D-Serine (DSR) is an endogenous amino acid involved in glia-synapse interactions that has unique neurotransmitter characteristics. DSR acts as obligatory coagonist at the glycine site associated with the N-methyl-D-aspartate subtype of glutamate receptors (NMDAR) and has a cardinal modulatory role in major NMDAR-dependent processes including NMDAR-mediated neurotransmission, neurotoxicity, synaptic plasticity, and cell migration. Since either over- or underfunction of NMDARs may be involved in the pathophysiology of neuropsychiatric disorders; the pharmacological manipulation of DSR signaling represents a major drug development target. A first generation of proof-of-concept animal and clinical studies suggest beneficial DSR effects in treatment-refractory schizophrenia, movement, depression, and anxiety disorders and for the improvement of cognitive performance. A related developing pharmacological strategy is the indirect modification of DSR synaptic levels by use of compounds that alter the function of main enzymes responsible for DSR production and degradation. Accumulating data indicate that, during the next decade, we will witness important advances in the understanding of DSR role that will further contribute to elucidating the causes of neuropsychiatric disorders and will be instrumental in the development of innovative treatments.

1. Introduction

Although the enzyme D-amino-acid oxidase (DAAO) has been identified in higher organisms in 1935 [1], historically, D-amino acids were thought to be absent in mammalian tissue. This dogma was revolutionized at the beginning of 1990’s when it was found that abundant quantities of free D-serine (DSR) occur in the mammalian brain, at concentrations comparable with those of classical neurotransmitters and higher than those of most essential amino acids [2, 3]. Presently, DSR is considered the most biologically active D-amino acid described in mammalian systems [4]. Phylogenetically, its concentrations appear to be extremely low in the brains of fish, amphibians, and birds, suggesting that endogenous DSR is specifically maintained at high levels in the mammalian brain among vertebrates [5].

In the late 1990s, it was demonstrated that DSR is an obligatory endogenous coagonist of the N-methyl-D-aspartate receptor (NMDAR), functioning in vivo as a specific and potent full agonist at the NMDAR-associated glycine (GLY) modulatory site (GMS). The NMDAR subtype of glutamate (GLU) receptors is widely expressed in the central nervous system (CNS) and has a cardinal role in activity-dependent changes in synaptic strength and connectivity underlying higher brain functions such as memory and learning [6, 7]. Unlike other neurotransmitter receptors, which are activated by individual neurotransmitters, NMDARs activation requires, in addition to the agonist GLU, the binding of a coagonist which was originally thought to be GLY [8, 9]. However, research over the last decade indicates that significant amounts of DSR are produced in the CNS [10–12], where DSR is converted from L-serine by serine racemase (SR) and is degraded by DAAO [11–13]. Furthermore, functional studies demonstrate that DSR represents the physiological ligand for the GMS in different brain areas including cortex and hippocampus [14–18], hypothalamus [19], and the retina [20, 21].

DSR has a cardinal modulatory role in major NMDAR-dependent processes, including NMDAR neurotransmission [22], neurotoxicity [23, 24], synaptic plasticity, [21] and cell
migration [25]. Either over- or underfunction of NMDAR neurotransmission may elicit neurotoxicity, leading to behavioral and cognitive dysfunction. NMDAR hyperactivity can cause cell death mediated by excitotoxic calcium overload [26, 27] in stroke and neurodegenerative disorders such as Alzheimer’s disease (AD) [28, 29]. By contrast, synaptic NMDAR hypoactivity leads to apoptosis [30, 31] and may contribute to the generation of psychotic symptoms and cognitive deficits. The long standing paradox that NMDARs can both promote neuronal health and kill neurons [32] highlights the importance of a strictly regulated optimal NMDAR function. Within this context, DSR modulation appears to play a critical role in the achievement of balanced NMDAR activity. Furthermore, compelling evidence suggests that dysfunctional DSR signaling may be involved in the pathophysiology of neuropsychiatric disorders.

