Published in final edited form as:

Adv Pharmacol. 2018 ; 82: 35–56. doi:10.1016/bs.apha.2017.10.002.

The Role of Serine Racemase in the Pathophysiology of Brain Disorders

Joseph T. Coyle^{*,†,1} and Darrick T. Balu^{*,†}

^{*}Harvard Medical School, Boston, MA, United States

[†]McLean Hospital, Belmont, MA, United States

Abstract

The *N*-methyl-D-aspartate receptor (NMDAR) is unique in requiring two agonists to bind simultaneously to open its cation channel: the neurotransmitter, glutamate, and the coagonists, glycine, or D-serine. The Snyder laboratory was the first to clone serine racemase (SR), the enzyme that synthesizes D-serine, and to localize it immunocytochemically. Our laboratory has focused on the role of D-serine in brain disorders. Silencing the expression of SR, a risk gene for schizophrenia (SCZ), in mice (SR^{-/-}), results in a phenotype that closely resembles SCZ including: cortical atrophy, reduced dendritic spine density and complexity, downregulation of parvalbumin-positive cortical GABAergic neurons, and cognitive impairments. This pathology can be reversed by treatment of SR^{-/-} mice with D-serine in adulthood. SR^{-/-} mice also exhibit abnormal response toward abusable substances, such as stimulants. They show reduced behavioral sensitization to D-amphetamine, but fail to extinguish it. Place preference to cocaine is altered, and the hedonic response to it is profoundly impaired as assessed by intracranial self-stimulation. D-cycloserine, a partial agonist at the NMDAR glycine modulatory site, shows therapeutic benefit for treating pathologic anxiety in combination with behavioral therapies. Studies in vitro with cortical culture and in vivo with middle cerebral artery occlusion show that silencing SR provides substantial protection against ischemic neuronal death. Finally, the switch of SR expression from neurons to reactive astrocytes after closed head trauma accounts for the reduced in vivo neuroplasticity, electroencephalogram abnormalities, and cognitive impairments.

1. INTRODUCTION

The Nishikawa laboratory was the first to report the presence of D-serine in the brain, thus upending the shibboleth that vertebrates utilize only L-amino acids (Hashimoto et al., 1992). This discovery also resolved a quarter of a century conundrum about the reason for the expression of D-amino acid oxidase (DAAO) in brain (Neims, Zieverink, & Smilack, 1966). Johnson and Ascher (1987) showed that the *N*-methyl-D-aspartate receptor (NMDAR) had the unusual characteristic of requiring a second ligand, glycine, to be bound to the receptor for glutamate to open the cation channel. Notably, Kleckner and Dingledine (1988)

¹Corresponding author: joseph_coyle@hms.harvard.edu.

CONFLICT OF INTEREST

J.T.C. reports holding a patent on the clinical use of D-serine that is owned by Massachusetts General Hospital but could yield royalties to J.T.C. D.T.B. reports no conflicts.

implicated D-serine as a potential coagonist at the NMDAR. They found D-serine to be more potent than glycine itself in the *Xenopus* expression system, although D-serine's presence in the brain awaited the Hashimoto et al. (1992) discovery.

Sol Snyder has had a deep interest in unusual signaling pathways in the nervous system such as nitric oxide and carbon monoxide (Bredt & Snyder, 1969; Verma, Hirsch, Glatt, Ronnett, & Snyder, 1993). This interest undoubtedly drew his attention to D-serine, which was known to be a potential coagonist at the NMDAR (Kleckner & Dingledine, 1988) and to be present in the brain (Hashimoto et al., 1992). Thus, his laboratory undertook the purification and characterization of serine racemase (SR), the enzyme that synthesizes D-serine, from rat brain (Wolosker, Blackshaw, & Snyder, 1999). They then cloned SR and showed that it was a member of the family of pyridoxal-5'-phosphate-dependent enzymes in eukaryotes (Wolosker, Sheth, et al., 1999). They also demonstrated that there was a close correspondence in the expression of SR, NMDARs, and D-serine levels in the forebrain of the rat (Schell, Molliver, & Snyder, 1995). They developed compelling evidence that endogenous D-serine in fact was a coagonist at synaptic receptors by showing loss of NMDAR activity when culture hippocampal neurons and hippocampal slice were perfused with purified DAAO to catabolize synaptic D-serine (Mothet et al., 2000). Finally, they showed that during development there was an inverse relationship between D-serine levels and DAAO expression in the cerebellum and hindbrain where glycine was found in high concentrations and was likely the coagonist. Thus, the Snyder laboratory laid the foundation by establishing D-serine as the coagonist at synaptic NMDARs in forebrain, which has been confirmed repeatedly since then (Li et al., 2013; Meunier et al., 2016).

This chapter will review research from the Coyle laboratory to define the potential role of D-serine in the pathophysiology of brain disorders. This effort was built upon the foundation laid by the Snyder laboratory on the role of D-serine as the forebrain coagonist at synaptic NMDARs.

2. SCHIZOPHRENIA

For over 40 years the "dopamine hypothesis" has dominated the thinking about the pathophysiology of schizophrenia due to the fact that antipsychotic drugs act at the dopamine D2 receptor (Snyder, 1976). Subsequent imaging research substantiated the relationship between excessive forebrain dopamine D2 receptor activation and psychosis in patients (Ginovart & Kapur, 2012). However, with the possible exception of clozapine, antipsychotic drugs have no effects on negative symptoms and cognitive impairments, the most robust predictors of disability in schizophrenia (Lim et al., 2016). Furthermore, cortical atrophy correlates with negative symptoms and cognitive impairments in chronic schizophrenia but not with the severity of psychosis (Davis et al., 1998; Johnstone et al., 1989). Thus, the core features of schizophrenia, which are primarily responsible for persistent disability, are linked to pervasive cortical pathology and are unlikely the consequence of simply dopamine dysfunction.

The implications of dysfunction of glutamatergic neurotransmission and specifically NMDAR function evolved primarily out of two research strategies: pharmacologic

challenges and postmortem neurochemical studies (for review, Coyle, Basu, Benneyworth, Balu, & Konopaske, 2012). Since dissociative anesthetics were first introduced to clinical practice 50 years ago, they were known to cause a syndrome clinically indistinguishable from schizophrenia (Anis, Berry, Burton, & Lodge, 1983). The demonstration that ketamine and phencyclidine are noncompetitive antagonists raised the question whether NMDARs might play a role in the pathophysiology of schizophrenia (Javitt & Zukin, 1991). Objective clinical challenge studies with ketamine confirmed that low-dose ketamine administered to normal volunteers caused negative symptoms and cognitive impairments, hypo-frontality and increased forebrain dopamine release similar to schizophrenia, while it exacerbated core symptoms in stabilized schizophrenic subjects (for review, Moghaddam & Krystal, 2012).

Our laboratory identified a neuropeptide, *N*-acetyl-L-aspartyl-L-glutamate (NAAG), which is concentrated in forebrain glutamatergic neurons but is also found in spinal motor neurons. We identified its catabolic enzyme, glutamate carboxypeptidase II (GCPII; also known as folate hydrolase I or *FOLH 1*, prostate-specific membrane antigen, and *N*-acetyl-L-aspartyl-L-glutamate peptidase or NAALADase) (for review, Bergeron & Coyle, 2012). NAAG has a dual role in inhibiting glutamatergic neurotransmission by activating metabotropic glutamate 3 receptors (mGluR3) that inhibit glutamate release (Jackson & Slusher, 2001) and by antagonizing NMDARs at the glycine modulatory site (GMS) (Bergeron, Coyle, Tsai, & Greene, 2005). We carried out a postmortem analysis of the levels of glutamate and NAAG, as well as the activity GCPII in the cortex and hippocampus in controls and in subjects with the diagnosis of schizophrenia, and found evidence for a significant increase in NAAG and reduced glutamate and GCPII activity. These findings were consistent with reduced glutamatergic neurotransmission, specifically through the NMDAR (Tsai et al., 1995).

