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Glial D-Serine Gates NMDA Receptors at Excitatory Synapses in Prefrontal Cortex

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***N*-methyl-D-aspartate receptors (NMDARs) subserve numerous neurophysiological and neuropathological processes in the cerebral cortex. Their activation requires the binding of glutamate and also of a coagonist. Whereas glycine and D-serine (D-ser) are candidates for such a role at central synapses, the nature of the coagonist in cerebral cortex remains unknown. We first show that the glycine-binding site of NMDARs is not saturated in acute slices preparations of medial prefrontal cortex (mPFC). Using enzymes that selectively degrade either D-ser or glycine, we demonstrate that under the present conditions, D-ser is the principle endogenous coagonist of synaptic NMDARs at mature excitatory synapses in layers V/VI of mPFC where it is essential for long-term potentiation (LTP) induction. Furthermore, blocking the activity of glia with the metabolic inhibitor, fluoroacetate, impairs NMDAR-mediated synaptic transmission and prevents LTP induction by reducing the extracellular levels of D-serine. Such deficits can be restored by exogenous D-ser, indicating that the D-amino acid mainly originates from glia in the mPFC, as further confirmed by double-immunostaining studies for D-ser and anti-glial fibrillary acidic protein. Our findings suggest that D-ser modulates neuronal networks in the cerebral cortex by gating the activity of NMDARs and that altering its levels is relevant to the induction and potentially treatment of psychiatric and neurological disorders.**

Keywords: astrocytes, coagonist, excitatory synapses, NMDA receptors, prefrontal cortex

Introduction

The *N*-methyl-D-aspartate receptor (NMDAR) subfamily of glutamate receptors is widely expressed in the central nervous system (CNS) where it is indispensable to many of the activity-dependent changes in synaptic strength and connectivity underlying higher brain functions such as memory formation and cognition (Malenka and Bear 2004; Rebola et al. 2010). Dysfunction of NMDA receptors is considered central to the pathophysiology of several neurologic and psychiatric disorders. Indeed, NMDAR hyperactivity can cause cell death in stroke and chronic neurodegenerative disorders such as Parkinson, Alzheimer's diseases, and HIV-associated dementia (Kemp and McKernan 2002; Hardingham and Bading 2003). By contrast, hypoactivity of NMDAR induces apoptosis during brain development and may contribute to psychotic and cognitive symptoms associated to schizophrenia (Millan 2005; Ross et al. 2006).

In addition to the agonist glutamate, activation of the NMDAR requires the binding of a coagonist which was originally thought to be glycine (Johnson and Ascher 1987; Paoletti and Neyton 2007). However, studies over the last decade have shown that the CNS produces significant amount of an atypical amino acid, D-serine (D-ser) (Hashimoto and Oka 1997; Martineau et al. 2006; Wolosker 2007). D-Ser is converted from L-ser by serine racemase (SR), an enzyme enriched in the CNS and is thought to be degraded by D-amino acid oxidase (DAAO) (Martineau et al. 2006; Wolosker 2007; Pollegioni and Sacchi 2010). Functional studies have demonstrated that the D-amino acid appears to be the physiological ligand for the coagonist site of NMDARs in different brain areas like the hippocampus (Mothet et al. 2000; Yang et al. 2003; Mothet et al. 2006; Zhang et al. 2008; Basu et al. 2009), the retina (Stevens et al. 2003; Kalbaugh et al. 2009), and the hypothalamus (Panatier et al. 2006).

Compelling evidence suggests that besides its physiological functions, D-ser may participate in excitotoxic events when released in excess (Martineau et al. 2006). Indeed, SR deletion confers neuronal protection to cerebral ischemia and excitotoxicity (Mustafa et al. 2010) and to β-amyloid 1–42 peptide injury in the forebrain (Inoue et al. 2008). By contrast, aging and schizophrenia are associated with decreased D-ser levels (Hashimoto et al. 2005; Mothet et al. 2006; Bendikov et al. 2007; Sacchi et al. 2008; Turpin et al. 2009).

As such, D-ser modulation appears to be central to many brain functions but also to play a role in the etiology of neurodegenerative disorders and psychiatric diseases affecting particularly the cerebral cortex. Nevertheless, whether D-ser serves as the endogenous coagonist of synaptic NMDARs in the cerebral cortex is unknown. Amongst the different cerebral areas, the prefrontal cortex (PFC) is critical for social cognition, conceptualization, and working memory whose disturbances are evident in Alzheimer's disease, stroke, or schizophrenia (Goto et al. 2010). In this study, we examined the respective roles of glycine versus D-ser in governing the activity of NMDARs localized in layers V/VI of PFC where they are strongly expressed (Wang et al. 2008). We have developed an acute brain slice preparation of medial prefrontal cortex (mPFC) for recordings from prelimbic area, an analog structure of primate PFC, from adult rat (Gabbott et al. 2005). We here demonstrate that mPFC of adult rats contains high levels of D-ser and that the coagonist site of NMDARs in layers V/VI is not saturated. Using enzymes that selectively degrade either D-ser

or glycine, we further report that under the present conditions D-ser is the major endogenous coagonist of synaptic NMDAR. Furthermore, we show that D-ser is produced partly by astrocytes in layers V/VI of mPFC and that D-ser and glia are both necessary for the induction of NMDAR-dependent long-term synaptic plasticity. Our findings thus underpin therapeutic strategies targeting D-ser availability in the treatment of brain disorders such as schizophrenia or stroke where the operation of NMDARs is disrupted.

Materials and Methods

All experiments were conducted with respect to European and French directives on animal experimentation.

Slice Preparation and Electrophysiological Recordings

Experiments were carried out on acute slices obtained from 45- to 60-old-day Wistar rats. The rats were anaesthetized with isoflurane and decapitated. The brain was then quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95% O_2 and 5% CO_2. Coronal slices (300 μm) of the mPFC including the infralimbic (area, 25) and the prelimbic (area 32) cortices were obtained and allowed to recover for at least 45 min at 31 °C in a submerged chamber containing ACSF before recording. After 30–60 min recovery at room temperature, one slice was transferred and submerged in a recording chamber where it was continuously perfused (1–2 mL/min) with ACSF composed of (in mM): NaCl, 123; KCl, 2.5; Na_2HPO_4, 1; $NaHCO_3$, 26.2; $MgCl_2$, 1.3; $CaCl_2$, 2.5; and glucose, 10 (pH 7.4; 295–300 mOsm kg^{-1}).

Pyramidal PFC neurons in layers V/VI of prelimbic cortices (area 32) were identified visually using infrared differential interference contrast microscopy (Olympus BX51). Synaptically evoked excitatory currents (EPSCs) were recorded under whole-cell voltage-clamp mode. The patch-clamp recording pipettes (borosilicate glass, Harvard Apparatus, 2.5–5 MΩ) were filled with cesium chloride (CsCl) solution containing (in mM): CsCl, 130; NaCl, 10; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10; QX-314, 5; ethyleneglycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetra acetic acid (EGTA), 1; and $CaCl_2$, 0.1 (adjusted to pH 7.1–7.3 with CsOH; 292–296 mOsm kg^{-1}). Neurons were clamped first at –70 mV for 5 min after breaking the seal and then depolarized at +40 mV. Membrane currents were recorded at 30 °C using a Multiclamp 700B amplifier (Axon Instruments, Inc.); signals were filtered at 2 kHz and digitized at 5 kHz via a DigiData 1440A interface (Molecular Devices). Series resistance (6–15 MΩ) and holding current were monitored throughout the experiment. Cells with Ra > 25 MΩ or holding current > –200 pA at resting potential were excluded from data analysis, as well as any cell for which a >20% change in those parameters occurred during the course of the experiment. The paired-pulse ratio (PPR) was calculated as the peak amplitude of the second evoked EPSC (2)/peak amplitude of the first EPSC (1). The pulse duration was 100 μs and the interval between the 2 pulses was 100 ms. Glass electrodes filled with ACSF were used to stimulate the layers I/II of the prelimbic area in the mPFC (Fig. 1*B,C*). Assuming that mean center-to-center distance between minicolumn in the prelimbic area is 45 μm in rats (Gabbott et al. 2005), the electrode of stimulation was shifted in order to avoid direct stimulation of the dendritic bundles of the recorded pyramidal neurons. For α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA-EPSCs, the pulse duration was 100 μs and the interval between 2 pulses was, respectively, 15 s for AMPA-EPSCs and 30 s for NMDA-EPSCs. In all figures, current curves are mean of at least 10 successive responses.

