israel

²Department of Psychiatry, Harvard Medical School, Boston, Massachusetts

³Translational Psychiatry Laboratory, McLean Hospital, Belmont MA 02478

⁴Laboratory of Psychiatric and Molecular Neuroscience, McLean Hospital, Belmont MA 02478

Abstract

D-Serine modulates NMDA receptors and regulates synaptic plasticity, neurodevelopment, and learning and memory. However, the primary site of D-serine synthesis and release remains controversial, with some arguing that it is a “gliotransmitter” and others defining it as a “neuronal co-transmitter”. Results from several laboratories using different strategies now show that the D-serine’s biosynthetic enzyme, serine racemase (SR), is expressed almost entirely by neurons, with few astrocytes appearing to contain D-serine. Cell-selective suppression of serine racemase expression demonstrates that neuronal, rather than astrocytic D-serine, modulates synaptic plasticity. Here we propose an alternative conceptualization whereby astrocytes affect D-serine levels by synthesizing L-serine that shuttles to neurons to fuel the neuronal synthesis of D-serine.

Keywords

D-Serine; serine racemase; gliotransmission; N-methyl D-aspartate receptor; glycine; synaptic plasticity

D-Serine: A physiological NMDA receptor co-agonist

NMDA receptors (NMDARs) play a central role in synapse formation, synaptic plasticity, and learning and memory [1]. A distinctive feature of these receptors is the requirement for a co-agonist binding simultaneously to the GluN1 subunit with glutamate binding to its recognition site on the GluN2-3 subunits for the activation of the receptor [2]. While glycine was originally thought to be the co-agonist at the “glycine-B site” on the NMDAR, studies over the last two decades have demonstrated that D-serine is at least as important in

Correspondence to: Herman Wolosker (hwolosker@tx.technion.ac.il), Darrick T. Balu (dbalu@mclean.harvard.edu) or Joseph T. Coyle (joseph_coyle@hms.harvard.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

modulating NMDARs, particularly in the forebrain [2–4]. Serine racemase (SR) synthesizes D-serine in the brain by converting L-serine to D-serine [4, 5].

Two different strategies are commonly employed to determine the roles played by glycine-B site agonists. First, in electrophysiologic studies, the extracellular agonists can be selectively eliminated by perfusing slices with purified D-amino acid oxidase (DAO) to degrade D-serine or purified glycine oxidase (GO) to eliminate glycine [3, 6–9]. Using this strategy, D-serine was shown to be the primary NMDAR co-agonist required for long-term potentiation (LTP) at the hippocampal Schaffer collateral-CA1 pyramidal neuron synapse [8–11]. A more specific approach is to genetically silence SR expression. SR^{−/−} mice have a 90% decrease in brain D-serine and show reduced NMDAR currents, impaired synaptic plasticity, and learning deficits, consistent with NMDAR hypofunction [12–14]. Importantly, the SR^{−/−} phenotype, including deficits in LTP and fear-conditioned learning, is reversed by restoring brain D-serine [14], strengthening the notion that D-serine is critical to forebrain NMDAR function.

D-Serine and the gliotransmission debate

Gliotransmission is a hypothesis that glia modulate neurotransmission by Ca²⁺-dependent vesicular release of neuroactive substances (e. g., glutamate, ATP and D-serine), which activate neuronal receptors [15–17]. There is ongoing debate, however, on whether gliotransmission occurs under physiological conditions [18–20]. The data that fostered the widespread notion that D-serine is a gliotransmitter can be summarized as follows: (i) D-Serine and its synthetic enzyme SR were initially thought to be exclusively present in astrocytes [5, 21, 22], and therefore the effects of D-serine on synaptic plasticity and NMDAR currents were attributed to astrocytic D-serine release [3, 6, 10, 11, 23]; (ii) D-serine immunoreactivity was detected in vesicular-like structures in cultured astrocytes that undergo exocytosis *in vitro* [24–26] and (iii) inhibition of astrocyte-mediated gliotransmitter release by “calcium clamping” or poisoning of astrocytes with fluoroacetate impaired synaptic plasticity in nearby synapses [11, 23].

We outline here how each of the aforementioned findings purporting D-serine to be a gliotransmitter arose from experimental artifacts that have been contradicted by recent findings from multiple laboratories.