Our hypothesis, which linked the observations from ketamine challenges and the postmortem findings on glutamatergic parameters in schizophrenia, proposed that the fundamental pathophysiology of schizophrenia resulted from NMDAR hypofunction in the cortex, causing the cognitive deficits and negative symptoms with psychosis being a downstream, subcortical complication (Coyle, 1996). The hypothesis was supported by the demonstration that both the GMS partial agonist, D-cycloserine (DCS); and GMS full agonist, D-serine, reduced negative symptoms, and enhanced cognition in schizophrenic patients stabilized on antipsychotic drugs (Goff, Tsai, Manoach, & Coyle, 1995; Tsai, Yang, Chung, Lange, & Coyle, 1998).

Although there were several pharmacologic models for the NMDAR hypofunction in schizophrenia (Bakshi & Geyer, 1995; Farber et al., 1995), we were concerned that they represented at best a transient receptor dysfunction, whereas schizophrenia likely resulted from a congenital impairment of NMDAR function since cognitive and behavioral abnormalities were often present in childhood long before the onset of psychosis (Erlenmeyer-Kimling et al., 2000). Therefore, based on the foundational research of the Snyder laboratory and evidence of reduced cerebrospinal fluid of D-serine in schizophrenia (Hashimoto et al., 2005), we decided to “knockout” SR since a deficiency in D-serine would likely impair, but not totally block NMDAR function because of the presence of the other coagonist glycine (Basu et al., 2009). Excising the first expressed exon of SR blocked

expression of SR and in the homozygote state (SR $-/-$), resulted in a >85% reduction of endogenous D-serine in cortex and hippocampus.

Extensive phenotyping of the SR $-/-$ mice have revealed striking homologies between them and the neuropathologic, neurochemical, and cognitive deficits associated with schizophrenia. The two most reproduced neuropathologic features of schizophrenia are downregulation of the cortical parvalbumin-positive GABAergic interneurons (Lewis, 2014) and reduced spine density and dendritic complexity of cortical pyramidal neurons, surrogates of glutamatergic synapses (Moyer, Shelton, & Sweet, 2015). In this regard, Selemon and Goldman-Rakic (1999) proposed that loss of neuropil, not loss of neurons, accounts for the cortical atrophy and ventricular enlargement in schizophrenia (1999). An in vivo MRI study revealed a significant 4% loss of cortical volume and a 23% increase of ventricular volume in adult SR $-/-$ mice, values similar to what has been reported in schizophrenia (Puhl et al., 2015). This loss of cortical gray matter in male SR $-/-$ mice reflected a wide-spread loss of dendritic complexity and spine density on pyramidal neurons in frontal cortex (DeVito et al., 2011), in the primary sensory cortex (Balu, Basu, Corradi, Cacace, & Coyle, 2012) and on the granule cells in the dentate gyrus of the hippocampus (Balu et al., 2013). In aggregate, the total reduction of cortical glutamatergic synapses (spine density $\times$ total dendritic length) is in the order of 30%, similar to schizophrenia.

Steullet et al. (2017) have summarized the evidence that an important pathologic feature of schizophrenia is the downregulation of the presynaptic markers for the cortical fast-firing PV + GABAergic neurons, which provide recurrent inhibition to the pyramidal neurons. They mounted evidence that these deficits result from oxidative stress, which damages the perineuronal net. Examination of these parameters in the SR $-/-$ mice revealed a 26% reduction in the number of PV + neurons and a 35% reduction of PV + neurons surrounded by perineuronal nets. These changes were accompanied by a fourfold increase in 8-oxo-2'-deoxyguanosine, a marker of oxidative stress. Notably, these pathologic alterations were present in the 8-month-old SR $-/-$ mice but not 2- to 3-month-old SR $-/-$ mice (Benneyworth, Roseman, Basu, & Coyle, 2011), consistent with the evidence that the pathology of schizophrenia evolves over the years from first episode to chronic state (Krystal & Anticevic, 2015).

NMDARs play a fundamental role in the functional neuroplastic response, long-term potentiation (LTP), which has been considered the molecular substrate of learning and memory (Malenka & Nicoll, 1993). The SR $-/-$ male mouse exhibited substantial impairments of LTP (-70% or more) at CA3–CA1 synapse in the hippocampus (Basu et al., 2009), at the thalamic synapse in the lateral nucleus of the amygdala (Li et al., 2013), and at the perforant path-dentate gyrus synapse (Balu et al., 2013). Notably, the deficit could be overcome by high-frequency stimulation, consistent with recruitment of glycine from astrocytes (Li et al., 2013), by perfusion with exogenous D-serine (Basu et al., 2009), or by treatment with D-serine to restore brain levels prior to sacrifice (Balu et al., 2013). Frantseva et al. (2008) used trans-cranial magnetic stimulation over the contralateral motor cortex to demonstrate impaired motor evoked potentials, which may represent LTP, and this deficit correlated with impaired motor-skilled learning. Postmortem studies of the neurochemistry and gene expression in schizophrenia have revealed alterations consistent with NMDAR

hypofunction, such as reduced BDNF/Akt/mammalian target of rapamycin (mTOR) signaling in the hippocampus, which is replicated in the male SR $-/-$ mouse (Balu et al., 2013).

The decision to choose knocking-out SR as a strategy to impair NMDAR function proved to be propitious. The largest genome-wide association study (GWAS) of risk genes for schizophrenia including over 100,000 controls and nearly 40,000 subjects with schizophrenia yielded 108 sites on the genome that achieved GWAS significance of $5 - 10^{-8}$, supporting the evidence that schizophrenia is a disorder of complex genetics with multiple risk alleles of modest effect interacting with the environment to cause the phenotype (Ripke, Neale, Corvin, et al., 2014). Notably, one of these sites contained the *SRR* gene; and the allelic variant in a noncoding region (as were the vast majority of the SNPs) reduced SR expression (Birnbaum, Zhang, Hyde, Kleinman, Weinberger, 2014). Furthermore, over a dozen other sites are associated with genes encoding proteins that are within 2 degrees of separation from the NMDAR, including *GRIN2A*, *GRIA1*, *Arc*, and *GRM3* (Balu & Coyle, 2015).

An important strategy for validating the hypothesized reduced NMDAR function in schizophrenia is to demonstrate that enhancing NMDAR function reduces core symptoms of the disorder. While there were no drugs developed to enhance NMDAR function, DCS, a drug developed to treat tuberculosis by inhibiting alanine racemase and D-alanine: D-alanine ligase, is also a partial agonist at the GMS on the NMDAR (Anon, 2008; Watson, Bolanowski, Baganoff, Deppeler, & Lanthorn, 1990). DCS was subsequently shown to have cognitive enhancing effects in animal models of dementia (Flood, Morley, & Lanthorn, 1992; Schuster & Schmidt, 1992). A double-blind dose finding study of DCS in patients with schizophrenia, who were on stable doses of antipsychotic drugs, was carried out to determine its effects on cognition and negative symptoms (Goff et al., 1995). The patients were maintained on antipsychotic drugs because the outcome was uncertain. Thus, there were ethical concerns about terminating an effective treatment and because negative and cognitive symptoms were unaffected by antipsychotic drugs. DCS exhibited a U-shaped dose response curve with decreased negative symptoms and improved cognition at a dose of 50 mg/day (Goff et al., 1995).