2. D-Serine Neurobiology: An Overview

Despite notable progress in the 20 years since DSR was first identified, many aspects of DSR neurobiology remain enigmatic and are presently the focus of intense research. In contrast to classic neurotransmitters, DSR was originally shown to be exclusively produced and released from astrocytes via a vesicular release mechanism [33–35]. However, although glial DSR is prominent, DSR presence was subsequently identified in neurons as well [23]. Some studies have found DSR in most or in a subset of neurons in the cerebral cortex [36], whereas others observed DSR mainly in hindbrain neurons [37, 38]. Recent data indicate that DSR is predominantly expressed in glutamatergic neurons further challenging the notion that DSR is exclusively released from astrocytes [23, 39–41]. Furthermore, it was reported that neurons robustly release endogenous DSR [42] and that neuronal DSR release via the amino acid transporter Asc-1 enhances NMDAR potentials and long term potentiation (LTP), a cellular mechanism that underlies learning and memory [43]. The presence of DSR in neurons led to an updated DSR signaling model (rev. in [34, 44]) which incorporates the release and uptake of DSR from both neurons and astrocytes (see Figure 1). This model emphasizes DSR role in the neuron-glia cross-talk relevant to NMDAR function modulation and allows for conceivably distinct roles of glial and neuronal DSR in both physiological and disturbed NMDAR function.

NMDAR is a tetramer composed of two NR1 subunits and two NR2 subunits or less commonly two NR3 subunits. NMDAR activation requires the binding of either GLY or DSR at the GMS on the NR1 subunit [45, 46]. DSR is enriched in corticobasal regions of the brain where its localization closely parallels that of NMDARs [46] and is thought to be the primary forebrain NMDAR coagonist. SR is considered the primary endogenous source of DSR (using L-serine as a substrate), while DAAO is generally regarded as its primary mechanism of degradation. This view is however confounded by the fact that SR also degrades both DSR and L-serine irreversibly to pyruvate and ammonia, appearing thus to provide a bidirectional regulation of free serine levels in vivo [4]. Synaptic DSR is taken up into glia and neurons differentially via Asc-1 and ASCT-2 transporters and is broken down mainly by peroxisomal DAAO forming the alpha-keto acid (AKA), ammonia, and hydrogen peroxide (Figure 1).

As a physiological coagonist of NMDARs, DSR may play a role in NMDAR-dependent neurodegeneration and can mediate neurotoxicity in primary cultures and hippocampal slices [23, 24]. Selective DSR degradation by DAAO markedly reduces NMDAR neurotransmission in cortical and hippocampal preparations [22, 47]. Moreover, DSR depletion in the medial prefrontal cerebral cortex diminishes NMDAR synaptic potentials and prevents LTP inhibition [48]. Recent data suggests that DSR release from astrocytes controls NMDAR-dependent plasticity and LTP in many thousands of excitatory synapses nearby [49], while in adulthood, neuronally-derived DSR regulates neuronal dendritic architecture in the somatosensory cortex [39].

Although they are both endogenous NMDAR coagonists, GLY and DSR seem to act at distinct receptor populations, with DSR present at synaptic NMDARs and GLY at their extrasynaptic counterparts [47], which may constitute a functionally distinct pool of receptors (rev. in [32, 47]). Synaptic NMDARs are responsible for inducing the most common forms of synaptic plasticity found in the brain, namely, LTP and long-term depression (LTD). Whether specific subsets of synaptic NMDARs mediate LTP or LTD [50–54] and whether extrasynaptic receptors also play a role in these processes [55] are controversial. Extrasympathetic NMDARs contribute to neuronal synchronization [56, 57] but have mostly been implicated in neurodegenerative disorders, including stroke, AD, and Huntington’s disease [58–60]. Recent evidence suggests that synaptic NMDARs are neuroprotective, whereas extrasynaptic receptors may promote cell death [32].

Overall, the complexities involved in the neurobiology of DSR-based signaling in the human brain are expected to further unravel during the coming decade and contribute to the understanding of a novel and complex neurotransmitter system and to the development of innovative pharmacotherapy for neuropsychiatric disorders.