D-Serine and SR are mainly neuronal

Early studies suggested that D-serine is exclusively synthesized and released by astrocytes [5, 21, 22], which exhibited seemingly robust SR immunoreactivity in tissue sections [5, 6]. In contrast, using more selective antibodies, Kartvelishvili et al [27] demonstrated prominent expression of SR and D-serine immunostaining in rat forebrain neurons and synthesis of D-serine by primary neuronal cultures (Figure 1). By using SR^{−/−} mice as negative controls to determine the specificity of staining, Miya et al demonstrated that SR was present in mouse forebrain glutamatergic and GABAergic neurons, but not in astrocytes [28] (Figure 1). In the cerebellum, SR was detected in Purkinje cells of wildtype but not, SR^{−/−} mice. No immunoreactivity was observed in Bergmann glia cells, confirming a

predominant neuronal expression. Moreover, astrocytic SR was either not detectable [28] or found predominantly in the magnocellular neurons of the rat supra-optic nucleus [29], where glia-derived D-serine had been incorrectly assumed to control synaptic plasticity [6].

A genetic approach was used by Benneyworth *et al.* [13], who generated mice with cell selective suppression of SR expression (Figure 1). Mice with exon 1 of SR flanked by loxP sites were crossed with mice transgenic for Cre-recombinase driven by promoters for CaMKII α and glial fibrillary acidic protein (GFAP), which are respectively expressed in forebrain glutamatergic neurons and astrocytes. Selective suppression of SR expression in forebrain glutamatergic neurons substantially (~65%) decreased SR expression in cortex and hippocampus, while suppression of SR expression in astrocytes caused a minimal (~10%) decrease in the hippocampus, a region known to contain GFAP-expressing astrocytes, but had no effects in the cortex or striatum [13] (Figure 1).

The apparent scarcity of astrocytic SR expression was not due to low sensitivity of the antibodies. *In situ* hybridization confirmed that SR mRNA is concentrated in neuronal populations in the forebrain [30]. Ehmsen *et al.* [31] analyzed transgenic mice where the SR coding region was replaced by enhanced green fluorescent protein (EGFP), so that EGFP could be used as a surrogate marker for SR expression sites. SR was virtually completely neuronal, with only trace astrocytic expression in the cortex and hippocampus [31]. Recently, low astrocytic SR staining was detectable in mouse hippocampus, only after the use of an amplification method [32], confirming the scarcity of astrocytic SR *in vivo*. Furthermore, Balu *et al.* used an SR antibody validated with brain tissue from SR $^{-/-}$ mice and found that SR was expressed in pyramidal and inhibitory neurons but not in S100 β -containing astrocytes in human primary motor cortex [33].

Intense immunohistochemical staining for D-serine in astrocytes has been reported by several groups [6, 21–26], with only two previous studies reporting higher levels of D-serine in rat forebrain neurons [27, 34]. In light of the demonstration of preferential expression of SR in neurons, Balu *et al.* and Ehmsen *et al.* [31, 33] used SR $^{-/-}$ mice, which have <15% of WT levels of D-serine, to optimize conditions for specific staining of D-serine and confirmed its predominant neuronal localization (Figure 2). Balu *et al.* [33] showed that astrocytes maintained intense staining for “D-serine” in SR $^{-/-}$ mice under standard blocking conditions with L-serine-bovine serum albumin conjugate. It was only after raising the L-serine conjugate concentration several-fold higher did the non-specific astrocyte staining disappear, revealing D-serine staining of pyramidal neurons and a subpopulation of GABAergic neurons (Figure 2). Likewise, Ehmsen *et al.* [31] reported predominant neuronal staining for D-serine and clarified the reason for the intense artifactual staining for D-serine in astroglia: astrocytes synthesize high concentrations of L-serine. They also showed that astrocytes are the primary source of L-serine for D-serine synthesis within SR-expressing neurons.

D-Serine release pathways in vitro differ from in vivo

Another source of confusion related to D-serine signaling comes from the misconception that primary astrocyte cultures are a legitimate model to study gliotransmission. Barres *et al.*

demonstrated that genes expressed in cultured astrocytes differ greatly from those expressed by astrocytes *in vivo* [35]. The former are more similar to reactive astrocytes observed under pathological conditions, such as stroke or neuroinflammation [36]. In fact, cultured astrocytes express SR, which further reinforced the belief that astrocytes are the primary source of D-serine *in vivo* [26, 37, 38]. Cultured astrocytes were reported to exhibit vesicular D-serine release that is abolished by addition of extracellular or intracellular Ca^{2+} chelators [37]. However, a subsequent study ascribed the D-serine release from astrocytes *in vitro* as non-vesicular, and at least in part, due to the opening of volume-regulated channels [39]. Furthermore, neuronal cultures also release D-serine by depolarization or by plasma membrane neutral amino acid antiporters [27, 39]. Therefore, a major question is whether it is possible to detect Ca^{2+} -dependent astrocytic release of D-serine from acute rat brain slices. In contrast to astrocyte cultures, endogenous D-serine release from rat cortical slices was not affected by chelating intracellular and extracellular Ca^{2+} , while impairing exocytotic glutamate release in the same slices [39]. Ca^{2+} -dependent vesicular D-serine release from astrocytes was not detectable in perfusates of acute brain slices.