For the induction of long-term potentiation (LTP), a 4 × 1 s pulse protocol was used at resting membrane potential (holding current = 0), with a 100-Hz stimulation period for each pulse. The interval between each pulse was 15 s. In some experiment, current-clamp mode was used to study the pattern of discharge of the pyramidal neurons from which EPSCs were recorded. In those cases, the recording electrodes were filled with (in mM): K-gluconate, 120; KCl, 20; HEPES, 10, EGTA, 1; $MgCl_2$, 1; $CaCl_2$, 0.1 (adjusted to pH = 7.2–7.4; 290 ± 5 mOsm kg^{-1}).

Spontaneous unitary excitatory postsynaptic currents (mEPSCs) were obtained in the presence of tetrodotoxin (TTX, 0.5 μM) and picrotoxin (50 μM), and in those case, the recordings electrodes were filled with K-gluconate-based solution. mEPSCs were recorded by using Axoscope, detected, and analyzed off-line using Axograph (Molecular Devices). At least 200 events were analyzed for each cell in each condition.

Data Analysis and Statistics

Data were collected and analyzed using pClamp10 software (Axon Instruments, Inc.). They are expressed as percentage values and are reported as mean ± the standard error of the mean of *n* cells. Statistical significance was evaluated via paired or unpaired Student's *t*-test. Significance was assessed at $P < 0.05$.

Determination of Tissue and Extracellular Amino Acid Levels

Levels of endogenous amino acids were determined on acute PFC slices from the same rats. Because of the limited amount of tissue per slice, 2 slices (approximately 1.5 mg) were pooled together. Analyses of the samples were performed using capillary electrophoresis with laser-induced fluorescence (CE-LIF) (CE: Beckman Coulter, P/ACE MDQ; LIF: Picometrics, LIF-UV-02, 410 nm, 15 mW) (Lapainis and Sweedler 2008). Briefly, tissue samples were first deproteinized by addition of cold trichloroacetic acid (TCA) to a 5% final concentration. The suspension was centrifuged at 16,800 × g for 10 min, and the TCA was extracted from the supernatant with water-saturated diethyl ether and stored at –80 °C until analysis. Extracellular levels of amino acids (glycine, L-ser, D-ser) were determined in control conditions and after exposure of acute PFC slices for 40 min to fluoroacetate (FAC), *Rhodotorula gracilis* DAAO (*Rg*DAAO), or *Bacillus subtilis* glycine oxidase (*Bs*GO) to evaluate the effects of such treatments. The extracellular media were retrieved and stored at –80 °C until analysis. Liquid phase of amino acids were processed for micellar CE-LIF (Zhao, Song, et al. 2005). Briefly, the samples were fluorescently derivatized at room temperature for 2 h with napthalene-2,3-dicarboxaldehyde (NDA) before being analyzed by CE using a hydroxypropyl-β-cyclodextrin (HP-β-CD) based chiral separation buffer. All data were collected and analyzed using Karat 32 software v8.0 (Beckman Coulter, Fullerton, CA).

The amount of amino acids in tissue was scaled to the protein content determined by the Lowry method using the Pierce BCA protein Assay kit (Thermo Scientific) assay with bovine serum albumin (BSA) as standards. The quantity of amino acids in the samples was determined from a standardized curve and peak identification was made by spiking the fraction with the appropriate amino acid and by evaluating the effect of selective peak removing for D-ser or glycine by enzymatic treatment with *Rg*DAAO or *Bs*GO on standards.

Immunohistochemistry

Wistar rats 45–60 old days were deeply anesthetized with sodium pentobarbital (50 mg/kg, intraperitonetally), transcardially perfused with an ice-cold solution containing 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4 supplemented with 0.25% glutaraldehyde, and brain were explanted and subjected to an overnight postfixation in the same solution and finally cryoprotected with 30% sucrose in 0.1 M PBS (pH 7.4). Brain coronal 30 μm sections were cut on a freezing microtome (Micron HM450). After several washes in PBS, free-floating brain sections were immunostained as described previously (Puyal et al. 2006). After a blocking/permeabilization step, sections were probed for 36 h at 4 °C with pairs of primary antibodies diluted in PBS plus 4% normal goat serum and 0.1% Triton X-100. After several washes, the slices were incubated for 1 h at room temperature with pairs of Alexa secondary antibodies (Alexa 488 anti-rabbit/Alexa 546 anti-mouse, Molecular Probes) at 1:2000 dilution in PBS plus 1.5% normal goat serum and 0.1% Triton X-100. Finally, slices were washed and mounted in Vectashield mounting medium (Vector Laboratories). Controls were performed by avoiding the primary antibodies. Immunofluorescence was analyzed using a laser scanning confocal microscope (Leica TCS SP2; Leica Microsystems). The confocal images were acquired using the Leica TCS software with a sequential mode to avoid interference between each channel and