3. D-Serine Therapeutic Potential

NMDARs crucial role in both physiological and pathophysiological processes has generated massive clinical interest in the development of novel pharmacological interventions aiming at NMDAR-related therapeutic targets. Since direct stimulation of NMDAR with GLU or aspartate, agonists of the primary GLU receptor site, is associated with neurotoxicity [68], most of the efforts to date have focused on the GMS. The main compounds directly acting at this site that have been so far assessed in clinical trials include GLY, DSR, and D-cycloserine (DCS), a tuberculostatic antibiotic having also complex agonist/antagonist action at the GMS [69]. In this context, a number of advantages are associated with DSR use, including better blood-brain-barrier penetration and stronger affinity at the GMS versus GLY [70] and, in contrast to DCS, specific and potent full agonist action at this site. Accordingly, during the last two decades, the first proof-of-concept animal and clinical studies have been performed with
DSR in the context of neuropsychiatric disorders including schizophrenia, Parkinson’s disease, depression, and anxiety disorders.

3.1. Schizophrenia. Over the last 20 years, glutamatergic models of schizophrenia have become increasingly accepted as etiopathological models of this disorder, mainly based on the observation that the cyclohexylamine “dissociative anesthetics” phencyclidine (PCP) and ketamine induce schizophrenia-like positive and negative symptoms and cognitive deficits by blocking NMDAR neurotransmission (rev. in [66, 71]). The PCP/NMDAR model implies that treatments which aimed at potentiating NMDAR function should be therapeutically beneficial. Furthermore, pharmacological manipulation of DSR signaling represents a particularly attractive candidate strategy since convergent lines of evidence suggest an involvement of dysfunctional DSR transmission in schizophrenia [72–75]. Single polymorphisms for SR and DAAO have been linked to schizophrenia [76–78], in rodents genetic loss of DAAO activity reverses schizophrenia-like phenotypes [79, 80] and reduced DSR serum and cerebrospinal fluid (CSF) levels were documented in chronic schizophrenia patients [81–85]. Moreover, supporting the hypo-NMDAR hypothesis of schizophrenia, DSR selectively blocks PCP-induced hyperactivity and stereotypic behavior [86, 87].

A number of clinical studies [62, 65, 88–90] have demonstrated that adjuvant DSR (30–120 mg/kg/day) added to ongoing treatment with non-clozapine antipsychotics results in significant symptom improvements in chronic schizophrenia patients. The most significant changes were registered in the negative symptom cluster (Figure 2). Nevertheless, two recent meta-analytic reviews indicate that additional dysfunction domains may be affected by DSR. S. P. Singh and V. Singh [91] reported medium effect sizes of DSR for negative symptoms (standardized mean difference (SMD), −0.53) and total symptomatology (SMD, −0.40). Tsai and Lin [92] found DSR effective for negative symptoms (effect size (ES), 0.48),
cognitive symptoms (ES, 0.42), and total psychopathology (ES, 0.40).

Two schizophrenia treatment issues stemming from these findings are the potential use of DSR for improving cognition and as stand-alone pharmacotherapy in this disorder. In a preliminary four week open-label study [65], it was shown that high dose DSR (≥60 mg/kg/day) improves neurocognitive functions as measured by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) battery. An additional controlled pilot investigation [93] compared the effectiveness of DSR (3 g/day) versus high-dose olanzapine (30 mg/day) as antipsychotic monotherapy in 18 treatment-resistant schizophrenia patients. The primary LOCF analysis indicated a lack of efficacy of DSR as compared to high-dose-resistant schizophrenia patients. However, DSR was not inferior to the prestudy antipsychotic drug treatment. Furthermore, among the patients who completed the nine study weeks, high dose olanzapine and DSR did not differ in their effectiveness, suggesting that a subgroup of patients may be successfully maintained on DSR.

In all clinical trials performed to date with DSR in schizophrenia, no significant adverse events have been observed at doses of ≤4 g/day. A potential concern with DSR use is nephrotoxicity [94, 95] which has been reported in one patient receiving 120 mg/kg/day and resolved following DSR discontinuation [65]. This apparent paucity of side effects seems remarkable in view of the fact that both acute [65, 96] and chronic [62, 88, 93] administration of 1-2 g DSR results in ≥100 times increases in DSR serum levels. Nevertheless, orally administered DSR is substantially metabolized by DAAO diminishing its bioavailability and necessitating the administration of gram level doses. In view of these limitations, the ideal dosage and mode of administration of DSR remain to be determined.