In vivo microdialysis experiments have demonstrated a significant decrease in hippocampal extracellular D-serine when SR expression is suppressed in forebrain glutamatergic neurons [40]. If the bulk of D-serine release is neuronal and Ca^{2+} -independent, which pathways are the main determinants of D-serine release? Recent data indicate that a large fraction of D-serine release occurs by the neuronal Asc-1 (SLC7A10) transporter, a plasma membrane antiporter that mediates tonic D-serine release [8]. Thus, blockade of Asc-1 by Lu AE00527 or its targeted deletion decreased the basal release of D-serine and impaired LTP at the hippocampal Schaffer collateral-CA1 synapse [41]. This finding is consistent with an earlier description of a tonic release of D-serine that would regulate synaptic plasticity under low levels of stimulation of glutamatergic afferents in the amygdala [7]. Thus, tonic neuronal non-vesicular D-serine release appears to be critical for synaptic NMDAR function, although these studies do not preclude other forms of release.

D-serine is not a gliotransmitter

A number of studies using different chemical and genetic manipulations of astrocytes in brain slices proposed that D-serine is a gliotransmitter [11, 42, 43]. However, the methodology and selectivity of the astrocytic perturbations used in these studies have been questioned [19, 20, 44], and recent data demonstrating that deletion of the neuronal, but not astrocytic SR impairs synaptic plasticity contradict their findings (Figure 3) [13].

Hennenberg *et al* [11] reported that LTP at the hippocampal Schaffer collateral-CA1 synapse was suppressed when Ca^{2+} increases were blocked in nearby astrocytes by a Ca^{2+} clamping technique, and suggested that astrocytic D-serine would mediate gliotransmission. In contrast, McCarthy and colleagues did not observe such regulation of synaptic plasticity by gliotransmission. They found no changes in short or long term plasticity in mouse lines engineered to selectively increase intracellular Ca^{2+} in astrocytes upon stimulation of Mas-Related G-protein coupled receptor member A1 (MRGA1) receptors or that are unable to mobilize intracellular Ca^{2+} due to the deletion of inositol triphosphate type 2 receptors (IP3R2) [18]. To account for McCarthy's negative results, it has been proposed that

astrocytes might not respond to the type of Ca^{2+} increase induced by MRGA1 activation or that IPR3R2 $^{-/-}$ mice may display alternative ways to increase intracellular Ca^{2+} in small astrocytic processes [45–47]. In line with McCarthy's results, the scarcity of D-serine and SR in astroglia (Figures 1 and 2) and the normal LTP observed after selective deletion of astrocytic SR (Figure 3) do not support a role of D-serine release from small astrocytic processes in synaptic plasticity.

A recent study by Shigetomi *et al* [42] reported that Transient Receptor Potential cation channel, subfamily A, member 1 knockout (TRPA1 $^{-/-}$) mice exhibit lower astrocytic basal intracellular Ca^{2+} levels and have reduced LTP presumably because of deficits in D-serine release, as monitored with a D-amino acid oxidase (DAO)-based D-serine biosensor. However, the results obtained with biosensors should be interpreted with caution. D-Serine biosensors also detect most neutral D-amino acids, including D-alanine, and might non-specifically oxidize other biological compounds [48]. Although D-alanine levels in the hippocampus are much lower than D-serine [49], the catalytic efficiency of DAO enzymes for D-alanine is about 40 times higher than of D-serine [50], so it is conceivable that DAO-based biosensors can detect trace amounts of D-alanine. Biosensors also do not distinguish the cellular origin of released D-serine. In this context, we argue that validation of D-serine biosensors should always be performed using cell-specific SR $^{-/-}$ mice as controls. Furthermore, TRPA1 activation by an agonist has no effect on LTP [42], and TRPA1 inhibition decreases interneuron inhibitory synapse efficacy [51], so indirect effects on neuronal D-serine cannot be excluded. Selective astrocytic localization of TRPA1 could not be directly confirmed by immunocytochemistry [51], and it is not known if SR or D-serine occurs in TRPA1-responsive astrocytes.

Haydon and coworkers used transgenic mice that express a dominant negative construct of the SNARE (dnSNARE) domain of synaptobrevin to reduce putative D-serine exocytosis from astrocytes. Using the same biosensor employed by Shigetomi *et al* [42], they found that the release of D-serine was normal in dnSNARE mice [52]. Furthermore, Nedergaard's laboratory has described widespread expression of the dnSNARE transgene in cortical neurons, undercutting the astrocyte specificity of the construct [44]. Despite these findings, the same dnSNARE mice were recently used to argue that D-serine released from astrocytes regulates dendritic spine plasticity of adult-born neurons in the dentate gyrus of the hippocampus [43]. The effects of dnSNARE expression were analyzed 30 days after the induction of the transgene and prolonged inhibition of exocytosis might have indirect effects on neuronal D-serine production. Most importantly, there has been no direct immunohistochemical evidence that SR and D-serine are found in dnSNARE transgenic astrocytes used in previous studies.