A subsequent 8-week, placebo-controlled clinical trial of 50 mg/day of DCS failed to demonstrate an effect on cognition, although negative symptoms were reduced by a significant 23% (Goff et al., 1999). In retrospect, such loss of DCS efficacy on cognition with chronic treatment was not surprising as it has been well documented in experimental animals (Parnas, Weber, & Richardson, 2005; Quartermain, Mower, Rafferty, Herting, & Lanthorn, 1994). The problem of NMDAR desensitization prompted Goff to carry-out a clinical trial with once a week treatment with DCS in patients with schizophrenia, who were stabilized on antipsychotic drugs (Goff et al., 2008). He replicated the effects of DCS on negative symptoms, but found that a cognitive task improved only after the first dose. Yurgelun-Todd et al. (2005) found that 8-week treatment of schizophrenic subjects stabilized on antipsychotic drugs with 50 mg/day of DCS also exhibited a significant reduction of negative symptoms that correlated significantly with increased activation of the left superior temporal gyrus when performing a memory task.

Javitt and his colleagues have carried out a series of clinical trials of glycine, a full agonist at the GMS on the NMDAR, which poorly crosses the blood–brain barrier and thus requires substantial doses (30–60 g/day) for efficacy (Javitt, 2006). Tsai has carried out clinical trials with D-serine, D-alanine, and sarcosine, which is an inhibitor of the glycine transporter, glycine transporter 1 (GlyT1), and a GMS agonist, and summarized the findings from his studies and those of others in a metaanalysis (Tsai & Lin, 2010). Their findings indicate that GMS agonists significantly reduce negative symptoms, positive symptoms, and improve cognition in patients with schizophrenia receiving antipsychotic drugs with effect sizes of 0.38–0.26. Finally, they have been studying the efficacy of sodium benzoate, an inhibitor of DAAO. The addition of 1 g per day of sodium benzoate to a stable regimen of antipsychotic drugs resulted in significant reduction in negative symptoms and improvement processing speed and visual learning (Lane et al., 2013). This finding strongly supports the primacy of D-serine as the GMS agonist critical to the core symptoms of schizophrenia.

3. ADDICTIONS

The cooccurrence of substance abuse and psychotic disorders, especially schizophrenia, presents serious challenges with treatment noncompliance, violence, confinement within the legal justice system, and poor clinical outcomes (Green, Canuso, Brenner, & Wojcik, 2003). The risk for substance abuse in individuals diagnosed with schizophrenia is three- to fourfold greater than in the general population, excluding nicotine dependence (Kavanagh, McGrath, Saunders, Dore, & Clark, 2002). In the case of nicotine dependence, up to 85% of individuals with schizophrenia have nicotine dependence, especially those with negative symptoms and chronicity (Cantor-Graae, Nordström, & McNeil, 2001). After nicotine, ethanol, cannabis, and stimulants appear to be the most commonly abused substances. In circumstances where cocaine is readily available, abuse can be high (Cantor-Graae et al., 2001). As recent research has revealed the central role of glutamatergic neurotransmission (for review, Carlezon & Thomas, 2009), especially mediated by NMDARs, in substance abuse and dependence, Coyle (2006) hypothesized that the high prevalence of comorbid substance abuse in schizophrenia result from share molecular mechanisms and not merely adverse social circumstances.

Substance abuse and dependence involve alterations in motivation, loss of behavioral inhibition, craving in the absence of withdrawal, and altered responsiveness to stressors and drug-associated stimuli (Carlezon & Thomas, 2009). Repeated administration of most drugs of abuse produces a state of behavioral sensitization that can be manifested in numerous behavioral tests (Carlezon & Nestler, 2002). Several studies now show that extinction of drug-mediated conditioned place preference (CPP) is NMDAR-dependent and is enhanced with treatment with DCS (Paolone, Botreau, & Stewart, 2009; Thanos, Bermeo, Wang, & Volkow, 2010). DCS also facilitates the extinction of morphine withdrawal-induced conditioned place aversion (Myers & Carlezon, 2010), suggesting that NMDAR function has a broad influence on how drug-related cues contribute to the development and maintenance of addictive behaviors. Interestingly, DCS can either weaken (by facilitating extinction) or strengthen (by facilitating reconsolidation) drug-related cues, depending on the timing of treatment (Myers & Carlezon, 2012).

In this context, Benneyworth and Coyle (2012) studied behavioral sensitization to D-amphetamine in mice with genetically altered GMS activation, the SR $-/-$ mouse, and the GlyT1 hypomorph mouse that exhibits increased NMDAR GMS activation (Tsai et al., 2004). Repeated psychostimulant treatment results in behavioral sensitization as measured as increased efficacy of the drug to stimulate locomotor activity (Vezina & Leyton, 2009). The acquisition of sensitization is facilitated by associative learning that links a specific context to drug exposure (Badiani, Anagnostaras, & Robinson, 1995). SR $-/-$ mice showed reduced expression of context-specific sensitization and conditioned hyperactivity. Nevertheless, this attenuated conditioned hyperactivity was completely resistant to extinction. Furthermore, while extinction in wild type (WT) mice with repeated context exposure reversed the increased spine density in the nucleus accumbens (NAc), the increased NAc spine density in SR $-/-$ mice was unaffected by this extinction protocol. The GlyT1 $+/-$ mice with enhanced GMS exhibited a more rapid acquisition of sensitization with amphetamine, but no apparent alteration in the extinction of conditioned hyperactivity. This finding is consistent with the report of Curcio et al. (2013), who found that administration of D-serine into the NAc enhanced behavioral sensitization to cocaine. Thus, based on the results with SR $-/-$ mice, it would appear that one possible explanation for the increased prevalence of substance abuse in schizophrenia may be impaired extinction that interferes with remaining abstinent.

CPP may be a homolog of drug craving in experimental animals. Recent studies indicate that enhancement of NMDAR GMS activity by treatment with DCS (Paolone et al., 2009; Thanos et al., 2010) or D-serine (Hammond, Seymour, Burger, & Wagner, 2013) accelerates extinction of cocaine-induced CPP and of cocaine self-administration. Accordingly, we studied CPP in the SR $-/-$ and GlyT1 $+/-$ mice. Compared with WT mice, GlyT1 $+/-$ mice displayed more rapid extinction of CPP and more robust cocaine-induced reinstatement than in WT mice (Puhl et al., 2015). SR $-/-$ appeared to immediately “forget” the learned cocaine-cued place preference because they did not exhibit cocaine-induced reinstatement and also exhibited attenuated locomotor sensitization, as also was observed with D-amphetamine (Benneyworth & Coyle, 2012). The lack of reinstatement in SR $-/-$ mice is consistent with critical role played by NMDARs in this process (Ma, Cepeda, & Cui, 2009). Interestingly, treatment of SR $-/-$ mice with 300 mg/kg/day of D-serine, a dose that normalizes cortical D-serine levels (Balu et al., 2013), caused SR $-/-$ mice to avoid the cocaine-paired side of the chamber during extinction. This counterintuitive behavior may reflect increased drug seeking after the cocaine reward was not obtained on the side previously rewarded with cocaine.

In ongoing studies, intracranial self-stimulation (ICSS) has been used to assess the hedonic properties of cocaine. In this test, rodents self-administer rewarding electrical stimuli to the medial forebrain bundle, which contains the NAc dopaminergic afferents (Markou & Koob, 1991). Drugs of abuse reduce the threshold for ICSS responding, suggesting that the rewarding effects of the drugs have additive effects with the rewarding effects of the stimulation. On the other hand, treatments that produce dysphoria (e.g., withdrawal and stress) increase thresholds for ICSS responding, suggesting that aversion can detract from reward in this test (Carlezon & Chartoff, 2007). Consistent with prior results, after stable self-stimulation thresholds were established, the addition of cocaine decreased the threshold

in a dose-dependent manner in WT mice. In contrast, at the doses found to be effective in WT mice, cocaine had no effect on self-stimulation thresholds in SR $-/-$ mice (Puhl et al., in press). Thus, the SR $-/-$ mice exhibit profound anhedonia and require much higher doses of cocaine to achieve a hedonic response.