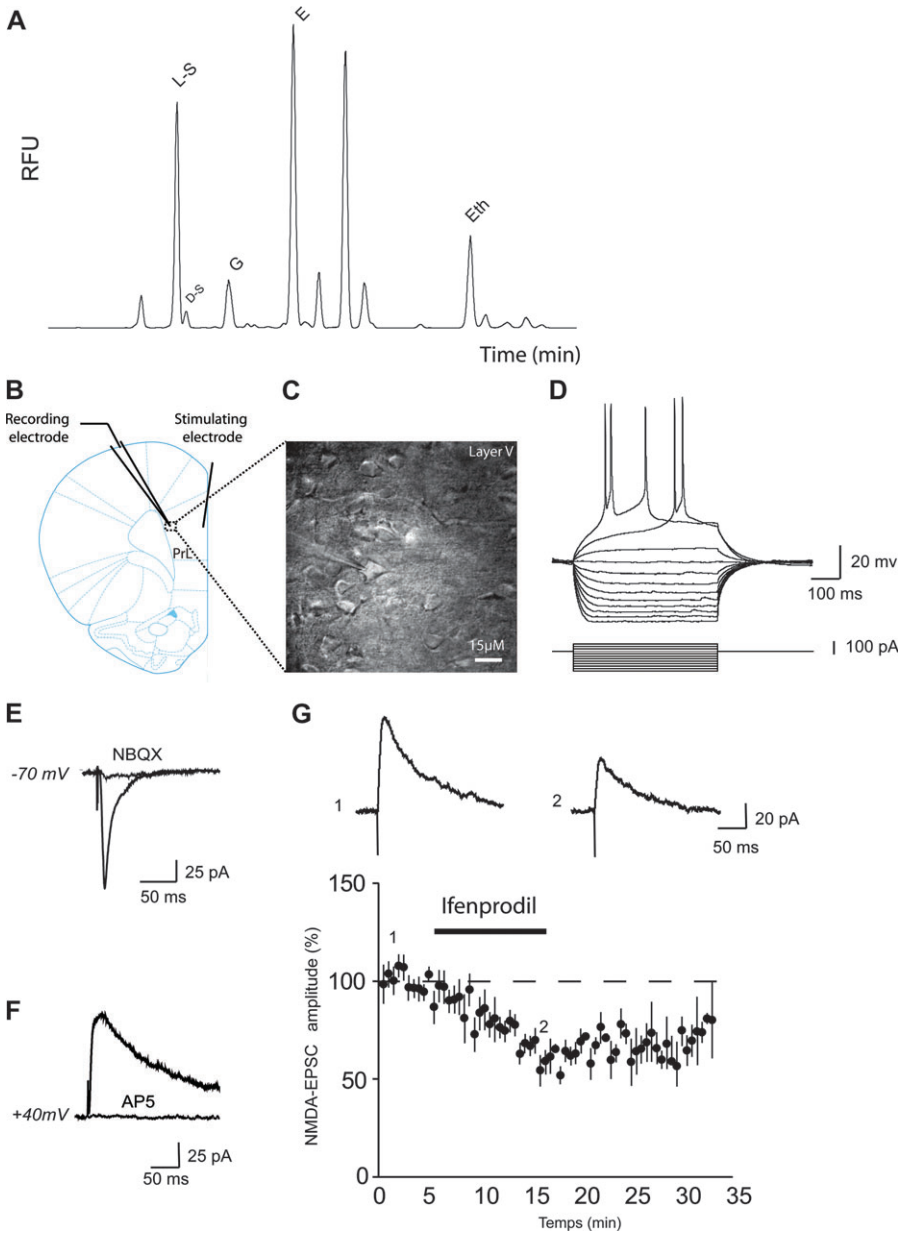

Figure 1. Properties of NMDA currents of layers V/VI pyramidal neurons in the mPFC. (*A*) Typical electropherogram (right) of NDA-derivatized amino acids showing the presence of D-ser in the mPFC of mature rats. L-S: L-serine, D-S: D-serine, E: glutamate, G: glycine, Eth: ethanolamine (internal standard). (*B* and *C*) Schematic representation (*B*) of the mPFC and micrograph (*C*) from the prelimbic area showing the patched pyramidal neurons in layers V/VI. Stimulating electrode is placed in layers I/II. (*D*) Characteristic *I/V* pyramidal cell response in current-clamp configuration. (*E*) In voltage clamp, pyramidal cells (lower trace) present at −70 mV a typical evoked AMPA current in presence of $GABA_A$ inhibitor picrotoxin; this current is blocked by application of 10 µM NBQX. (*F*) A typical outward NMDA current (upper trace) is revealed at +40 mV under both picrotoxin and NBQX. This current is blocked by application of AP5. An AMPA/NMDA ratio was measured by dividing the NMDA peak current and the AMPA peak current. This ratio is close to 1 in control conditions. (*G*) Time course of the effect of ifenprodil on NMDA-EPSCs. Layers V/VI pyramidal cell NMDA receptors contain a mixed NR2A/NR2B subunits. Application of 3 µM ifenprodil reduced NMDA currents by about 30%.

without saturation of any pixel. Moreover, emission windows were fixed for each fluorophore in conditions where no signal is detected from the other fluorophore. Stack images were taken in the PFC area, and a Z-projection was made with ImageJ 1.43 software (http://rsb.info.nih.gov/ij) in standard deviation projection mode.

Drugs, Enzymes, and Antibodies

All drugs were bath applied. Appropriate stock solutions were made, stored at −20 °C and diluted to the final concentration with ACSF containing vehicle (<1/1000) just before application. Picrotoxin, 2,3-dihydroxy-6-nitro-7-sulfonyl-benzo[f]quinoxaline (NBQX), D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5), ifenprodil, sarcosine, D-ser, glycine, and TTX were obtained from TOCRIS bioscience. Sodium fluoroacetate (FAC) was obtained from Fluka. D-Cycloserine was from Sigma-Aldrich. NDA was obtained from Molecular Probes (Eugene, OR). D-Cycloserine and HP-β-CD were from Sigma-Aldrich.

Recombinant *Rg*DAAO (EC 1.4.3.3) was overexpressed in *Escherichia coli* cells and purified as reported earlier (Fantinato et al. 2001); the final enzyme preparation had a specific activity of 100 ± 15 U/mg protein on D-ser as substrate. Recombinant *Bs*GO (EC 1.4.3.19) was overexpressed in *E. coli* cells as well (Job et al. 2002); the final enzyme preparation had a specific activity of 0.9 ± 0.2 U/mg protein on glycine as substrate. These flavoenzymes specifically degrade D-ser (*Rg*DAAO) and glycine (*Bs*GO), as demonstrated by the corresponding apparent kinetic efficiency (k_{cat}/K_m ratio) values: k_{cat}/K_m ratios of 3.0 and 0.058 mM^{-1} s^{-1} were

determined for *Rg*DAAO on D-ser and glycine, respectively (Fantinato et al. 2001; Pollegioni et al. 2007), while the k_{cat}/K_m ratios determined for *Bs*GO were 0.00025 and 0.867 mM^{-1} s^{-1} on D-ser and glycine, respectively (Job et al. 2002). To degrade D-ser or glycine, slices were incubated for at least 45 min and then continuously perfused with aCSF containing *Rg*DAAO (0.2–0.4 U/mL) or *Bs*GO (0.1–0.2 U/mL), respectively.

Affinity-purified rabbit polyclonal antibody against conjugated D-ser was from GemacBio (France) and affinity-purified mouse monoclonal anti-glial fibrillary acidic protein (GFAP) antibody (clone G-A-5) and mouse monoclonal S100β antibody (clone 1B2) were from Sigma.

Results

Evoked NMDA Receptor–Mediated EPSCs in Layers V/VI mPFC Neurons

We first showed that both D-ser and glycine are present in mPFC in significant amounts (D-ser: 0.144 nmol/mg prot; glycine: 0.805 nmol/mg prot) as revealed by capillary electrophoresis (Fig. 1*A*). The retrieved amounts and their ratio are comparable to the ones found in others brain areas such as the hypothalamus (Panatier et al. 2006), the hippocampus (Mothet et al. 2006), or the retina (Stevens et al. 2003). This result indicates that both amino acids, glycine, and D-ser could serve as NMDAR endogenous coagonist in the prelimbic area of the mPFC.

Synaptic currents in layers V/VI pyramidal cortical neurons were recorded in the whole-cell patch-clamp configuration (Fig. 1*B,C*). These neurons displayed a mean resting membrane potential of –64 ± 0.4 mV and were able to sustain firing when depolarized above spike threshold (Fig. 1*D*) We studied glutamatergic transmission in layers V/VI mPFC neurons by stimulating excitatory afferents through a glass electrode placed in layers I/II (Fig. 1*B,C*). When recorded at –70 mV and under picrotoxin (50 μM), EPSCs were entirely mediated by AMPA/kainate receptors since they were insensitive to AP5 (50 μM) and blocked by NBQX (10 μM) (Fig. 1*E*). To isolate the NMDA component of the EPSCs, we clamped the cells at +40 mV in the presence of NBQX. Under these conditions, the evoked current had much slower rise time and decay time as expected for NMDARs (Wang et al. 2008) and was inhibited by AP5 (Fig. 1*F*), confirming that it was mediated by NMDARs. These responses were also partially blocked with ifenprodil (3 μM; 73.5 ± 7.4% of control, $n = 7$, $P = 0.012$) (Fig. 1*G*), a NR2B subunit–containing NMDAR antagonist (Panatier et al. 2006; Paoletti and Neyton 2007; Wang et al. 2008) indicating that synaptic NMDARs are mainly composed of NR2A subunits (Massey et al. 2004). No changes in the holding current and in the series resistance (Ra) were observed during the time course of ifenprodil application arguing against a possible effect of ifenprodil on the integrity of the membrane (Supplementary Fig. 1).