In order to more definitively test the D-serine gliotransmitter hypothesis, Benneyworth *et al* [13] monitored LTP at the hippocampal Schaffer collateral-CA1 synapse in mice with conditional SR deletion in hippocampal astrocytes (aSR $^{-/-}$) as compared to neurons (nSR $^{-/-}$). The aSR $^{-/-}$ mice displayed normal LTP, while the LTP was significantly reduced in nSR $^{-/-}$ mice (Figure 3). Furthermore, isolated NMDAR currents were lower in nSR-KOs, and restored by D-serine addition to the perfusate [13]. To ensure complete knockout in GFAP-positive hippocampal astrocytes, the authors analyzed control GFAP-LacZ reporter mice and

observed a knockout efficiency greater than 80%. Therefore, lack of changes in LTP in aSR^{-/-} mice was not due to incomplete Cre expression. Finally, adult nSR^{-/-} mice have reduced dendritic complexity and spine density in pyramidal neurons at the somatosensory cortex, demonstrating that neuronally derived D-serine regulates dendrite plasticity [53]. These observations negate the hypothesis that D-serine is a gliotransmitter, and confirm the neuronal origin of D-serine signaling. While we argue that astrocytic D-serine does not regulate synaptic plasticity, these experiments do not apply to other types of proposed gliotransmitters, such as adenosine, ATP or glutamate.

Role of glial metabolism in D-serine homeostasis: The Serine Shuttle

Hypothesis

Although we demonstrate that adult astrocytes *in vivo* do not produce enough D-serine to affect synaptic plasticity, they are by no means unimportant for D-serine production. Most of the SR substrate, L-serine, is synthesized *de novo* in the brain in astrocytes, which express all the enzymes required to convert glucose into L-serine [54, 55]. Astrocytes exclusively express 3-phosphoglycerate dehydrogenase (Phgdh), the committed step in the L-serine synthesis pathway [54]. Mutations in Phgdh cause severe neurodevelopmental problems, microcephaly and seizures in children, and are associated with low levels of L-serine and D-serine in the CSF [56]. Deletion of Phgdh in mouse brain astrocytes dramatically reduces brain L- and D-serine by about 80% [55], with D-serine deficits localized to neurons [31], indicating that astrocytic synthesis of L-serine is required for D-serine synthesis by neurons. These observations motivated the proposal of the “serine shuttle model”, whereby L-serine synthesized by astrocytes is shuttled to neurons to fuel the synthesis of D-serine [57] (Figure 4). This mechanism adds to other forms of possible metabolic interchanges between glia and neurons [58, 59]. Subsequent neuronal release of D-serine then modulates NMDAR function (Figure 4). Termination of D-serine signaling is attained by re-uptake into neurons that express D-serine transporters [4, 57]. Some neuronally produced D-serine might be transported into astrocytes, which in some parts of the brain, metabolize D-serine via the peroxisomal DAO enzyme, known to be enriched in astrocytes [60]. Several questions regarding D-serine signaling still remain to be answered (See Outstanding Questions Box).

OUTSTANDING QUESTIONS

- Which pathways are the main determinants of neuronal release of D-serine?
- How is D-serine signaling terminated given the low expression of the catabolic enzyme D-amino acid oxidase in the adult forebrain?
- Is D-serine important for synaptic NMDAR transmission in forebrain brain regions outside the hippocampus and amygdala?
- What are the relative role of D-serine and glycine as NMDAR co-agonists at different synapses?
- How is SR activity regulated *in vivo*?

- How does dysregulation of D-serine signaling contribute to neuropsychiatric disorders that involve NMDAR hypofunction?

The serine shuttle provides an alternative explanation for why poisoning astrocytes impairs NMDAR function, a major element in the gliotransmitter hypothesis. Previous studies reported that metabolic poisoning of astrocytes with fluoroacetate impairs LTP and decreases NMDAR currents presumably by blocking astrocytic D-serine release [11, 23]. We propose that fluoroacetate effects are not due to selective blockade of gliotransmission, but rather to the disruption of the serine shuttle among other metabolic reactions [61]. In agreement with this possibility, Billard and Mothet's groups recently confirmed that fluoroacetate poisoning of astrocytes indeed impairs LTP in slices, an effect that can be reversed by the addition of L-serine to the perfusate [9]. Thus, restoring D-serine synthesis in neurons can overcome the metabolic blockade of fluoroacetate.

How to test if a substance is a glial or neuronal transmitter/modulator?