In summary, the role of the NMDARs in substance abuse behaviors observed with the SR $-/-$ and GlyT1 $+/-$ mice are consistent with the results of prior studies in which NMDAR function was manipulated pharmacologically (Ma et al., 2009). Given how closely the SR $-/-$ mouse mimics the neuropathology and cognitive deficits of schizophrenia (Balu et al., 2013), these findings suggest that the very high prevalence of substance abuse in schizophrenia results from their lower hedonic responses to abused substances including nicotine, stimulants, opiates, and alcohol so that they require much higher doses to achieve a pleasurable response comparable to controls. As a consequence, they become much more physically dependent on the addictive substance and thus suffer substantial withdrawal symptoms if they cease taking the drug. The results also suggest that pharmacologic interventions to normalize NMDAR function might not only diminish cognitive and negative symptoms, but also reduce the risk for substance abuse in schizophrenia.

4. ANXIETY DISORDERS

Anxiety disorders include several disparate conditions including generalized anxiety disorder, panic disorder, and posttraumatic stress disorder. Michael Davis and colleagues have played a major role in implicating NMDAR function in anxiety disorders by studying the acquisition and extinction of conditioned fear using a Pavlovian strategy (Pavlov, 1927; for review, Davis, 2011). Classical fear conditioning occurs when an affectively neutral stimulus (unconditioned stimulus; UC) is coupled with a noxious aversive stimulus (conditioned stimulus; CS) such as a foot-shock. The fear-eliciting effects of the CS, often measured in rodents as the duration of “freezing” can be extinguished by repeatedly presenting the CS without the accompanying UC. Extinction does not result from “unlearning,” but rather the learning of a new association (Falls & Davis, 1995). The amygdala is the brain structure that sits at the epicenter of fear conditioning as demonstrated by lesions of the amygdala (Falls & Davis, 1995), direct injection of NMDAR antagonists (Falls, Miserendino, & Davis, 1992), and functional brain imaging (Fredrikson & Faria, 2013).

However, the relative contribution of glycine or D-serine to GMS occupancy at critical NMDARs in the amygdala mediating LTP coupled to the CS–UC association remained ill-defined. Experiments using SR $-/-$ mice and in vitro enzymatic degradation of D-serine with DAAO demonstrated the importance of this amino acid for the NMDAR-mediated component of excitatory postsynaptic currents (EPSCs) at thalamo-LA (lateral nucleus of the amygdala) synapses and NMDAR-dependent thalamo-BLA LTP (Li et al., 2013). Specifically, tonic activation of NMDARs at synapses in the amygdala under low-activity conditions is supported by ambient D-serine as demonstrated by its elimination with perfusion of the slice with DAAO. In contrast, strong afferent stimulation was insensitive to DAAO, but was abolished by perfusion with glycine oxidase. Consistent with these in vitro slice findings, SR $-/-$ mice displayed impairments in fear memory retrieval when reexposed

to the conditioning context 24 h after trace-fear conditioning (Balu et al., 2013, 2016; Basu, Puhl, & Coyle, 2016). That this impairment was specifically related to loss of D-serine was demonstrated by the fact that treatment of SR $-/-$ with 150 mg/kg D-serine for 3 weeks restored contextual-related freezing to WT levels.

Preclinical animal studies using Pavlovian fear conditioning have been used to test ability of drugs to facilitate extinction learning. For example, intraamygdalar infusion of DCS facilitated the extinction of fear conditioning in a dose-dependent way that was prevented by coinjection of the GMS antagonist, HA-966 (Walker, Ressler, Lu, & Davis, 2002). Enhancement of NMDAR function with DCS appears to act specifically on the consolidation of the memory (Richardson, Ledgerwood, & Cranney, 2004).

Demonstrating the translational potential of these animal studies, Ressler and colleagues pioneered the use of DCS to augment exposure therapy to treat anxiety disorders building upon the animal studies with extinction in fear conditioning paradigms (Morrison & Ressler, 2014). His first demonstration of the efficacy of DCS with exposure therapy cleverly exploited virtual glass Hyatt elevators in patients with acrophobia (Ressler et al., 2004). Coupling exposure therapy with DCS resulted in those acrophobics being able to tolerate significantly higher virtual floors as compared to those receiving placebo and exposure. Importantly, this reduction in anxiety persisted for 3 months. In their metaanalysis, Norberg, Krystal, and Tolin (2008) found that DCS significantly facilitated fear extinction and exposure therapy for anxiety disorders, that the effects generally persisted, that desensitization to the effects of DCS appeared with chronic treatment, and that DCS administration was most effective when closely timed to the intervention. A more recent metaanalysis by Mataix-Cols et al. (2017) found that DCS treatment was associated with a small augmentation effect on exposure-based cognitive behavior therapy for anxiety, obsessive-compulsive, and posttraumatic stress disorders, an effect that was not moderated by the concurrent use of antidepressants.

5. BRAIN DAMAGE

Excessive activation of glutamate ionotropic receptors, especially NMDARs, has long been known to cause oxidative stress, thereby compromising neuronal function and ultimately causing neuronal death (Coyle & Puttfarcken, 1993). The GMS on the NMDAR is thus a target for neuroprotection, as GMS antagonists prevent NMDAR-mediated excitotoxic neuronal degeneration (Wu, Lee, & Schwarcz, 2000). However, the relative roles of D-serine or glycine as the endogenous coagonist-mediating excitotoxic neuronal degeneration were unexplored. Mustafa et al. (2010) used SR $-/-$ mice to define the role of D-serine in excitotoxicity in vitro and in vivo. As reviewed by Jaffrey and Snyder (1995), nitric oxide appears to be the proximate mediator of excitotoxic neuronal degeneration in stroke and ischemia. Consistent with this, cerebral cortex cultures from SR $-/-$ mice produce ~60% less NO than do those from litter mate WT mice, although there is no difference in the expression of neuronal nitric oxide synthase. When subject to glucose-oxygen deprivation, an in vitro model of ischemia, the SR $-/-$ culture exhibited a ~50% reduction in neuronal degeneration. Similarly, after middle cerebral artery occlusion, the SR $-/-$ adult mice had a ~50% reduction in infarct volume.

Traumatic brain injury results in neuronal dysfunction and even excitotoxic neuronal degeneration. The Liebl laboratory has been studying a model of closed head injury that results in compromised hippocampal neuronal function and cognitive impairment, without neuronal degeneration (Perez et al., 2016). After controlled cortical impact (CCI), hippocampal neurons downregulated their expression of SR and D-serine levels, whereas the reactive astrocytes that propagated in the damaged hippocampus after CCI expressed SR and synthesized D-serine (Perez et al., 2017). Notably, Western blots showed little change in the overall expression of SR in the hippocampus, demonstrating the importance of documenting cellular localization of SR immunohistochemically. Previous studies have demonstrated that cultured astrocytes, which assume the features of reactive astrocytes, express SR (Zhuang et al., 2010). The critical role that astrocytic D-serine release plays in impairing synaptic plasticity and cognition after CCI was established by showing that mice with SR selectively eliminated in glial fibrillary associated protein (GFAP)-containing astrocytes were protected from the deleterious effects of CCI. Thus, the tonic release of D-serine from postinjury proliferating reactive astrocytes, which would preferentially act at extrasynaptic NMDARs known to mediate excitotoxicity, may be largely responsible for the symptoms of concussive closed head injury (Perez et al., 2017). Notably, cultured astrocytes exhibit characteristics of A1 reactive astrocytes that have neurotoxic as opposed to A2 neurotrophic astrocytes (Liddelow et al., 2017) effects and express SR (S. Li, T. Anderson, J.T. Coyle, et al., unpublished).