We next checked for the level of occupancy of the NMDAR glycine-binding site. Exogenous application of D-ser (100 μM) to the bathing solution induced a significant and reversible increase in the amplitude of the NMDAR-mediated EPSCs (125.0 ± 6.2% of control, $n = 17$, $P = 0.001$, Fig. 2*A,F*). D-ser had no effect on AMPAR-mediated EPSCs (Fig. 2*B*). Surprisingly, glycine by itself (10–500 μM) failed to potentiate NMDA-EPSCs (104.6 ± 8.7% of control, $n = 10$, $P = 0{,}43$, Fig. 2*C,F*) and only a modest but significant potentiating effect (115.6 ± 1.9% of control, $n = 7$, $P < 0.001$) was also observed when glycine transporters (GlyTs) were blocked with 0.5 mM sarcosine (Fig. 2*D,F*). Preincubation of mPFC slices with D-serine (100 μM) occluded the potentiating effect of sarcosine (Fig. 2*E,F*) arguing that D-serine and not glycine is the most effective coagonist in the PFC. Finally, we used another different specific partial agonist of the glycine site, D-cycloserine whose application (500 μM) significantly increases the NMDA component to 121.3 ± 5.1% (Supplementary Fig. 2). A dose–response curve shows that D-ser is the most effective coagonist at lower doses (EC_{50} for D-ser: 26.5 μM, D-cycloserine: 202.3 μM). Taken together, these results indicate that the glycine site of NMDARs in layers V/VI mPFC pyramidal neurons is not fully saturated by the endogenous coagonist at least in acute brain slices.

D-Serine is the Endogenous Coagonist of NMDARs in Layers V/VI mPFC Neurons

To identify whether D-ser, glycine, or both amino acids acted as endogenous coagonists of synaptic NMDARs in layers V/VI mPFC pyramidal neurons, we investigated the action of 2 enzyme scavengers, yeast DAAO (*Rg*DAAO) and bacterial glycine oxidase (*Bs*GO), that selectively degrade D-ser and glycine, respectively (Fantinato et al. 2001; Job et al. 2002; Panatier et al. 2006). To assess the action of these enzymes on synaptic NMDARs, slices were incubated for at least 45 min with either *Rg*DAAO or *Bs*GO (0.2 U/mL), and the AMPA/NMDA ratios of peak currents were compared between these different conditions (Fig. 3*A,B*). In slices treated with *Rg*DAAO, this ratio was largely increased (control vs. *Rg*DAAO: 1.09 ± 0.18 vs. 2.20 ± 0.26, $n = 6$, $P = 0.006$) compared with control conditions, whereas inactive catalytic mutant *Rg*DAAO had no effect (data not shown). While NMDA-EPSCs were decreased by *Rg*DAAO, AMPAR-mediated EPSCs were not impacted (control vs. *Rg*DAAO: –118 ± 11 vs. –132 ± 24 pA, $n = 27$ for control and $n = 10$ for *Rg*DAAO, $P = 0.57$, Fig. 3*A,C*). The specific action of *Rg*DAAO on NMDAR-EPSCs was further investigated by the analysis of spontaneous EPSCs (mEPSCs) that are driven by AMPAR (Supplementary Fig. 3). *Rg*DAAO did not alter the amplitude nor the frequency of mEPSCs thus confirming the specific action of DAAO on NMDA-EPSCs as reported earlier (Mothet et al. 2000; Yang et al. 2003). Subsequent application of exogenous D-ser restored the AMPA/NMDA peak current ratio (1.40 ± 0.15, Fig. 3*A,D*) to control values indicating that these changes induced by *Rg*DAAO on NMDAR-mediated currents are most likely due to the degradation of D-ser by the enzyme. To ensure that this is the case, we next evaluated the effect of *Rg*DAAO on extracellular levels of D-ser. Using CE-LIF, we measured the levels of D-ser before and after exposure of PFC slices to *Rg*DAAO (Supplementary Fig. 4*B*). As expected, *Rg*DAAO (0.2 U/mL, 40 min) decreased extracellular D-ser levels by 77.03 ± 9.51% ($P = 0.0098$) without impacting significantly glycine levels (–14.40 ± 12.29% of control, $P = 0.139$). Collectively, these findings strongly support the idea that in the mPFC, D-ser is an endogenous coagonist of synaptic NMDARs as reported for other brain areas (Stevens et al. 2003; Yang et al. 2003; Panatier et al. 2006; Zhang et al. 2008).

We next investigated the contribution of endogenous glycine to NMDARs activity at the same synapses using *Bs*GO. Unlike what we observed following *Rg*DAAO incubation, we did not detect any significant changes in the AMPA/NMDA ratio (control vs. *Bs*GO: 1.24 ± 0.25, $n = 11$, $P = 0.62$, Fig. 3*B,C*) although *Bs*GO (0.2 U/mL, 40 min) is effective in reducing significantly and specifically the extracellular levels of glycine by 64.1 ± 7.5% ($P < 0.001$, Supplementary Fig. 4*C*).

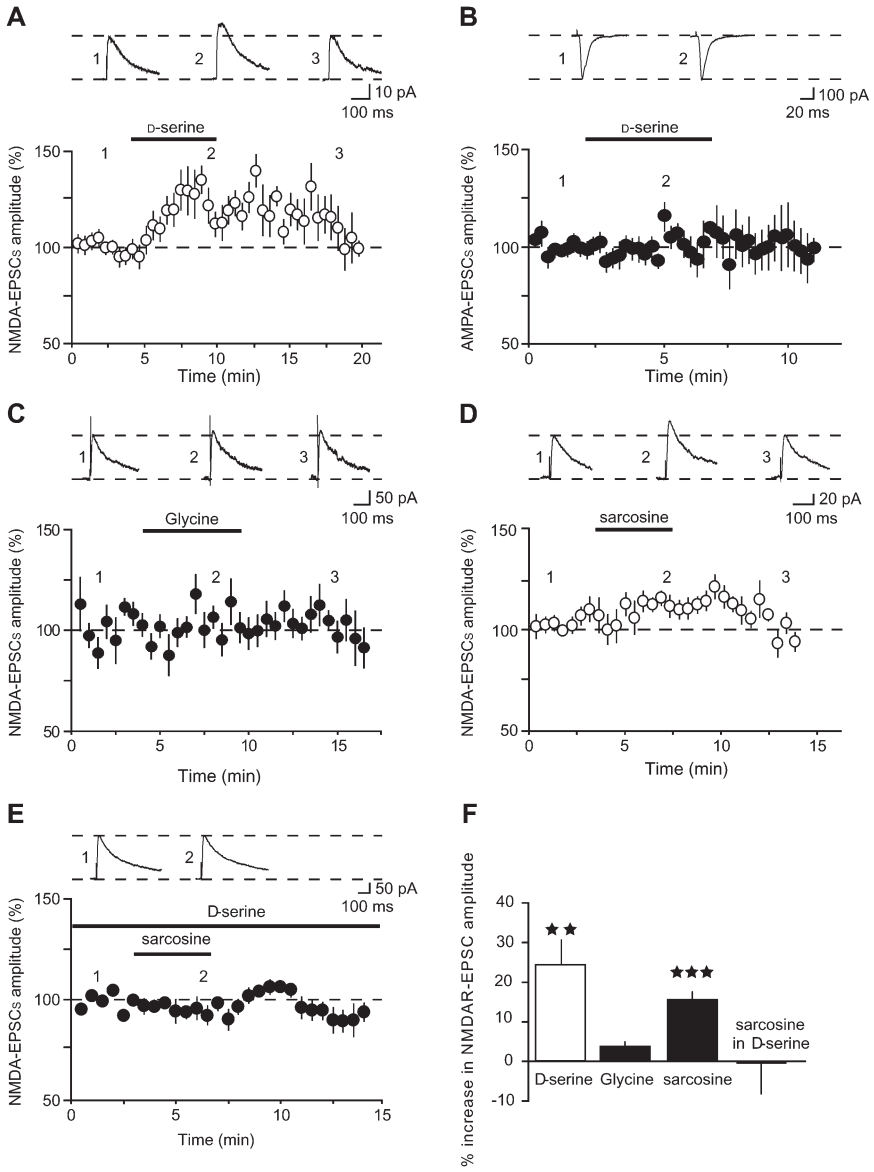

Figure 2. Saturation of NMDA coagonist site. (*A* and *B*) Glycine site of NMDA receptors is not saturated under control conditions. Indeed, application of 100 μM D-ser reversibly increases NMDA currents of about 20% while no effect is observed on AMPAR-mediated EPSCs (panel *B*). (*C*) The application of 100 μM glycine has no effect on NMDA-EPSCs while, (*D*) the application of 0.5 mM sarcosine (a specific GlyT1 inhibitor) induces a slow but significant increase in NMDA currents. (*E*) The potentiating effect of sarcosine was abolished when sarcosine was applied in presence of 100 μM D-ser. Numbers in panels *A*–*E* represent the example of currents for the corresponding condition. (*F*) Bar graphs indicate mean ± standard error of the mean for the different experimental conditions. All NMDA-EPSCs and AMPA-EPSCs were recorded at +40 and −70 mV holding potentials, respectively. $^{**}P < 0.01$, $^{***}P < 0.001$, comparison with Student's *t*-test.