3.2. Parkinsonism, Drug-Induced Dyskinesia, and Parkinson’s Disease. Idiopathic Parkinson’s disease (PD) is an age-dependent neurodegenerative disorder characterized by intertwined motor and behavioral and cognitive dysfunctions. Current pharmacological approaches to PD predominantly target the dopamine system. Although dopaminergic medications are effective, a significant number of patients show continued motor symptoms, drug-induced dyskinesia, and “on/off” phenomena, even during treatment. Furthermore, the treatment of nonmotor symptoms represents an additional major therapeutic challenge in PD. Among these manifestations, apathy and cognitive impairment respond poorly to presently available medications, pose increased management difficulties, and contribute significantly to caregiver burden. An innovative pharmacological approach for PD presently under investigation is the modulation of NMDAR-mediated glutamatergic neurotransmission (rev. in [97]).

We have hypothesized that direct or indirect augmentation of synaptic GLY or DSR levels may represent a novel type of treatment for PD [63]. This line of thought stems mainly from the clinical data obtained in schizophrenia research, indicating GLY and DSR efficacy against negative symptoms (rev. in [91, 92]). To the extent that negative symptoms of schizophrenia overlap with components of the apathy syndrome characteristic of PD (e.g., reduced motivation/initiative/volition and anhedonia) and have similar underlying etiology, that is, prefrontal dopaminergic deficit [98], the beneficial effect of DSR on negative symptoms would predict potential beneficial effects against apathy in PD. Moreover, therapeutic effects of NMDAR agonists are not confined to behavioral symptoms of schizophrenia but extend to motor symptoms as well. Some GLY and DSR schizophrenia clinical trials have included subjects that had...
significant antipsychotic drugs-induced parkinsonian [62, 64] and tardive dyskinesia [61, 62, 64, 65] symptoms. In these studies, highly significant, large effect size improvements were registered in these symptom domains (Figures 4 and 5). Thus, since GMS agonists affect motor and nonmotor clinical domains that overlap significantly with PD phenomenology, it is hypothesized that NMDAR neurotransmission modulation specifically via DSR administration may represent an innovative treatment approach in PD.

This hypothesis accords well with current theories on the role of NMDAR in modulation of brain dopaminergic systems relevant to PD [99]. NMDARs are divided into
subtypes based upon the presence of specific modulatory subunits. In adults, NMDARs are primarily of types NR2A and NR2B and research in PD has focused predominantly upon development of NR2B antagonists based upon the observation that dopaminergic denervation leads to specific upregulation of striatal NR2B versus NR2A receptors [100]. Significantly, in animal models, NR2B selective antagonists have proven more effective than nonselective drugs, such as MK-801, that target both NR2A and NR2B receptors [101], indicating that NR2A blockade, in fact, may be deleterious, but that NR2A stimulation might be beneficial. NMDAR subtypes show differing sensitivity to GMS agonists such that NR2B receptors are saturated under physiological conditions, whereas NR2A are not [102]. Thus, GMS agonists may function in vivo as selective NR2A agonists. Consequently, activation of NR2A versus NR2B receptors may restore the balance between NR2A- and NR2B-containing NMDARs similarly to the effects of NR2B antagonists.

We recently addressed the hypothesis that GMS agonists might be beneficial for motor and negative-like symptoms in PD in a 6-week controlled adjuvant treatment trial of DSR (30 mg/day) versus placebo in advanced PD patients (age, 64.3 ± 7.4 years; disease duration, 8.9 ± 5.4 years; Hoehn & Yahr staging II–IV) [67]. The ~2 g/day DSR regimen was well tolerated and resulted in significant reductions in Unified Parkinson’s Disease Rating Scale (UPDRS) and Simpson-Angus Scale for Extrapyramidal Symptoms (SAS) total scores. Five of 10 completers had >20% improvement in total UPDRS scores during DSR treatment versus 1 of 10 subjects during placebo administration ($\chi^2 = 4.07, P = 0.04$). For SAS scores, 7 subjects had >20% improvement during DSR treatment versus 2 during placebo administration ($\chi^2 = 5.3, P = 0.02$) (Figure 6). Significant benefits relative to placebo were also observed in the Positive and Negative Syndrome Scale (PANSS) and in both the motor (III) and mental (I) UPDRS subscales. In view of these novel concepts and findings, additional larger-scale studies are presently warranted to further determine whether motor and nonmotor PD components are significantly affected by GMS modulators.