In addition to a possible disruption of the serine shuttle, there are other nonspecific effects associated with the use of gliotoxins. These include general blockade of astrocytic metabolic activity [62], dysregulation of extracellular K^+ levels [40] and profound disruption of the glutamate/glutamine shuttle [62], all of which may indirectly affect neurotransmission [19]. Likewise, the injection of intracellular Ca^{2+} chelators and inhibition of vesicular fusion by dnSNARE may also generate nonspecific effects. Ca^{2+} chelation may inhibit the activity of a wide variety of Ca^{2+} -binding proteins, which could affect additional astrocytic functions. To avoid these caveats, we propose that the gold-standard for evaluating the cellular source should be the selective deletion of the candidate molecule itself in astrocytes and neurons. Restoration of the levels of the candidate transmitter should reverse the phenotype. In the case of D-serine, the cell-selective deletion of SR revealed a role of neuronal, rather than astrocytic D-serine in synaptic plasticity. This strategy may be difficult to implement with other transmitter candidates. Unlike D-serine, some gliotransmitter candidates are important for the intermediary metabolism, making their selective deletion undesirable. Deletion of their membrane transporters in select cell populations may provide a more specific way to disrupt their signaling and minimize changes to other important cellular activities.

Concluding remarks

Although astrocytes play an important role in modulating L-serine availability through the serine shuttle, the evidence is incontrovertible that neurons, not astrocytes, are the major sources of D-serine. The misperception of the astrocytic source of D-serine resulted from a series of unfortunate and re-enforcing artifacts. The SR expression in cultured astrocytes provided support for the artifactual demonstration of SR and D-serine by immunohistochemistry in forebrain astroglia. The remarkably high concentration of L-serine in astroglia may have subverted the specificity of antiserum against D-serine. Therefore, immunocytochemical studies should be validated by molecular methods to suppress the expression of the moiety of interest. Efficacy of glial toxins, such as fluoroacetate, in blocking LTP likely derives from their disruption of the serine shuttle, interfering with

neuronal synthesis of D-serine from astrocyte synthesized L-serine. Deletion of SR in a cell-selective manner demonstrates that neuronal D-serine is the main regulator of NMDAR-dependent synaptic plasticity. If extrapolated to the general glia-neuron communication field, the lesson learned is that, when possible, cell-selective deletion of the molecule of interest in neurons and astrocytes should be the gold-standard for evaluating the cellular origin of the candidate transmitter.

Acknowledgments

The authors thank Ms. Thea Anderson for art work and helpful suggestions. Writing of this review was supported by grants from the Israel Science Foundation, Legacy Heritage Fund, and the Allen and Jewel Prince Center for Neurodegenerative Processes of the Brain (H.W.), and NIH grants 5R00MH099252-04 (D.T.B.), RO1 MH051290 and P50 MH060450 (J.T.C.).

References

1. Traynelis SF, et al. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev.* 2010; 62:405–496. [PubMed: 20716669]
2. Balu DT, Coyle JT. The NMDA receptor ‘glycine modulatory site’ in schizophrenia: D-serine, glycine, and beyond. *Current opinion in pharmacology.* 2015; 20:109–115. [PubMed: 25540902]
3. Mothet JP, et al. D-serine is an endogenous ligand for the glycine site of the N-methyl-D- aspartate receptor. *Proc Natl Acad Sci U S A.* 2000; 97:4926–4931. [PubMed: 10781100]
4. Wolosker H, et al. D-amino acids in the brain: D-serine in neurotransmission and neurodegeneration. *Febs J.* 2008; 275:3514–3526. [PubMed: 18564180]
5. Wolosker H, et al. Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. *Proc Natl Acad Sci U S A.* 1999; 96:13409–13414. [PubMed: 10557334]
6. Panatier A, et al. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell.* 2006; 125:775–784. [PubMed: 16713567]
7. Li Y, et al. Identity of endogenous NMDAR glycine site agonist in amygdala is determined by synaptic activity level. *Nat Commun.* 2013; 4:1760. [PubMed: 23612301]
8. Rosenberg D, et al. Neuronal D-serine and glycine release via the Asc-1 transporter regulates NMDA receptor-dependent synaptic activity. *J Neurosci.* 2013; 33:3533–3544. [PubMed: 23426681]
9. Le Bail M, et al. Identity of the NMDA receptor coagonist is synapse specific and developmentally regulated in the hippocampus. *Proc Natl Acad Sci U S A.* 2015; 112:E204–213. [PubMed: 25550512]
10. Mothet JP, et al. A critical role for the glial-derived neuromodulator d-serine in the age-related deficits of cellular mechanisms of learning and memory. *Aging Cell.* 2006; 5:267–274. [PubMed: 16842499]
11. Henneberger C, et al. Long-term potentiation depends on release of D-serine from astrocytes. *Nature.* 2010; 463:232–236. [PubMed: 20075918]
12. Basu AC, et al. Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. *Mol Psychiatry.* 2009; 14:719–727. [PubMed: 19065142]
13. Benneyworth MA, et al. Cell selective conditional null mutations of serine racemase demonstrate a predominate localization in cortical glutamatergic neurons. *Cell Mol Neurobiol.* 2012; 32:613–624. [PubMed: 22362148]
14. Balu DT, et al. 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.* 2013; 110:E2400–2409. [PubMed: 23729812]
15. Araque A, et al. Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. *J Neurosci.* 1998; 18:6822–6829. [PubMed: 9712653]