Furthermore, the application of 100 μM D-ser had an effect similar to that observed in the absence of *Bs*GO (120.4 ± 10.1% of control, $n = 10$, $P < 0.05$) indicating that degradation of glycine did not affect NMDA-EPSCs (Fig. 3*C*,*D*). Interestingly, glycine (10–500 μM) failed to induce any change in NMDA-EPSCs (Fig. 2*C* for 100 μM and data not shown), an effect that may be related to the presence of highly efficient GlyTs for this amino acid that quickly clear up glycine from the synaptic cleft (Betz et al. 2006). Indeed, inhibition of GlyTs by sarcosine increased modestly NMDA-EPSCs (Fig. 2*D*,*F*) in the absence of added D-ser (Fig. 2*E*,*F*), an effect that was prevented by *Bs*GO (Supplementary Fig. 5*A*,*D*) further demonstrating that the enzyme is active and not degraded by peptidase during incubation. Still, because sarcosine has been reported to be an excellent substrate for *Bs*GO (Job et al. 2002), we conducted another set of controls to ascertain that the occluding effect of *Bs*GO was due to degradation of glycine rather than sarcosine. When glycine was first enzymatically removed by *Bs*GO (Supplementary Fig. 5*B*,*D*), sarcosine no longer potentiated NMDA-EPSCs. These results support the idea that GlyTs do remove efficiently glycine preventing it to enter synaptic cleft and also show that the observed potentiating effect of sarcosine was not due to a direct agonist action of sarcosine on NMDARs as reported (Zhang et al. 2009). These data support the idea that glycine, unlike D-ser, is not an endogenous coagonist at synaptic NMDARs on layers V/VI mPFC pyramidal neurons and that glycine and D-ser compete at synaptic NMDARs. Accordingly, glycine induced a significant potentiation of NMDA-EPSCs (+37.0 ± 9.6% vs. control, $n = 4$, $P < 0.03$) only when D-ser has been removed enzymatically by

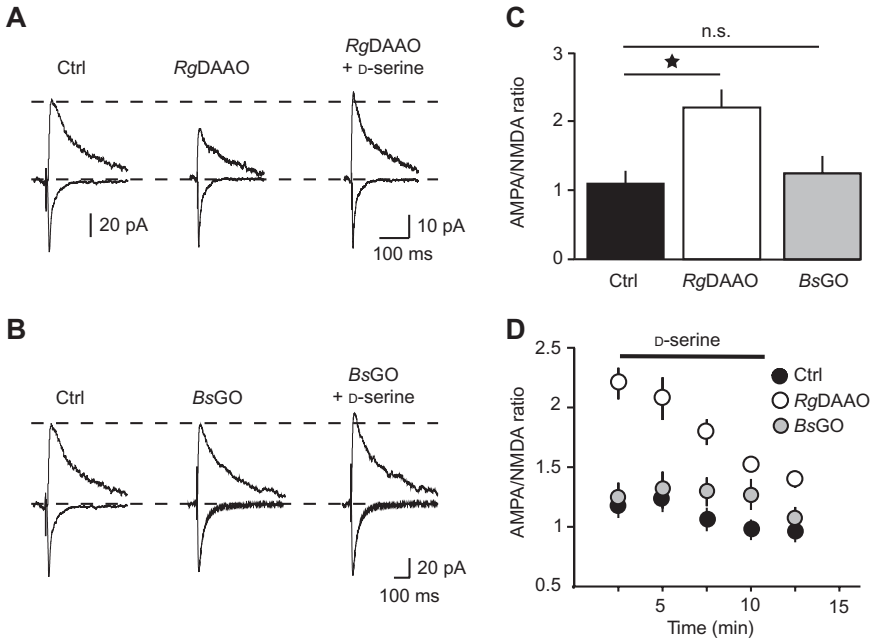

Figure 3. D-serine but not glycine is an endogenous coagonist of NMDA receptors in layers V/VI pyramidal cells of mPFC. (*A–D*) AMPA/NMDA ratio after 45 min incubation of slices in *Rg*DAAO or *Bs*GO. (*A*) After *Rg*DAAO, NMDA current, but not AMPA current, was strongly decreased. As a consequence, the AMPA/NMDA ratio was significantly increased (*Rg*DAAO, open bar and circle in panels *C* and *D*, respectively). Subsequent application of 100 μM D-ser increased NMDA current (panel *A*, right). *Rg*DAAO was not completely washed out since AMPA/NMDA ratio was brought back only to control value (panel *D*, compare control in black circle to *Rg*DAAO in open circles). (*B*) By contrast, NMDA current was not reduced in slices incubated in *Bs*GO. Thus, AMPA/NMDA ratio of slices incubated in *Bs*GO was not modified (panels *C* and *D*, compare control to *Bs*GO; gray circle). Subsequent application of D-ser slightly increased NMDA current (~20% see text) as in control conditions (panel *D*, gray circle). All NMDA-EPSCs and AMPA-EPSCs were recorded at +40 and −70 mV holding potentials, respectively. $*P < 0.05$, comparison with Student's *t*-test.

*Rg*DAAO (Supplementary Fig. 5*C,D*) as described previously in the hippocampus (Shleper et al. 2005).

Because endogenous D-ser regulates the activity of NMDARs in mPFC, we made the assumption that the D-amino acid may contribute to the induction of long-term changes in synaptic plasticity notably the LTP whose induction in the cerebral cortex like in others brain areas requires NMDAR activation (Massey et al. 2004; Banerjee et al. 2009). LTP was induced by high-frequency stimulation of the excitatory inputs in layers I/II. This protocol reliably induced a long-lasting increase in the amplitude of the evoked EPSCs (162.4 ± 18.3% of control, $n = 7$, $P = 0.007$, Fig. 4*A*). Such an LTP was abolished in the presence of D-AP5 in the bath (96.1 ± 18.7% of control, $n = 5$, $P = 0.44$, Fig. 4*A*), as expected from an NMDAR-dependent process. Interestingly, the same induction protocol yielded no potentiation in *Rg*DAAO-incubated slices (81.0 ± 13.0% of control, $n = 5$, $P = 0.12$). Such impairment of synaptic plasticity could be rescued by providing exogenous glycine to the brain slices (163.4 ± 19.8%, $n = 5$, $P = 0.016$, Fig. 4*B*) in line with our above observations that glycine can modulate NMDA-EPSCs when D-ser is not present at the synaptic cleft (Supplementary Fig. 5). These results support the notion that D-ser is governing the plasticity of excitatory synapses in the CNS as observed in the hippocampus (Yang et al. 2003; Mothet et al. 2006) and the hypothalamus (Panatier et al. 2006).

Glia as a Main Source for D-Ser in the mPFC

Recent data revealed that in the cerebral cortex and other brain regions, neurons in addition to astrocytes are an important source of D-ser (Kartvelishvily et al. 2006; Miya et al. 2008; Rosenberg et al. 2010). To test whether PFC D-ser originates from astrocytes or neurons in layers V/VI mPFC, we performed immunostaining for D-ser (Martineau et al. 2006). We showed that the amino acid was evenly present in all layers of the mPFC of adult rat with a strong staining evident in astrocyte-like cells (Fig. 5*A*). Double immunostaining for D-ser with GFAP in layers V/VI of mPFC further identified astrocytes as the cells where the amino acid is strongly present (Fig. 5*B*) with labeling in the soma and processes of those cells. No particular laminar staining for GFAP was observed in the mPFC (data not shown). We also found moderate and infrequent staining of D-ser in the cell bodies of neurons throughout the mPFC layers (Fig. 5*B*).