6. CONCLUSION

It appears that SR through its product, D-serine, plays a dominant role in modulating NMDAR function in forebrain. In fact, it would not be an overstatement that D-serine is the “gate-keeper” for NMDAR function in forebrain and can be linked to most pathologic processes known to involve forebrain NMDARs. That the GMS, where D-serine acts in forebrain, might be a target for pharmacologic intervention to modify NMDAR activity has been addressed by the Schwarcz laboratory (Schwarcz, Bruno, Muchowski, & Wu, 2012). Kynurenic acid (Kyna) blocks the GMS on the NMDAR, but also is a noncompetitive inhibitor at an allosteric site on the α -7-nicotinic receptor. Altering the levels of Kyna, an endogenous metabolite of tryptophan, shifts vulnerability to excitotoxic neuronal degeneration and seizures. The derivative of Kyna, 7-chlorokynurenic acid, is a potent and selective GMS antagonist (Croucher & Bradford, 1990).

Of course, strategies to manipulate the effects of D-serine in disease processes presume that the site of synthesis and release are well understood. Many publications have indicated that D-serine is a “glio-transmitter,” the release of which is coordinated by synaptic glutamate to ensure NMDAR activation (Gundersen, Storm-Mathisen, & Bergersen, 2015). As reviewed by Wolosker, Balu, and Coyle (2016), this concept arose out of a series of unforeseen artifacts: lack of specificity of SR antibodies, expression of SR in cultured “reactive” astrocytes, and high concentrations of L-serine in astrocytes causing deceptive immune cross-reactivity with D-serine antiserum in immunocytochemical studies. These artifacts were uncovered by the use of SR $-/-$ mice to verify specificity of SR antibodies (Balu, Takagi, Puhl, Benneyworth, & Coyle, 2014; Miya et al., 2008) and of L-serine blocking conditions to ensure D-serine immune-specificity (Balu et al., 2014), as well as conditional

knock-out methods that showed negligible effects on SR expression and NMDAR-dependent neurotransmission when silencing SR in astrocytes (Benneyworth & Coyle, 2012; Ishiwata, Umino, Balu, Coyle, & Nishikawa, 2015; Perez et al., 2017).

The neuronal localization of SR and D-serine raised the possibility that D-serine was a “cotransmitter” with glutamate, much like neuropeptides are exocytotically released with simple neurotransmitters like GABA and dopamine. However, immunohistochemical studies indicated that SR and D-serine are also localized to GABAergic neurons, in addition to glutamatergic neurons across the forebrain (Balu et al., 2014; Miya et al., 2008). Initially, this localization to GABAergic neurons seemed puzzling since postsynaptic GABA receptors do not possess a GMS. However, Lin, Jacobi, Anderson, and Lynch (2016) found that endogenous D-serine and SR colocalize with the PSD-95 in glutamatergic synapses on both excitatory and inhibitory neurons, but not with the presynaptic vesicular glutamate transporter-1, VGluT1. Thus, D-serine appears to function in an autocrine fashion, being released from the postsynaptic neuron, thereby ensuring that the postsynaptic NMDARs’ GMS are occupied with D-serine to respond immediately to released glutamate (Fig. 1).

Our 20-year pursuit of the hypothesis that NMDAR hypofunction is a fundamental pathophysiologic mechanism in schizophrenia (Coyle, 1996) has led to many surprises and counter-intuitive discoveries. But the route was laid out by the lessons of my mentor, Sol Snyder: try to ask important questions, do good basic science, keep your eye on clinical relevance, and follow the evidence.

Acknowledgments

Some of the research described in this review was supported by NIH research grants including R01MH05190 and 1R21DA035427 and K99MH099252 and R00MH099252 to D.T.B.

ABBREVIATIONS

Arc	activity-regulated cytoskeleton-associated protein
BDNF	brain-derived neurotrophic factor
CCI	controlled cortical impact
CPP	conditioned place preference
CS	conditioned stimulus
DAAO	D-amino acid oxidase
DCS	D-cycloserine
FOLH1	gene encoding folate hydrolase 1
GABAergic	T-amino-butyric acidergic
GCPII	glutamate carboxypeptidase II
GlyT1	glycine transporter 1

GMS	glycine modulatory site
GRIA1	gene for glutamate ionotropic receptor AMPA type subunit 1
GRIN2A	gene for glutamate ionotropic receptor NMDA type subunit 2A
GRM3	gene for glutamate metabotropic receptor 3
GWAS	genome-wide association study
ICSS	intracranial self-stimulation
Kyna	kynurenic acid
LTP	long-term potentiation
mTOR	mammalian target of rapamycin
NAAG	<i>N</i> -acetyl-L-aspartyl-L-glutamate
NAc	nucleus accumbens
NAALADase	<i>N</i> -acetyl-L-aspartyl-L-glutamate peptidase
NMDAR	<i>N</i> -methyl-D-aspartate receptor
PSD-95	postsynaptic density with mass of 95 Da
SR	serine racemase
UC	unconditioned stimulus
VGluT1	vesicular glutamate transporter-1
WT	wild type

References

- Anis NA, Berry SC, Burton NR, Lodge D. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by *N*-methyl-aspartate. *British Journal of Pharmacology*. 1983; 79(2):565–575. [PubMed: 6317114]
- Cycloserine. Tuberculosis (Edinburgh, Scotland). 2008; 88(2):100–101.
- Badiani A, Anagnostaras SG, Robinson TE. The development of sensitization to the psychomotor stimulant effects of amphetamine is enhanced in a novel environment. *Psychopharmacology (Berlin)*. 1995; 117(4):443–452. [PubMed: 7604146]
- Bakshi VP, Geyer MA. Antagonism of phencyclidine-induced deficits in prepulse inhibition by the putative atypical antipsychotic olanzapine. *Psychopharmacology*. 1995; 122(2):198–201. [PubMed: 8848537]
- Balu DT, Basu AC, Corradi JP, Cacace AM, Coyle JT. The NMDA receptor co-agonists, D-serine and glycine, regulate neuronal dendritic architecture in the somatosensory cortex. *Neurobiology of Disease*. 2012; 45(2):671–682. [PubMed: 22024716]
- 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]
- Balu DT, Li Y, Puhl MD, Benneyworth MA, Basu AC, Takagi S, et al. Multiple risk pathways for schizophrenia converge in serine racemase knockout mice, a mouse model of NMDA receptor