16. Bezzi P, et al. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature*. 1998; 391:281–285. [PubMed: 9440691]
17. Parpura V, et al. Glutamate-mediated astrocyte-neuron signalling. *Nature*. 1994; 369:744–747. [PubMed: 7911978]
18. Agulhon C, et al. Hippocampal short- and long-term plasticity are not modulated by astrocyte Ca²⁺ signaling. *Science (New York, N Y)*. 2010; 327:1250–1254.
19. Nedergaard M, Verkhratsky A. Artifact versus reality--how astrocytes contribute to synaptic events. *Glia*. 2012; 60:1013–1023. [PubMed: 22228580]
20. Sloan SA, Barres BA. Looks can be deceiving: reconsidering the evidence for gliotransmission. *Neuron*. 2014; 84:1112–1115. [PubMed: 25521372]
21. Schell MJ, et al. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. *Proc Natl Acad Sci U S A*. 1995; 92:3948–3952. [PubMed: 7732010]
22. Schell MJ, et al. D-serine as a neuromodulator: regional and developmental localizations in rat brain glia resemble NMDA receptors. *J Neurosci*. 1997; 17:1604–1615. [PubMed: 9030620]
23. Fossat P, et al. Glial D-Serine Gates NMDA Receptors at Excitatory Synapses in Prefrontal Cortex. *Cereb Cortex*. 2012; 22:595–606. [PubMed: 21690263]
24. Williams SM, et al. Immunocytochemical analysis of D-serine distribution in the mammalian brain reveals novel anatomical compartmentalizations in glia and neurons. *Glia*. 2006; 53:401–411. [PubMed: 16342169]
25. Bergersen LH, et al. Immunogold detection of L-glutamate and D-serine in small synaptic-like microvesicles in adult hippocampal astrocytes. *Cereb Cortex*. 2012; 22:1690–1697. [PubMed: 21914633]
26. Martineau M, et al. Storage and uptake of D-serine into astrocytic synaptic-like vesicles specify gliotransmission. *J Neurosci*. 2013; 33:3413–3423. [PubMed: 23426669]
27. Kartvelishvili E, et al. Neuron-derived D-serine release provides a novel means to activate N-methyl-D-aspartate receptors. *J Biol Chem*. 2006; 281:14151–14162. [PubMed: 16551623]
28. Miya K, et al. Serine racemase is predominantly localized in neurons in mouse brain. *J Comp Neurol*. 2008; 510:641–654. [PubMed: 18698599]
29. Wang YF, et al. Hyposmolality differentially and spatiotemporally modulates levels of glutamine synthetase and serine racemase in rat supraoptic nucleus. *Glia*. 2013; 61:529–538. [PubMed: 23361961]
30. Yoshikawa M, et al. The serine racemase mRNA is predominantly expressed in rat brain neurons. *Arch Histol Cytol*. 2007; 70:127–134. [PubMed: 17827670]
31. Ehmsen JT, et al. D-serine in glia and neurons derives from 3-phosphoglycerate dehydrogenase. *J Neurosci*. 2013; 33:12464–12469. [PubMed: 23884950]
32. Suzuki M, et al. Glycolytic flux controls D-serine synthesis through glyceraldehyde-3-phosphate dehydrogenase in astrocytes. *Proc Natl Acad Sci U S A*. 2015; 112:E2217–2224. [PubMed: 25870284]
33. Balu DT, et al. D-serine and serine racemase are localized to neurons in the adult mouse and human forebrain. *Cell Mol Neurobiol*. 2014; 34:419–435. [PubMed: 24436034]
34. Yasuda E, et al. Immunohistochemical evidences for localization and production of D-serine in some neurons in the rat brain. *Neuroscience letters*. 2001; 299:162–164. [PubMed: 11166963]
35. Cahoy JD, et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J Neurosci*. 2008; 28:264–278. [PubMed: 18171944]
36. Zamanian JL, et al. Genomic analysis of reactive astrogliosis. *J Neurosci*. 2012; 32:6391–6410. [PubMed: 22553043]
37. Mothet JP, et al. Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. *Proc Natl Acad Sci U S A*. 2005; 102:5611–5606.
38. Yang Y, et al. Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. *Proc Natl Acad Sci U S A*. 2003; 100:15194–15199. [PubMed: 14638938]