The presence of D-ser in mPFC glia cells strongly suggested that those cells contribute to the regulation of NMDAR functions in the mPFC through the release of D-ser. Thus, to test this hypothesis, we next assessed the effect of FAC (5 mM, 40 min), a glia-specific metabolic inhibitor (Hassel et al. 1997; Hülsmann et al. 2003; Andersson et al. 2007; Zhang et al. 2008; Okada-Ogawa et al. 2009) on synaptic transmission and LTP. As expected if glia was the source of D-ser, FAC produced a significant reduction in D-ser (FAC vs. control: −55.46 ± 18.80%, $n = 2$, $P = 0.02$) but not in glycine (FAC vs. control: −12.32 ± 12.39%, $n = 2$, $P = 0.20$) extracellular levels measured when acute PFC slices were exposed to the toxin (Supplementary Fig. 4*B,C*). Accordingly, FAC increases the AMPA/NMDA ratio (FAC vs. control: 3.15 ± 0.70, $n = 6$, $P = 0.03$, Fig. 6*A*) while it did not affect AMPA-EPSCs (data not shown). This result was similar to that observed when D-ser was degraded with *Rg*DAAO (Fig. 3*A,C,D*). At the same time, release probability was not impaired by FAC as revealed by its lack of action on PPR of AMPAR-EPSCs (control: 100.1 ± 7.9% vs. FAC: 110.8 ± 10.9%, $n = 5$, $P = 0.19$, Fig. 6*B*). Furthermore, we did not observe any effect of FAC on the membrane properties of the

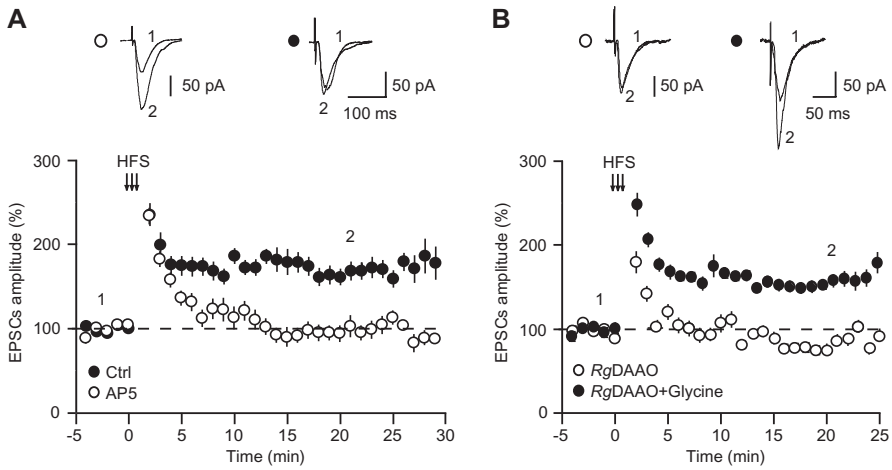

Figure 4. Pyramidal cell LTP depends on the saturation level of glycine site of NMDA receptors. (*A*) LTP is induced in layers V/VI pyramidal cells by high-frequency stimulation (HFS). This LTP is NMDA dependent since it is blocked by application of AP5. (*B*) LTP is suppressed if slices are incubated in *Rg*DAAO and is maintained in slices incubated in *Rg*DAAO and glycine.

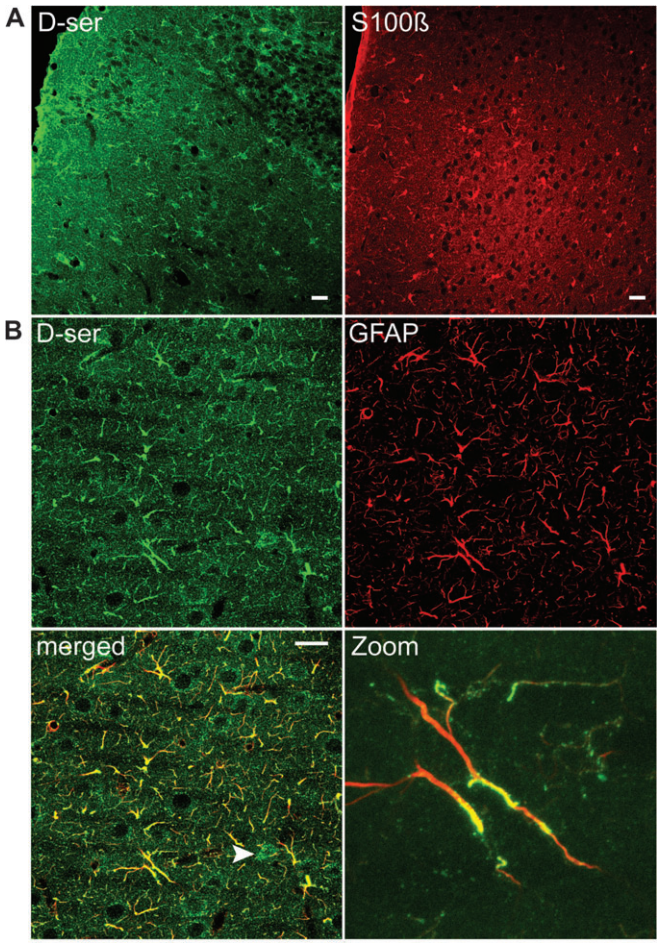

Figure 5. Cellular distribution of D-serine in the mPFC layers V/VI. (*A*) Low-magnification view of the distribution of D-ser (1/1000, Alexa 488) and S100β (1/2000, Alexa 546) throughout the PFC layers. (*B*) Double immunostainings showing the cellular distribution of GFAP (1/1 000, Alexa 546) versus D-serine (1/1 000; Alexa 488) in mPFC layers V/VI. Scales: 25 μm. Arrowheads highlight the presence of labeled soma of neurons.

cortical neurons as indicated by the absence of changes in membrane resting potential (−65.3 ± 1.3 mV at t = 0 min and −65.36 ± 1.8 mV at t = +45 min, n = 11), amplitude of action potential (77.2 ± 2.6 mV at t = 0 min and 73.5 ± 2.4 mV at t = +45 min, n = 11), and spike threshold (−36.5 ± 1.7 mV at t = 0 min and −37.7 ± 2.2 mV at t = +45 min, n = 11). The increase in AMPA/NMDA ratio induced by FAC application could be completely restored to its control values by adding exogenous D-ser (100 μM) to the slice (FAC + D-ser vs. control: 1.60 ± 0.25, P = 0.14) (Fig. 6*A*). These data strongly suggest that glia, through the release of D-ser, gates NMDARs in layers V/VI mPFC pyramidal neurons of the prelimbic area.

Given the contribution of glia to synaptic transmission, we next examined their role in the induction of LTP which relies on D-ser and which is NMDA dependent (Fig. 4). No LTP could be induced in FAC-treated slices (91.4 ± 14.3% of control, n = 6, P = 0.54, Fig. 6*C*), a result similar to what was observed in the presence of AP5 or *Rg*DAAO and in agreement with the requirement of glia and D-ser to activate NMDARs. Exogenous applications of D-ser reversed this LTP impairment allowing the induction of a potentiation equivalent to that obtained under control conditions (158.4 ± 31.2% of control, n = 7, P = 0.03, Fig. 6*B*). Therefore, these data indicate that glia, through the release of D-ser, regulates activity of excitatory synapses in the mPFC.