3.3. Depression and Anxiety. The antidepressant potential of NMDAR antagonism has been unambiguously established during the last decade. Already in the early 1990s, preclinical studies indicated that several types of NMDAR antagonists exert antidepressant-like effects in animal models of depression [103–105]. Subsequently, a series of animal studies demonstrated that long-term antidepressant treatment produces adaptive changes in the binding profile of NMDARs [106]. The translational confirmation of these findings was achieved by the demonstration of a robust, rapid, and long-lasting antidepressant effect of ketamine in, usually treatment-resistant, unipolar or bipolar depression patients (rev. in [107, 108]). Furthermore, in addition to ketamine which acts as a noncompetitive antagonist at the intranMDAR channel PCP site, similar effects seem achievable with mechanistically diverse NMDAR antagonists. Recently, we reported that treatment with high-dose DCS, potentially having a net antagonistic effect at the GMS, also improves major depression symptomatology in treatment-resistant patients [109]. Nevertheless, an apparently contradictory body of data advocates in favor of NMDAR agonists efficacy as antidepressants. GLY and DSR adjuvant treatment results in alleviation of depressive symptoms in schizophrenia patients [91,
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92], and SSR504734, a reversible GLY-transporter inhibitor, as well as DSR, has been shown to have antidepressant/anti-anxiety effects in depression/anxiety models [110, 111]. Moreover, expression of NMDAR 1 and 2A subunit is decreased in postmortem brains of patients with major depression [112], and NMDAR binding is also reduced in suicide victims [113]. Taken together, these findings imply that NMDAR hypofunction may contribute to the pathophysiology of depression. Moreover, this hypothesis is supported by recent clinical data, although systematic investigation of antidepressant effects of NMDAR enhancement is still in an early phase. Acute administration of 2.1 g DSR to 35 healthy university students was reported to reduce, in a placebo-controlled challenge paradigm study, subjective feelings of depression and anxiety as measured by Visual Analog Scales [114]. The GLY-transporter-1 (GLYT1) inhibitor sarcosine [115] and the DDAO inhibitor sodium benzoate [116] were reported to be beneficial in depressed patients who were drug-naïve for at least 3 months and had no history of treatment-resistance.

Present explanations for the discordant observations of antidepressant effect of both NMDAR agonists and antagonists include, theoretically, common α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated mechanisms [117] and similar net effects achieved by differential action at synaptic versus extrasynaptic NMDARs [29, 32]. A stratified model of psychiatric phenomenology, as function of suboptimal/decreased versus overactive/increased NMDAR function, may also contribute to the conceptualization of available data by taking into account the vast heterogeneity underlying the overinclusive concept of depression. While schizophrenia is a typical NMDAR hypofunction disorder, responsive to treatment with NMDAR agonists, the opposite may be characteristic of treatment-resistant depression. Nevertheless, milder, nonrefractory forms of depression may represent predominately suboptimal NMDAR functioning and could be responsive to GMS agonism. Interestingly, depression feelings are improved in both schizophrenia [91, 92] and normal subjects [114] by DSR and in schizophrenia [92] and nonrefractory depression [115] by sarcosine. On the other hand, ketamine characteristically exacerbates schizophrenia manifestations [118], while it has antidepressant, mood stabilizing and procognitive effects in treatment-resistant depression [119].

Anxiety disorders represent an additional domain in which treatment aiming at augmentation of DSR synaptic levels may prove beneficial. Brain regions extensively implicated in the mediation of fear and anxiety (i.e., amygdala, hippocampus, and prefrontal cortex) are characterized by high NMDAR levels [120] and may show morphological changes as a result of stress-related disorders [121, 122]. NMDARs play a central role in stress response [123] and are critically involved in learning and memory formation which may be impaired in anxiety disorder (e.g., post-traumatic stress disorder, PTSD) [124].