39. Rosenberg D, et al. Neuronal release of D-serine: a physiological pathway controlling extracellular D-serine concentration. *Faseb J*. 2010; 24:2951–2961. [PubMed: 20371631]
40. Largo C, et al. The effect of depressing glial function in rat brain in situ on ion homeostasis, synaptic transmission, and neuron survival. *J Neurosci*. 1996; 16:1219–1229. [PubMed: 8558250]
41. Sason H, et al. Asc-1 Transporter Regulation of Synaptic Activity via the Tonic Release of d-Serine in the Forebrain. *Cereb Cortex*. 2016
42. Shigetomi E, et al. TRPA1 channels are regulators of astrocyte basal calcium levels and long-term potentiation via constitutive D-serine release. *J Neurosci*. 2013; 33:10143–10153. [PubMed: 23761909]
43. Sultan S, et al. Synaptic Integration of Adult-Born Hippocampal Neurons Is Locally Controlled by Astrocytes. *Neuron*. 2015; 88:957–972. [PubMed: 26606999]
44. Fujita T, et al. Neuronal transgene expression in dominant-negative SNARE mice. *J Neurosci*. 2014; 34:16594–16604. [PubMed: 25505312]
45. Volterra A, et al. Astrocyte Ca(2+)(+) signalling: an unexpected complexity. *Nat Rev Neurosci*. 2014; 15:327–335. [PubMed: 24739787]
46. Srinivasan R, et al. Ca(2+) signaling in astrocytes from *Ip3r2*(–/–) mice in brain slices and during startle responses in vivo. *Nat Neurosci*. 2015; 18:708–717. [PubMed: 25894291]
47. Bazargani N, Attwell D. Astrocyte calcium signaling: the third wave. *Nat Neurosci*. 2016; 19:182–189. [PubMed: 26814587]
48. Polcari D, et al. Disk-shaped amperometric enzymatic biosensor for in vivo detection of D-serine. *Anal Chem*. 2014; 86:3501–3507. [PubMed: 24650010]
49. Morikawa A, et al. Determination of free D-aspartic acid, D-serine and D-alanine in the brain of mutant mice lacking D-amino acid oxidase activity. *J Chromatogr B Biomed Sci Appl*. 2001; 757:119–125. [PubMed: 11419736]
50. Pollegioni L, et al. Specificity and kinetics of *Rhodotorula gracilis* D-amino acid oxidase. *Biochim Biophys Acta*. 1992; 1120:11–16. [PubMed: 1348188]
51. Shigetomi E, et al. TRPA1 channels regulate astrocyte resting calcium and inhibitory synapse efficacy through GAT-3. *Nat Neurosci*. 2012; 15:70–80.
52. Clasadonte J, et al. Astrocyte control of synaptic NMDA receptors contributes to the progressive development of temporal lobe epilepsy. *Proc Natl Acad Sci U S A*. 2013; 110:117545–7540.
53. Balu DT, Coyle JT. Neuronal D-serine regulates dendritic architecture in the somatosensory cortex. *Neuroscience letters*. 2012; 517:77–81. [PubMed: 22521586]
54. Yamasaki M, et al. 3-Phosphoglycerate dehydrogenase, a key enzyme for l-serine biosynthesis, is preferentially expressed in the radial glia/astrocyte lineage and olfactory ensheathing glia in the mouse brain. *J Neurosci*. 2001; 21:7691–7704. [PubMed: 11567059]
55. Yang JH, et al. 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*. 2010; 285:41380–41390. [PubMed: 20966073]
56. Klomp LW, et al. Molecular characterization of 3-phosphoglycerate dehydrogenase deficiency--a neurometabolic disorder associated with reduced L-serine biosynthesis. *Am J Hum Genet*. 2000; 67:1389–1399. [PubMed: 11055895]
57. Wolosker H, Radziszewsky I. The serine shuttle between glia and neurons: Implications for neurotransmission and neurodegeneration. *Biochem Soc Trans*. 2013:41550–1546. 1.
58. Bak LK, et al. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J Neurochem*. 2006; 98:641–653. [PubMed: 16787421]
59. Belanger M, et al. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab*. 2011; 14:724–738. [PubMed: 22152301]
60. Horiike K, et al. D-amino-acid oxidase is confined to the lower brain stem and cerebellum in rat brain: regional differentiation of astrocytes. *Brain Res*. 1994; 652:297–303. [PubMed: 7953743]
61. Hassel B, et al. Trafficking of amino acids between neurons and glia in vivo. Effects of inhibition of glial metabolism by fluoroacetate. *J Cereb Blood Flow Metab*. 1997; 17:1230–1238. [PubMed: 9390655]