Discussion

The data documented in this study suggest that, under the present experimental conditions at least, in layers V/VI PFC pyramidal neurons of prelimbic area, D-ser, but not glycine, is the endogenous ligand of glycine-binding site of synaptic NMDARs. This D-amino acid appears to be mainly derived from astrocytes rather than neurons and is required for long-term synaptic plasticity induction in the mature PFC of rodents.

Relevance of the Rat Prelimbic Area as an Analog Structure of Primate PFC

We focused our study on the prelimbic area (area 32) inside the mPFC of rats (Gabbott et al. 2005). Although the existence of a proper prefrontal cortex in rodents is controversial, there

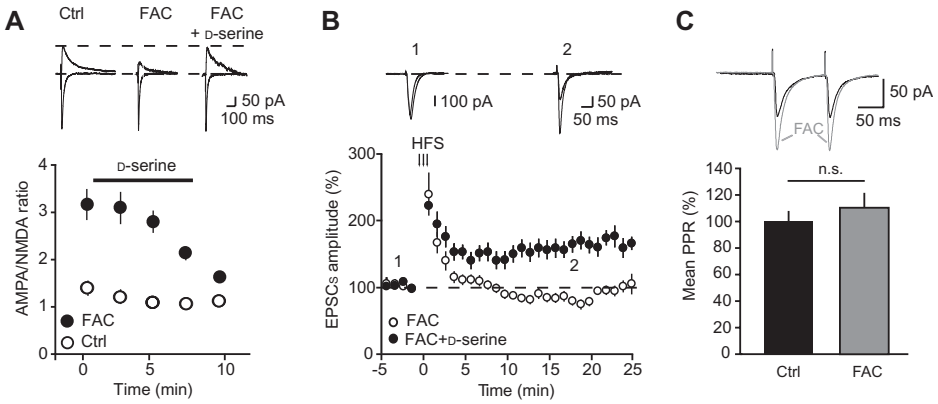

Figure 6. Glial cells control NMDA receptors in layers V/VI pyramidal cells. (*A*) In slices incubated for 40 min in FAC (a metabolic glial inhibitor), AMPA/NMDA peak current ratio is strongly increased. This effect was abolished by further application of 100 μM D-ser (black bar). All NMDA-EPSCs and AMPA-EPSCs were recorded at +40 and −70 mV holding potentials, respectively. (*B*) NMDA-dependent LTP is under glial control. Indeed, LTP is suppressed in slices incubated in FAC and maintained in slices incubated in FAC plus D-ser. (*C*) PPR is not affected when glia is functionally eliminated by FAC.

is strong evidence that prelimbic and infralimbic areas of mPFC in rats are homologue of the dorsolateral prefrontal cortex of primates (Uylings et al. 2003). First, anatomical studies have shown that projections of mPFC in rats to subcortical target nuclei are very similar to that of ventromedial PFC in primates in particular for monoaminergic and cholinergic systems (Uylings and van Eden 1990; Jodo and Aston-Jones 1997; Hajós et al. 1998; Jodo et al. 1998; Carr and Sesack. 2000). Second, the mPFC in rats and the dorsolateral PFC in primates share similar structural and laminar organizations and functions (Gabbott et al. 2005). Indeed, prelimbic area is implicated in working memory, selection of information, response selection and implementation, and dynamic goal-directed behavior (Uylings et al. 2003; Gabbott et al. 2005). Then, we can conclude that although rat PFC is not as differentiated as it is in primates, prelimbic area is centrally involved in some dorsolateral-like characteristics allowing using it as an analog structure of primates PFC.

A Role for D-Ser in NMDAR-Dependent Functions at Excitatory Synapses

Activation of NMDARs requires binding of a coagonist which was first proposed to be glycine (Johnson and Ascher 1987). It has, however, become clear over the last decade that D-ser can play an equivalent role (Wolosker 2007). In hippocampal neurons cocultured with astrocytes, degradation of D-ser with DAAO affected NMDAR-mediated responses (Mothet et al. 2000). This finding was subsequently extended to acute slice preparations from hippocampus (Yang et al. 2003; Mothet et al. 2006; Zhang et al. 2008) and to other structures such as the retina (Stevens et al. 2003; Kalbaugh et al. 2009) and the hypothalamus (Panatier et al. 2006). Moreover, studies in SR-knockout (KO) mice also suggest that D-ser is an endogenous coagonist of synaptic NMDARs in the hippocampus (Basu et al. 2009). Correspondingly, NMDAR-mediated synaptic currents are impaired and LTP is blunted in SR-KO mice. We here report that selectively degrading D-ser with *Rg*DAAO causes a pronounced but incomplete inhibition of NMDAR-mediated EPSCs, an effect that is neutralized by application of exogenous D-ser. This finding indicates that D-ser is an endogenous coagonist of NMDARs expressed by layers V/VI PFC pyramidal neurons (Wang et al. 2008). The incomplete blockade of synaptic transmission by *Rg*DAAO may reflect incomplete depletion of synaptic D-ser by *Rg*DAAO or a complementary contribution of endogenous glycine to the gating of synaptic NMDARs in the PFC.

In fact, although several studies have examined the possibility that D-ser is the endogenous coagonist of synaptic NMDARs, few have investigated whether or not glycine plays such a role. Using *Bs*GO, an enzyme that specifically degrades glycine, it was established that this amino acid was not an endogenous ligand of the NMDAR glycine-binding site in supraoptic neurons of the rat hypothalamus (Panatier et al. 2006). We report here similar findings at intrinsic excitatory synapses in layers V/VI pyramidal PFC neurons since *Bs*GO, unlike *Rg*DAAO, did not affect NMDAR-mediated EPSCs although the levels of glycine were significantly reduced by the enzyme as we confirmed it by CE-LIF analysis. These findings are consistent with previous observations that D-ser is the dominant endogenous ligand of synaptic NMDARs during adulthood in the CNS (Mothet et al. 2000; Yang et al. 2003; Shleper et al. 2005; Panatier et al. 2006). They also suggest that glycine might not usually be present at sufficient concentrations within the synaptic cleft to serve as an endogenous coagonist of synaptic NMDARs, in contradiction to what is often described in the literature. CE-LIF measurements of the PFC content of amino acids revealed a significantly higher concentration of glycine than D-ser that may question this assertion. However, it is plausible that high affinity glycine transporters GlyT1 and GlyT2 expressed by PFC glia and neurons (Jursky and Nelson 1996; Chen et al. 2003; Cubelos et al. 2005) suppress glycine levels in the local microenvironment of pyramidal synaptic NMDARs well below their affinity for the coagonist (range 0.36–3.7 μM) (Laurie and Seeburg 1994). Coexpression of both GlyT1 and NMDARs in *Xenopus* oocytes dramatically reduces the level of glycine capable of reaching the coagonist site (Supplisson and Bergman 1997). Similarly, inhibition of GlyTs potentiates NMDA-mediated responses in the prefrontal cortex (Chen et al. 2003). Accordingly, we observed a modulatory effect of endogenous glycine on NMDA-EPSCs only when GlyTs were initially inhibited with sarcosine. Although the potentiating effect of sarcosine on synaptic NMDA-EPSCs was only modest, this is

likely due to the fact that endogenous D-ser competes with glycine for the coagonist site of synaptic NMDARs (Paoletti and Neyton 2007). Indeed, we showed in the present study that endogenous glycine could exert its modulatory action on synaptic NMDARs only if D-ser has been first removed (Figs 4 and 5). In other cases, glycine has no modulatory effect when D-ser is present in the synaptic cleft under basal conditions or at saturating levels (Fig. 2). It is conceivable that GlyTs prevent the amino acid from entering the synaptic cleft under physiological conditions (Betz et al. 2006). Nevertheless, we cannot discard the possibility that glycine may reach the synapse and then gates the synaptic NMDARs in pathological conditions wherein activity of GlyTs is altered/disrupted. The likelihood of this scenario is supported by studies showing that GlyTs inhibitor displays antipsychotic profiles in rodents (Tsai et al. 2004). Furthermore, genetically induced reduction of GlyTs expression in mice leads to impairment in glutamatergic neurotransmission in the hippocampus (Tsai et al. 2004; Martina et al. 2005) and retina (Reed et al. 2009). Thus, the relative contribution of glycine versus D-ser at synaptic NMDARs implies that the release of D-ser is operating locally at synaptic sites to underpin synaptic functions (Schell et al. 1997; Mothet et al. 2000; Henneberger et al. 2010).