Following a series of studies indicating that extinction learning is NMDAR-dependent, Davis and colleagues first demonstrated that DCS can enhance retention of fear extinction in rats and subsequently showed that DCS enhances the outcome of extinction-based therapy (i.e., virtual reality exposure therapy) for height phobia [125, 126]. These findings were replicated and are cardinal for the concept that DCS may enhance the outcomes of exposure-based cognitive behavioral therapy (CBT). Furthermore, clinical trials accumulated across a range of anxiety-related disorders including specific phobia, social phobia, panic disorders, obsessive-compulsive disorder, and PTSD confirm that single dose (25–500 mg) DCS acutely administered prior to the psychotherapeutic sessions shows promise in augmenting the effects of exposure-based therapy (rev. in [127]). However, the efficacy of DCS has been variable across studies, with several evidencing strong augmentative effects and several showing either relatively weak or no effects [128].

The potential of acute DSR administration in conjunction with CBT interventions has not yet been explored, although it may hold several advantages. DCS is a partial agonist at the GMS of NMDARs bearing the GluN2A and GluN2B subunits (previously NR2A and NR2B subunits) and a full agonist of NMDARs containing the GluN2C and GluN2D subunits [69, 129]. Furthermore, its net effect is affected by the concentration of endogenous GMS modulators (e.g., GLY, DSR, and kynurenic acid), which may be differentially altered in pathophysiological states. In contrast, DSR acts as a specific and potent full agonist at GMS and DSR-induced improvements in cognition parameters have been reported in healthy subjects [114] and schizophrenia patients [65]. Furthermore, Horio et al. [130] recently proposed that DCS may act as a prodrug for DSR in the brain. In an in vivo microdialysis study using free-moving mice, these researchers reported significantly increased extracellular DSR levels in mouse hippocampus following oral or intracerebroventricular (ICV) administration of DCS. Therefore, it was proposed that the DSR produced in the brain after DCS treatment may play at least a partial role in the therapeutic effects of DCS seen in patients with anxiety disorders [130].

While chronic use of DCS for facilitation of exposure sessions is known to lead to negative effects [131, 132], the use of DSR as continuous pharmacology unrelated to CBT interventions may prove rewarding. In long-standing PTSD, in which learning deficits may impair normal extinction of aversive memories, NMDAR agonists may hold a therapeutic potential [133–135]. Accordingly, the demonstrated efficacy of DSR against negative and cognitive symptoms of chronic treatment-resistant schizophrenia patients [91, 92] may also be of relevance to PTSD therapeutics, since PTSD impairments include cognitive dysfunction and features such as affective numbing, anhedonia, and withdrawal from social/vocational activities. Consequently, we have conducted a 6-week controlled proof-of-concept trial that examined the effects of 30 mg/kg/day DSR used as mono- or add-on pharmacotherapy with twenty-two chronic PTSD patients [136]. Compared with placebo administration, DSR treatment resulted in significantly reduced Hamilton Rating Scale for Depression (HAM-D) (P = 0.007) and Mississippi Scale for Combat-Related PTSD-civilian version (MISS-PTSD-CV) (P = 0.001) scores and a trend towards improved Clinician-Administered PTSD Scale (CAPS) total scores. These preliminary findings suggest that GMS-based
pharmacotherapy may be effective in PTSD and warrant larger-sized clinical trials with optimized DSR dosages.

Obsessive-compulsive disorder (OCD) represents an additional clinical entity for which enhancement of GMS-mediated neurotransmission via continuous pharmacological treatment is presently assessed [137]. A case report of a young adult male patient who was disabled with OCD and body dysmorphic disorder illustrates the use of high-dose GLY (0.8 mg/kg), with gradual improvement of clinical status [138]. In a placebo-controlled double-blind trial including 24 adult outpatients with OCD, who were treated with adjunctive GLY 60 g/day for 12 weeks, 14 patients completed the study and two patients in the GLY group were considered responders [139]. The Hoffmann-LaRoche GLYT1 inhibitor bitopertin is presently assessed as add-on treatment in conjunction with selective serotonin reuptake inhibitors in OCD in a Phase II multicenter study [140]. The use of DSR or indirect elevation of DSR levels in OCD has not yet been reported.

3.4. Cognitive Impairment and Dementia. Cognitive impairment is a cardinal feature of dementia and NMDAR dysfunction is hypothesized to play a cardinal role in AD which is the most common type of dementia [141]. NMDAR overactivation by GLU leads to cell death mediated by calcium overload [31, 32]. This process, known as excitotoxicity, is one of the accepted neurochemical models of AD. Furthermore, there are mutual interactions between NMDAR and Amyloid-β peptide (Aβ) which is a hallmark of AD pathogenesis [142]. Aβ increases NMDAR activity [143, 144] and induces inward Ca2+ current and neurotoxicity [145]. Reciprocally, NMDAR activation stimulates Aβ production [146-148] and Aβ associated synaptic loss may be NMDAR-dependent [149].