- hypofunction. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110(26):E2400–E2409. <https://doi.org/10.1073/pnas.1304308110>. [PubMed: 23729812]
- Balu DT, Li Y, Takagi S, Presti KT, Ramikie TS, Rook JM, et al. An mGlu5-positive allosteric modulator rescues the neuroplasticity deficits in a genetic model of NMDA receptor hypofunction in schizophrenia. *Neuropsychopharmacology*. 2016; 41(8):2052–2061. [PubMed: 26741285]
- Balu DT, Takagi S, Puhl MD, Benneyworth MA, Coyle JT. D-serine and serine racemase are localized to neurons in the adult mouse and human forebrain. *Cellular and Molecular Neurobiology*. 2014; 34(3):419–435. [PubMed: 24436034]
- Basu AC, Puhl MD, Coyle JT. Endogenous co-agonists of the NMDA receptor modulate contextual fear in trace conditioning. *Neurobiology of Learning and Memory*. 2016; 136:244–250. [PubMed: 27633914]
- Basu AC, Tsai GE, Ma CL, Ehmsen JT, Mustafa AK, Han L, et al. Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. *Molecular Psychiatry*. 2009; 14(7):719–727. [PubMed: 19065142]
- Benneyworth MA, Coyle JT. Altered acquisition and extinction of amphetamine-paired context conditioning in genetic mouse models of altered NMDA receptor function. *Neuropsychopharmacology*. 2012; 37(11):2496–2504. [PubMed: 22763616]
- Benneyworth MA, Roseman AS, Basu AC, Coyle JT. Failure of NMDA receptor hypofunction to induce a pathological reduction in PV-positive GABAergic cell markers. *Neuroscience Letters*. 2011; 488(3):267–271. [PubMed: 21094213]
- Bergeron R, Coyle JT. NAAG, NMDA receptor and psychosis. *Current Medicinal Chemistry*. 2012; 19(9):1360–1364. [PubMed: 22304714]
- Bergeron R, Coyle JT, Tsai G, Greene RW. NAAG reduces NMDA receptor current in CA1 hippocampal pyramidal neurons of acute slices and dissociated neurons. *Neuropsychopharmacology*. 2005; 30(1):7–16. [PubMed: 15354184]
- Birnbaum R, Zhang F, Hyde TM, Kleinman JE, Weinberger DR. Serine racemase (SRR) and schizophrenia risk: Functional genomic characterization of SCZ GWAS risk SNP rs4523957 by RNA sequencing. *American College of Neuropsychopharmacology*. 2014 (Abstracts).
- Bredt DS, Snyder SH. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96(22):12363–12368. [PubMed: 10555111]
- Cantor-Graae E, Nordström LG, McNeil TF. Substance abuse in schizophrenia: A review of the literature and a study of correlates in Sweden. *Schizophrenia Research*. 2001; 48(1):69–82. [PubMed: 11278155]
- Carlezon WA Jr, Chartoff EH. Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. *Nature Protocols*. 2007; 2(11):2987–2995. [PubMed: 18007634]
- Carlezon WA Jr, Nestler EJ. Elevated levels of GluR1 in the midbrain: A trigger for sensitization to drugs of abuse? *Trends in Neurosciences*. 2002; 25(12):610–615. [PubMed: 12446127]
- Carlezon WA Jr, Thomas MJ. Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis. *Neuropharmacology*. 2009; 56(Suppl 1):122–132. [PubMed: 18675281]
- Coyle JT. The glutamatergic dysfunction hypothesis for schizophrenia. *Harvard Review of Psychiatry*. 1996; 3(5):241–253. [PubMed: 9384954]
- Coyle JT. Substance use disorders and Schizophrenia: A question of shared glutamatergic mechanisms. *Neurotoxicity Research*. 2006; 10(3–4):221–233. [PubMed: 17197372]
- Coyle JT, Basu A, Benneyworth M, Balu D, Konopaske G. Glutamatergic synaptic dysregulation in schizophrenia: Therapeutic implications. *Handbook of Experimental Pharmacology*. 2012; 213:267–295.
- Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science*. 1993; 262(5134):689–695. [PubMed: 7901908]
- Croucher MJ, Bradford HF. 7-Chlorokynurenic acid, a strychnine-insensitive glycine receptor antagonist, inhibits limbic seizure kindling. *Neuroscience Letters*. 1990; 118(1):29–32. [PubMed: 2175409]

- Curcio L, Podda MV, Leone L, Piacentini R, Mastrodonato A, Cappelletti P, et al. Reduced D-serine levels in the nucleus accumbens of cocaine-treated rats hinder the induction of NMDA receptor-dependent synaptic plasticity. *Brain*. 2013; 136:1216–1230. [PubMed: 23518710]
- Davis M. NMDA receptors and fear extinction: Implications for cognitive behavioral therapy. *Dialogues in Clinical Neuroscience*. 2011; 13(4):463–474. [PubMed: 22275851]
- Davis KL, Buchsbaum MS, Shihabuddin L, Spiegel-Cohen J, Metzger M, Frecska E, et al. Ventricular enlargement in poor-outcome schizophrenia. *Biological Psychiatry*. 1998; 43(11):783–793. [PubMed: 9611667]
- DeVito LM, Balu DT, Kanter BR, Lykken C, Basu AC, Coyle JT, et al. Serine racemase deletion disrupts memory for order and alters cortical dendritic morphology. *Genes, Brain, and Behavior*. 2011; 10(2):210–222.
- Erlenmeyer-Kimling L, Rock D, Roberts SA, Janal M, Kestenbaum C, Cornblatt B, et al. Attention, memory, and motor skills as childhood predictors of schizophrenia-related psychoses: The New York High-Risk Project. *The American Journal of Psychiatry*. 2000; 157(9):1416–1422. [PubMed: 10964857]
- Falls WA, Davis M. Lesions of the central nucleus of the amygdala block conditioned excitation, but not conditioned inhibition of fear as measured with the fear-potentiated startle effect. *Behavioral Neuroscience*. 1995; 109(3):379–387. [PubMed: 7662148]
- Falls WA, Miserendino MJ, Davis M. Extinction of fear-potentiated startle: Blockade by infusion of an NMDA antagonist into the amygdala. *Journal of Neuroscience*. 1992; 12(3):854–863. [PubMed: 1347562]
- Farber NB, Wozniak DF, Price MT, Labruyere J, Huss J, St Peter H, et al. Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: Potential relevance to schizophrenia? *Biological Psychiatry*. 1995; 38(12):788–796. [PubMed: 8750036]
- Flood JF, Morley JE, Lanthorn TH. Effect on memory processing by D-cycloserine, an agonist of the NMDA/glycine receptor. *European Journal of Pharmacology*. 1992; 221(2–3):249–254. [PubMed: 1330624]
- Frantseva MV, Fitzgerald PB, Chen R, Möller B, Daigle M, Daskalakis ZJ. Evidence for impaired long-term potentiation in schizophrenia and its relationship to motor skill learning. *Cerebral Cortex*. 2008; 18(5):990–996. [PubMed: 17855721]
- Fredrikson M, Faria V. Neuroimaging in anxiety disorders. *Modern Trends in Pharmacopsychiatry*. 2013; 29:47–66. [PubMed: 25225017]
- Genovart N, Kapur S. Role of dopamine D(2) receptors for antipsychotic activity. *Handbook of Experimental Pharmacology*. 2012; 212:27–52.
- Goff DC, Cather C, Gottlieb JD, Evins AE, Walsh J, Raeke L, et al. Once-weekly D-cycloserine effects on negative symptoms and cognition in schizophrenia: An exploratory study. *Schizophrenia Research*. 2008; 106(2–3):320–327. [PubMed: 18799288]
- Goff DC, Tsai G, Levitt J, Amico E, Manoach D, Schoenfeld DA, et al. A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. *Archives of General Psychiatry*. 1999; 56(1):21–27. [PubMed: 9892252]
- Goff DC, Tsai G, Manoach DS, Coyle JT. Dose-finding trial of D-cycloserine added to neuroleptics for negative symptoms in schizophrenia. *The American Journal of Psychiatry*. 1995; 152(8):1213–1215. [PubMed: 7625475]
- Green AI, Canuso CM, Brenner MJ, Wojcik JD. Detection and management of comorbidity in patients with schizophrenia. *The Psychiatric Clinics of North America*. 2003; 26(1):115–139. [PubMed: 12683263]
- Gundersen V, Storm-Mathisen J, Bergersen LH. Neuroglial transmission. *Physiological Reviews*. 2015; 95(3):695–726. [PubMed: 26084688]
- Hammond S, Seymour CM, Burger A, Wagner JJ. D-Serine facilitates the effectiveness of extinction to reduce drug-primed reinstatement of cocaine-induced conditioned place preference. *Neuropharmacology*. 2013; 64:464–471. [PubMed: 22728761]
- Hashimoto K, Engberg G, Shimizu E, Nordin C, Lindström LH, Iyo M. Reduced D-serine to total serine ratio in the cerebrospinal fluid of drug naive schizophrenic patients. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. 2005; 29(5):767–769. [PubMed: 15939521]