62. Fonnum F, et al. Use of fluorocitrate and fluoroacetate in the study of brain metabolism. *Glia*. 1997; 21:106–113. [PubMed: 9298853]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

TRENDS BOX

- Serine racemase (SR) and D-serine are predominantly localized to neurons. Previous observations of astrocytic localization in brain tissue were due to non-specific antibody immunoreactivity, demonstrated using SR^{-/-} mice as controls.
- D-Serine is tonically released from neurons by antiporters in a non-vesicular-dependent mechanism.
- Neuronal SR deletion impairs NMDAR function and synaptic plasticity, while astrocytic SR deletion has no effect.
- Astrocytes contribute to D-serine homeostasis by synthesizing and exporting L-serine, which fuels the neuronal synthesis of D-serine. This model provides a mechanism by which glial toxins impair NMDAR-dependent synaptic plasticity.

Fig. 1. Neuronal localizations of SR

Robust neuronal SR staining in rat cerebral cortex (A) and hippocampal CA1 (B). SR staining in the cerebral cortex of WT mice (C) that was absent in SR $-/-$ (SR-KO) controls (D). Neuronal nucleus marker (E and F) co-localizes with SR in WT mice (G), with no signal in SR-KO (H). Cell-selective SR knockout in mice demonstrate predominant expression of SR in neurons (I to K). SR staining at the cerebral cortex, corpus callosum, and hippocampal regions of astrocytic-selective SR-KO (aSR-KO, panel I) was virtually the same as in SR-WT (panel J), but strongly reduced in neuronal SR-KO (nSR-KO, panel K). Reproduced with permission from references [27], panels A and B; [28], panels C to H; [13], panels I to K. CA1, region 1 of the cornus ammonis of the hippocampus; CTX, cerebral cortex.

Fig. 2. Neuronal localizations of D-serine

D-Serine staining in neuronal cells bodies of the cerebral cortex (A) was reduced in SR-KO mice (B). Very few astrocytes stain for D-serine in the hippocampus and cerebral cortex, though most astrocytes in the corpus callosum contained D-serine (C). Improved L-serine pre-absorption technique revealed specific neuronal staining in the primary somatosensory cortex, and no co-localization with astrocytic marker S100 β (D–E). Highest immunoreactivity for D-serine was found in GABAergic neurons stained with GAD67 marker (G–H). Reproduced with permission from references [31] (A–C); [33] (D–I). CTX, cerebral cortex; CC, corpus callosum; GAD67; glutamic acid decarboxylase 67; HIPP, hippocampus; S100 β , S100 calcium-binding protein beta.

Fig. 3. Neuronal SR, but not astrocytic, is required for synaptic plasticity

LTP at the Schaffer collateral-CA1 synapse in the hippocampus is unaffected in (A) mice lacking SR in astrocytes (aSRCKO), but significantly reduced in (B) mice lacking SR in forebrain glutamatergic neurons (nSRCKO). LTP was induced by a single 1-s train of high-frequency stimulation (100 Hz, at arrow). Insets show the average of fEPSPs recorded before (black traces) and 45 min after (gray traces) the induction of LTP. Diagrams below panels A and B show the cell-selective knockout strategies used to generate aSRCKO and nSRCKO at the hippocampus. Panel C shows a 70% decrease in LTP level in nSR^{-/-} mice. Reproduced with permission from reference [13]. GFAP-Cre, glial fibrillary acidic protein-cre recombinase; CaMKII α Calcium/calmodulin-dependent protein kinase II alpha-Cre recombinase.

Fig. 4. Serine shuttle model of D-serine metabolism

This model proposes that neuronal D-serine production requires astrocytic export of L-serine from glia to neurons. Astrocytes obtain glucose from blood vessels through glucose transporter 1 (GLUT1, step 1). Glucose is converted into L-serine in several steps, with the committed step catalyzed by the 3-phosphoglycerate dehydrogenase enzyme (step 2). L-Serine exits the astrocytes by neutral amino acid exchangers, such as ASCT1 (Slc1a4) and system N transporters (T, step 3). Neurons take up L-serine by neutral amino acid antiporters or system A transporter (T, step 4). Neuronal SR converts L-serine into D-serine (step 5). D-Serine is subsequently released from neurons by Asc-1 (Slc7a10) or other transporters (step 6). Released D-serine allows synaptic NMDAR activation (step 7). D-Serine signaling may terminate through neuronal reuptake (step 8) or low-affinity astrocytic uptake and subsequent metabolism by the peroxisomal D-amino acid oxidase (step 9). It is still unclear which neuronal compartment releases D-serine for synaptic NMDAR activation. The scheme was modified from reference [57]. Dao, D-amino acid oxidase; glut1, glucose transporter 1; 3-P-glycerate, 3-phosphoglyceric acid; 3-P-OH-pyruvate, 3-phosphohydroxypyruvate.