On the contrary, NMDAR signaling pathways in the cerebral cortex and hippocampus are impaired in the aging brain [150]. NMDAR neurotransmission is crucial to neuronal survival and NMDAR hypofunction is known to lead to apoptosis [29, 30]. Blockade of NMDAR function by gene deletion or using NMDAR antagonists (e.g., ketamine) may worsen them in the healthy human organism. Furthermore, it was recently reported that sodium benzoate administration, which hypothetically can block NMDAR overactivation by preventing excessive influx of calcium without affecting physiological NMDAR activity [166, 167]. Consistently, therapeutically relevant plasma concentrations of memantine produce only 30% NMDAR occupancy [168]. Pharmacological intervention at GMS may represent an additional therapeutic mechanism for AD. NMDAR function enhancement via GMS may avoid the excitotoxicity mediated through the GLU site. Furthermore, in mouse models, the learning deficits caused by NMDAR hypofunction in mice with point mutations in GMS can be rescued by administration of DSR [169, 170]. Supporting neurotrophic/cognitive effects, DCS can improve cognitive functions in animal studies [171, 172] and is used clinically in conjunction with CBT interventions [127]. However, cognition-enhancing effects of DCS in AD have not been conclusively demonstrated [173-175].

The potential efficacy of DSR for the treatment of cognitive impairments has not yet been assessed. Nevertheless, in preliminary investigations, DSR was shown to improve recognition and working memory parameters in mice [176] and cognitive tasks performance in healthy subjects [114]. Thus, DSR may improve cognitive parameters, while NMDAR antagonists (e.g., ketamine) may worsen them in the healthy human organism. Furthermore, it was recently reported that sodium benzoate administration, which hypothetically results in increased synaptic DSR levels is beneficial in patients with MCI or mild AD [177]. In contrast, memantine is approved for use in moderate to severe AD [178], but its efficacy in MCI or mild AD is questionable [179].

3.5. Amyotrophic Lateral Sclerosis. Recent data suggest that DSR is involved in the neurodegenerative processes associated with amyotrophic lateral sclerosis (ALS). This neurodegenerative disease targets motor neurons in the spinal cord, brain stem, and cerebral cortex, leading to death within a few years of onset [180, 181]. In ALS, DSR may mediate motoneuron cell death caused by excessive NMDAR stimulation [182]. A missense mutation that inactivates DAAO results in increased DSR levels in patients with MCI or mild AD [177]. In contrast, memantine is approved for use in moderate to severe AD [178, 182]. Thus, both anabolic and catabolic DSR-related abnormalities may lead to increased...
synaptic DSR levels and contribute to disease pathogenesis. Pharmacological interventions aiming at inhibiting DSR synthesis or release may represent an innovative treatment strategy in ALS and potentially other neurodegenerative disorders characterized by NMDAR overactivation.

4. Indirect Augmentation of DSR Function

Regulation of GMS function via pharmacological manipulation of GLYT1 and DAAO represents presently an important research and development target. Although DSR may be effective for treatment of various psychiatric symptom domains, DSR is substantially neutralized by DAAO, diminishing its oral bioavailability and necessitating the administration of high doses. Moreover, a concern with high doses may be potential nephrotoxicity, although no significant adverse events have yet been observed at DSR doses of ≤4 g/day [65, 91, 92].

High levels of DAAO expression and enzyme activity are found in the mammalian liver, kidney, and brain although the expression pattern can vary between species. Humans express DAAO in both liver and kidney, whereas mice, for example, express DAAO in the kidney but not the liver [184]. The physiological role of DAAO in the kidney and liver is detoxification of accumulated D-amino acids [185]. Collectively, the limited preclinical experience with a small number of structurally diverse DAAO inhibitors indicates that extensive inhibition of peripheral and central DAAO has a limited effect on brain or extracellular DSR concentration. Furthermore, in contrast to the fairly robust effects reported with DSR administration in animal models, the reported behavioral effects of DAAO inhibitors are fairly modest and inconsistent (rev. in [186]).