- Hashimoto A, Nishikawa T, Hayashi T, Fujii N, Harada K, Oka T, et al. The presence of free D-serine in rat brain. *FEBS Letters*. 1992; 296(1):33–36. [PubMed: 1730289]
- Ishiwata S, Umino A, Balu DT, Coyle JT, Nishikawa T. Neuronal serine racemase regulates extracellular D-serine levels in the adult mouse hippocampus. *Journal of Neural Transmission* (Vienna). 2015; 122(8):1099–1103.
- Jackson PF, Slusher BS. Design of NAALADase inhibitors: A novel neuro-protective strategy. *Current Medicinal Chemistry*. 2001; 8(8):949–957. [PubMed: 11375762]
- Jaffrey SR, Snyder SH. Nitric oxide: A neural messenger. *Annual Review of Cell and Developmental Biology*. 1995; 11:417–440.
- Javitt DC. Is the glycine site half saturated or half unsaturated? Effects of glutamatergic drugs in schizophrenia patients. *Current Opinion in Psychiatry*. 2006; 19(2):151–157. [PubMed: 16612195]
- Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *The American Journal of Psychiatry*. 1991; 148(10):1301–1308. [PubMed: 1654746]
- Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature*. 1987; 325(6104):529–531. [PubMed: 2433595]
- Johnstone EC, Owens DG, Bydder GM, Colter N, Crow TJ, Frith CD. The spectrum of structural brain changes in schizophrenia: Age of onset as a predictor of cognitive and clinical impairments and their cerebral correlates. *Psychological Medicine*. 1989; 19(1):91–103. [PubMed: 2727213]
- Kavanagh DJ, McGrath J, Saunders JB, Dore G, Clark D. Substance misuse in patients with schizophrenia: Epidemiology and management. *Drugs*. 2002; 62(5):743–755. [PubMed: 11929329]
- Kleckner NW, Dingledine R. Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes. *Science*. 1988; 241(4867):835–837. [PubMed: 2841759]
- Krystal JH, Anticevic A. Toward illness phase-specific pharmacotherapy for schizophrenia. *Biological Psychiatry*. 2015; 78(11):738–740. [PubMed: 26542740]
- Lane HY, Lin CH, Green MF, Helleman G, Huang CC, Chen PW, et al. Add-on treatment of benzoate for schizophrenia: a randomized, double-blind, placebo-controlled trial of D-amino acid oxidase inhibitor. *JAMA Psychiatry*. 2013; 70(12):1267–1275. [PubMed: 24089054]
- Lewis DA. Inhibitory neurons in human cortical circuits: Substrate for cognitive dysfunction in schizophrenia. *Current Opinion in Neurobiology*. 2014; 26:22–26. [PubMed: 24650500]
- Li Y, Sacchi S, Pollegioni L, Basu AC, Coyle JT, Bolshakov VY. Identity of endogenous NMDAR glycine site agonist in amygdala is determined by synaptic activity level. *Nature Communications*. 2013; 4:1760. <https://doi.org/10.1038/ncomms2779>.
- Liddelaw SA, Gattenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017; 541(7638):481–487. [PubMed: 28099414]
- Lim J, Lee SA, Lam M, Rapisarda A, Kraus M, Keefe RS, et al. The relationship between negative symptom subdomains and cognition. *Psychological Medicine*. 2016; 46(10):2169–2177. [PubMed: 27087476]
- Lin H, Jacobi AA, Anderson SA, Lynch DR. D-Serine and serine racemase are associated with PSD-95 and glutamatergic synapse stability. *Frontiers in Cellular Neuroscience*. 2016; 10:34. [PubMed: 26941605]
- Ma YY, Cepeda C, Cui CL. The role of striatal NMDA receptors in drug addiction. *International Review of Neurobiology*. 2009; 89:131–146. [PubMed: 19900618]
- Malenka RC, Nicoll RA. NMDA-receptor-dependent synaptic plasticity: Multiple forms and mechanisms. *Trends in Neurosciences*. 1993; 16(12):521–527. [PubMed: 7509523]
- Markou A, Koob GF. Postcocaine anhedonia. An animal model of cocaine withdrawal. *Neuropsychopharmacology*. 1991; 4(1):17–26. [PubMed: 2003866]
- Mataix-Cols D, et al. D-Cycloserine augmentation of exposure-based cognitive behavior therapy for anxiety, obsessive-compulsive, and posttraumatic stress disorders: A systematic review and meta-analysis of individual participant data. *JAMA Psychiatry*. 2017; 74(5):501–510. [PubMed: 28122091]

- Meunier CN, Dallérac G, Le Roux N, Sacchi S, Levasseur G, Amar M, et al. D-Serine and glycine differentially control neurotransmission during visual cortex critical period. *PLoS One*. 2016; 11(3):e0151233. [PubMed: 27003418]
- Miya K, Inoue R, Takata Y, Abe M, Natsume R, Sakimura K, et al. Serine racemase is predominantly localized in neurons in mouse brain. *The Journal of Comparative Neurology*. 2008; 510(6):641–654. [PubMed: 18698599]
- Moghaddam B, Krystal JH. Capturing the angel in “angel dust”: Twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans. *Schizophrenia Bulletin*. 2012; 38(5):942–949. [PubMed: 22899397]
- Morrison FG, Ressler KJ. From the neurobiology of extinction to improved clinical treatments. *Depression and Anxiety*. 2014; 31(4):279–290. [PubMed: 24254958]
- Mothet JP, Parent AT, Wolosker H, Brady RO Jr, Linden DJ, Ferris CD, et al. D-serine is an endogenous ligand for the glycine site of the *N*-methyl-D-aspartate receptor. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97(9):4926–4931. [PubMed: 10781100]
- Moyer CE, Shelton MA, Sweet RA. Dendritic spine alterations in schizophrenia. *Neuroscience Letters*. 2015; 601:46–53. [PubMed: 25478958]
- Mustafa AK, Ahmad AS, Zeynalov E, Gazi SK, Sikka G, Ehmsen JT, et al. Serine racemase deletion protects against cerebral ischemia and excitotoxicity. *The Journal of Neuroscience*. 2010; 30(4):1413–1416. [PubMed: 20107067]
- Myers KM, Carlezon WA Jr. Extinction of drug- and withdrawal-paired cues in animal models: Relevance to the treatment of addiction. *Neuroscience & Biobehavioral Reviews*. 2010; 35(2):285–302. [PubMed: 20109490]
- Myers KM, Carlezon WA Jr. D-Cycloserine effects on extinction of conditioned responses to drug-related cues. *Biological Psychiatry*. 2012; 71(11):947–955. [PubMed: 22579305]
- Neims AH, Zieverink WD, Smilack JD. Distribution of D-amino acid oxidase in bovine and human nervous tissues. *Journal of Neurochemistry*. 1966; 13(3):163–168. [PubMed: 4380208]
- Norberg MM, Krystal JH, Tolin DF. A meta-analysis of D-cycloserine and the facilitation of fear extinction and exposure therapy. *Biological Psychiatry*. 2008; 63(12):1118–1126. [PubMed: 18313643]
- Paolone G, Botreau F, Stewart J. The facilitative effects of D-cycloserine on extinction of a cocaine-induced conditioned place preference can be long lasting and resistant to reinstatement. *Psychopharmacology*. 2009; 202(1–3):403–409. [PubMed: 18695929]
- Parnas AS, Weber M, Richardson R. Effects of multiple exposures to D-cycloserine on extinction of conditioned fear in rats. *Neurobiology of Learning and Memory*. 2005; 83(3):224–231. [PubMed: 15820858]
- Pavlov, IP. *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. Oxford, United Kingdom: Oxford University Press; 1927.
- Perez EJ, Cepero ML, Perez SU, Coyle JT, Sick TJ, Liebl DJ. EphB3 signaling propagates synaptic dysfunction in the traumatic injured brain. *Neurobiology of Disease*. 2016; 94:73–84. [PubMed: 27317833]
- Perez EJ, Tapanes SA, Loris ZB, Sick TJ, Coyle JT, Liebl DJ. Enhanced astrocytic D-serine underlies synaptic damage after traumatic brain injury. *The Journal of Clinical Investigation*. 2017; 127:3114–3125. [PubMed: 28714867]
- Puhl MD, Desai RI, Takagi S, Presti KT, Doyle MR, Donahue RJ, et al. N-methyl-D-aspartate receptor co-agonist availability affects behavioral and neurochemical responses to cocaine: Insights into comorbid schizophrenia and substance abuse. *Addiction Biology*. in press.
- Puhl MD, Mintzopoulos D, Jensen JE, Gillis TE, Konopaske GT, Kaufman MJ, et al. In vivo magnetic resonance studies reveal neuroanatomical and neurochemical abnormalities in the serine racemase knockout mouse model of schizophrenia. *Neurobiology of Disease*. 2015; 73:269–274. [PubMed: 25461193]
- Quartermain D, Mower J, Rafferty MF, Herting RL, Lanthorn TH. Acute but not chronic activation of the NMDA-coupled glycine receptor with D-cycloserine facilitates learning and retention. *European Journal of Pharmacology*. 1994; 257(1–2):7–12. [PubMed: 8082709]

- Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, et al. Cognitive enhancers as adjuncts to psychotherapy: Use of D-cycloserine in phobic individuals to facilitate extinction of fear. *Archives of General Psychiatry*. 2004; 61(11):1136–1144. [PubMed: 15520361]
- Richardson R, Ledgerwood L, Cranney J. Facilitation of fear extinction by D-cycloserine: Theoretical and clinical implications. *Learning & Memory*. 2004; 11(5):510–516. [PubMed: 15466301]
- Ripke S, Neale BM, Corvin A, et al. Biological insights from 108 schizophrenia-associated genetic loci. *Nature*. 2014; 511(7510):421–427. [PubMed: 25056061]
- Schell MJ, Molliver ME, Snyder SH. D-Serine, an endogenous synaptic modulator: Localization to astrocytes and glutamate-stimulated release. *Proceedings of the National Academy of Sciences of the United States of America*. 1995; 92(9):3948–3952. [PubMed: 7732010]
- Schuster GM, Schmidt WJ. D-Cycloserine reverses the working memory impairment of hippocampal lesioned rats in a spatial learning task. *European Journal of Pharmacology*. 1992; 224(1):97–98. [PubMed: 1451747]
- Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: When physiology meets pathology. *Nature Reviews. Neuroscience*. 2012; 13(7):465–477. [PubMed: 22678511]
- Selemon LD, Goldman-Rakic PS. The reduced neuropil hypothesis: A circuit based model of schizophrenia. *Biological Psychiatry*. 1999; 45(1):17–25. [PubMed: 9894571]
- Snyder SH. The dopamine hypothesis of schizophrenia: Focus on the dopamine receptor. *The American Journal of Psychiatry*. 1976; 133(2):197–202. [PubMed: 1251927]
- Steullet P, Cabungcal JH, Coyle J, Didriksen M, Gill K, Grace AA, et al. Oxidative stress-driven parvalbumin interneuron impairment as a common mechanism in models of schizophrenia. *Molecular Psychiatry*. 2017; 22(7):936–943. [PubMed: 28322275]
- Thanos PK, Bermeo C, Wang GJ, Volkow ND. D-Cycloserine facilitates extinction of cocaine self-administration in rats. *Synapse*. 2010; 65(9):938–944.
- Tsai GE, Lin PY. Strategies to enhance *N*-methyl-D-aspartate receptor-mediated neurotransmission in schizophrenia, a critical review and meta-analysis. *Current Pharmaceutical Design*. 2010; 16(5): 522–537. [PubMed: 19909229]
- Tsai G, Passani LA, Slusher BS, Carter R, Baer L, Kleinman JE, et al. Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. *Archives of General Psychiatry*. 1995; 52(10):829–836. [PubMed: 7575102]
- Tsai G, Ralph-Williams RJ, Martina M, Bergeron R, Berger-Sweeney J, Dunham KS, et al. Gene knockout of glycine transporter 1: Characterization of the behavioral phenotype. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101(22):8485–8490. [PubMed: 15159536]
- Tsai G, Yang P, Chung LC, Lange N, Coyle JT. D-serine added to anti-psychotics for the treatment of schizophrenia. *Biological Psychiatry*. 1998; 44(11):1081–1089. [PubMed: 9836012]
- Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH. Carbon monoxide: A putative neural messenger. *Science*. 1993; 259(5093):381–384. [PubMed: 7678352]
- Vezina P, Leyton M. Conditioned cues and the expression of stimulant sensitization in animals and humans. *Neuropharmacology*. 2009; 56(Suppl 1):160–168. [PubMed: 18657553]
- Walker DL, Ressler KJ, Lu KT, Davis M. Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. *The Journal of Neuroscience*. 2002; 22(6):2343–2351. [PubMed: 11896173]
- Watson GB, Bolanowski MA, Baganoff MP, Deppeler CL, Lanthorn TH. D-Cycloserine acts as a partial agonist at the glycine modulatory site of the NMDA receptor expressed in *Xenopus* oocytes. *Brain Research*. 1990; 510(1):158–160. [PubMed: 2157524]
- Wolosker H, Balu DT, Coyle JT. The rise and fall of the D-Serine-mediated gliotransmission hypothesis. *Trends in Neurosciences*. 2016; 39(11):712–721. [PubMed: 27742076]
- Wolosker H, Blackshaw S, Snyder SH. Serine racemase: A glial enzyme synthesizing D-serine to regulate glutamate-*N*-methyl-D-aspartate neurotransmission. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96(23):13409–13414. [PubMed: 10557334]

- Wolosker H, Sheth KN, Takahashi M, Mothet JP, Brady RO Jr, Ferris CD, et al. Purification of serine racemase: Biosynthesis of the neuromodulator D-serine. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96(2):721–725. [PubMed: 9892700]
- Wu HQ, Lee SC, Schwarcz R. Systemic administration of 4-chlorokynurenine prevents quinolinate neurotoxicity in the rat hippocampus. *European Journal of Pharmacology*. 2000; 390(3):267–274. [PubMed: 10708733]
- Yurgelun-Todd DA, Coyle JT, Gruber SA, Renshaw PF, Silveri MM, Amico E, et al. Functional magnetic resonance imaging studies of schizophrenic patients during word production: Effects of D-cycloserine. *Psychiatry Research*. 2005; 138(1):23–31. [PubMed: 15708298]
- Zhuang Z, Yang B, Theus MH, Sick JT, Bethea JR, Sick TJ, et al. EphrinBs regulate D-serine synthesis and release in astrocytes. *The Journal of Neuroscience*. 2010; 30(47):16015–16024. [PubMed: 21106840]

Fig. 1.

Schematic representation of D-serine pathways in health and disease. Resting astrocytes synthesize L-serine from glucose, which is then transported into neurons to be converted to D-serine by serine racemase, a process known as the “serine shuttle” (Wolosker et al., 2016). Serine racemase and D-serine are concentrated at the postsynaptic densities in glutamatergic and serine racemase-expressing GABAergic neurons (Li et al., 2013), where D-serine binds to the glycine modulatory sites on synaptic NMDA receptors, rendering them “primed” to respond to synaptic glutamate. D-serine is transported into astrocytes where it is catabolized by D-amino acid oxidase (DAAO). Inflammatory insults such as traumatic brain injury cause the proliferation of reactive or A1 “toxic” astrocytes (Liddelow et al., 2017) that express serine racemase and release D-serine, which activates extrajunctional receptors causing neurotoxicity (Perez et al., 2017).