Given the moderate efficacy of DAAO inhibitors on brain DSR and behavior, several authors have investigated the effects of coadministering DAAO inhibitors in conjunction with DSR. Ferraris et al. [187] showed that the 6-chloro analog (CBIO) had quite pronounced effects on brain and plasma DSR when coadministered with 30 mg/kg DSR, relative to either CBIO or DSR administered alone. Hashimoto et al. [188] extended this finding by showing effects on cortical DSR and also demonstrated that coadministration of DSR (30 mg/kg) and CBIO reversed an MK-801-induced deficit in prepulse inhibition (PPI), whereas the 30 mg/kg dose of DSR had no effect on its own. Smith et al. [186] showed that coadministration of compound 4 in conjunction with DSR elevates CSF and cortical DSR levels to a greater extent than administration of DSR alone in male rats. Overall, these findings suggest that DAAO inhibitors could be useful clinically for reducing the dose of DSR necessary for symptom improvement. Moreover, the coadministration of DAAO inhibitors with DSR could ameliorate potential side effects associated with the administration of high DSR doses, for example, nephrotoxicity [189].

Recently, the first results of clinical research with sodium benzoate have been reported. Benzoic acid and its salts, including sodium benzoate, exist in many plants and are widely used as food preservatives [190]. Sodium benzoate also acts as a DAAO inhibitor and has favorable effects in NMDAR-based models such as pain relief [191, 192] and glial cell death [193]. The potential molecular mechanisms of action of sodium benzoate remain to be determined. Since DAAO activity is high in the adult brain cerebellum, it is possible that DSR cerebellar levels may be increased following sodium benzoate administration. Furthermore, recent findings suggest that sodium benzoate may upregulate brain-derived neurotrophic factor (BDNF) via protein kinase A-(PKA-)mediated activation of cAMP response element binding (CREB) protein [194].

In two controlled trials, the administration of up to 1 g/day sodium benzoate proved beneficial for schizophrenia [195] and MCI or mild AD [177] patients. Furthermore, partial remission within 6 weeks was reported with a major depressive disorder patient treated with 500 g/day sodium benzoate [116]. These preliminary findings show promise for DAAO inhibition as a novel treatment approach. Nevertheless, at present, the therapeutic potential of DAAO inhibitors is still relatively unexplored and preclinical studies have primarily addressed the relevance of these compounds mainly for schizophrenia. Further research is warranted given that the few published studies characterizing novel DAAO inhibitors have yielded conflicting results.

5. Conclusions and Future Directions

The scientific view about DSR has changed drastically during the last decade. Converging data strongly suggest a complex and unique neurotransmitter function of DSR which is likely to include an important role in glia-synapse interactions. Furthermore, the demonstration of a DSR modulatory role in cardinal NMDAR-dependent processes has been a driving force for the conceptualization of novel treatment strategies involving the direct or indirect manipulation of DSR signaling. These concepts are likely to undergo further integration and development in the context of the need for strictly balanced NMDAR functioning, with either over- or under-NMDAR function potentially involved in the pathogenesis of neuropsychiatric dysfunctions. A first generation of proof-of-concept animal and clinical studies indicate beneficial DSR effects in refractory schizophrenia, movement, depression, and anxiety disorders and for the improvement of cognitive performance. An additional presently developing strategy is the indirect modulation of DSR synaptic levels by use of compounds that alter the function of main enzymes responsible for DSR production and degradation.

Future research on DSR is likely to further develop along three main axes: (1) characterization of the DSR neurotransmitter system and its role throughout lifespan; (2) the implication of DSR in pathological states characterized by either hypo- or hyper-NMDAR function; and (3) direct or indirect pharmacological manipulation of DSR signaling. The accumulated data suggest that, during the next decade, we will witness important advances in DSR research that will further contribute to elucidate the causes of neuropsychiatric disorders and will be instrumental in the development of innovative treatments.
Conflict of Interests

Dr. Heresco-Levy is inventor in patents for the use of NMDAR-glycine site modulators in movement disorders and in patent applications for the use of NMDAR-glycine site modulators in depression and autoimmune-induced NMDAR dysfunctions. Dr. Durrant reports no conflict of interests.

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