Our demonstration that D-ser is the endogenous ligand for the glycine site of synaptic NMDARs prompted an evaluation of its participation in long-term synaptic plasticity since LTP at excitatory synapses relies on NMDAR in particular in the cerebral cortex (Feldman 2009). Not surprisingly perhaps, we observed a complete blockade of LTP when D-ser was depleted by *Rg*DAAO. As LTP and its counterpart long-term depression have been proposed as cellular substrate of information processing and memory (Malenka and Bear 2004; Feldman 2009; Rebola et al. 2010), the observation that LTP depends on D-ser further expands the notion that D-ser plays an important function in the cognitive processes. In the present study, we observed that the blockade of NR2B-containing synaptic NMDARs with ifenprodil reduced the total synaptic NMDA current by ~30%. These results indicate that NMDARs at excitatory synapses at PFC layers V/VI pyramidal neurons are preferentially composed of NR2A subunits as previously shown (Zhao, Toyoda, et al. 2005) and that NR2B subunits represent only a small fraction (Wang et al. 2008). Noteworthy, both NR2B and NR2A NMDAR subunits contribute to the formation of LTP in the PFC where NR2B-containing NMDARs are engaged in the formation of contextual memory (Zhao, Toyoda, et al. 2005).

Astrocytes Are Involved in the Control of NMDARs

Many lines of evidence suggest that D-ser is synthesized and released from glia. Immunostaining for D-ser and SR revealed the presence of the D-amino acid and its synthesizing enzyme in astrocytes from different brain regions in adulthood (Schell et al. 1997; Stevens et al. 2003; Panatier et al. 2006; Williams et al. 2006). Using neuron–glia cocultures and pure neuronal cultures treated with glia medium, it was originally established that glia was the source of D-ser in the hippocampus (Mothet et al. 2000; Ribeiro et al. 2002; Yang et al. 2003; Zhang et al. 2008). Release of D-ser from (Mothet et al. 2005) and trafficking within (Martineau et al. 2008) cortical glial cells in culture indicated that astrocytes could release D-ser through a Ca^{2+}-dependent vesicular pathway. The idea that glia represents an important source for D-ser was strengthened by observations made in the hypothalamus where a physiological withdrawal of astrocytic processes was found to be associated with a deficiency in D-ser-mediated NMDAR EPSCs (Panatier et al. 2006). Still, it has been also established that in brain areas such as the hippocampus, the cerebral cortex, or the vestibular nuclei, D-ser may originate from neurons (Puyal et al. 2006; Kartvelishvily et al. 2006; Miya et al. 2008; Rosenberg et al. 2010).

In the present study, we show by immunostaining that D-ser is mainly present in astrocytes (see Fig. 5). By contrast, PFC neurons seem to represent only a minor source of D-ser in a physiological context. Nevertheless, the localization of SR remains controversial. In some areas like the hypothalamus (Panatier et al. 2006), SR could be found localized mainly to glia which is consistent with the fact that only glia and notably astrocytes have the ability to synthesize L-serine, the substrate of SR (Yamasaki et al. 2001). But SR is also found in neurons in the cerebral cortex or in the hippocampus (Kartvelishvily et al. 2006; Miya et al. 2008; Rosenberg et al. 2010). In an elegant model developed recently, Herman Wolosker proposed that glia synthesizes and exports L-ser which is used by neuronal SR for the synthesis of D-ser. Once released by neurons, D-ser will be uptaken, stored, and released in an activity-dependent manner by glia (Wolosker 2011). According to this model, neurons and not glia cells represent the main locus for the synthesis of D-ser. Although neurons express ASCT1 and ASCT2 allowing for the potential shuttling of L-ser from glia to neurons (Gliddon et al. 2009; Shao et al. 2009), PFC pyramidal neurons are probably unable to synthesize large amount of D-ser. Thus, the low amount of D-ser in PFC neurons could be attributed to the presence of DAAO which degrades the amino acid once taken up by neurons (data not shown). We cannot exclude that PFC neurons may synthesize and release D-ser when depolarized (Rosenberg et al. 2010) through a nonexocytotic mechanism. Nevertheless, the relative contribution of neuronal versus glial-derived D-ser on NMDARs-mediated EPSCs and LTP could be unmasked pharmacologically using FAC, an astroglial aconitase inhibitor (Hassel et al. 1997; Hülsmann et al. 2003; Andersson et al. 2007; Okada-Ogawa et al. 2009). In addition, we have shown by CE-LIF that FAC specifically reduced D-ser levels, supporting the idea of a control of NMDA-EPSCs by glial D-ser. FAC poisoning of glia reduces NMDA-EPSCs and completely blocks LTP induction, in line with the idea that astrocytes are involved in the control of NMDARs activity through the release of D-ser (Stevens et al. 2003; Yang et al. 2003; Zhang et al. 2008). Still, we cannot rule out fully a contribution of neuronal D-ser in regulating synaptic NMDARs as FAC does not totally block NMDA-EPSCs. One possible explanation is that the residual currents are modulated by the neuronal reserve of D-ser released through a sodium dependent antiport as proposed by the group of Wolosker (Ribeiro et al. 2002; Kartvelishvily et al. 2006; Rosenberg et al. 2010). It is also conceivable that FAC indirectly affects the release of D-ser from neurons rather than inhibiting the release of the coagonist from glia. Indeed, inhibition of the glial aconitase by FAC leads to accumulation of citrate and to a reduction in the formation of glutamine, an important precursor for neurotransmitter glutamate and γ-aminobutyric acid (GABA) (Hamilton and Attwell 2010).

Under conditions of sustained $GABA_A$ receptor blockade, FAC might theoretically inhibit glial glutamine levels thereby affecting the neuronal synthesis of glutamate and in turn the release of D-ser from glia or neurons (Kartvelishvily et al. 2006).

However, this latter scenario can be ruled out since FAC did not affect the release of neuronal glutamate as suggested by the absence of any changes in the amplitude of AMPA-EPSCs or PPR as expected if FAC was impacting the glutamine–glutamate cycle (Fig. 6*C*). If there was a neural source of D-ser in the PFC, functionally eliminating glia with FAC would not be expected to impact synaptic transmission as neuronal D-ser will compensate (at least partially) the pharmacologically induced deficit in extracellular D-ser and then result in unaffected synaptic transmission, but the opposite was observed. These observations indicate that the use of FAC selectively targeted the glial contribution to synaptic transmission and unmasked the role of glia-derived D-ser. It has been shown that in situ administration of FAC decreases the level of D-ser by 25% in the PFC (Kanematsu et al. 2006) supporting our observations that FAC impacts synaptic transmission by selectively reducing the levels of extracellular D-ser and not glycine (Supplementary Fig. 3). Interestingly, we observed that FAC also impacts the levels of extracellular glutamate indicating that glutamate is also released by glia in the PFC as demonstrated in the hippocampus (Fellin et al. 2006). Still, the inhibitory effect of FAC on synaptic transmission and plasticity is fully prevented when D-ser is added showing that D-ser is the limiting extracellular factor for NMDA receptors activity in the PFC. Nevertheless, we cannot rule out the possibility that neuronal D-ser may also be important for the function of NMDARs when neuronal DAAO activity is impaired as observed in schizophrenia (Schumacher et al. 2004; Millan 2005; Almond et al. 2006; Ross et al. 2006; Verrall et al. 2007; Sacchi et al. 2008; Labrie et al. 2010). The nature of the interplay between neuronal and glia-derived D-ser will require further explorations.

Physiological and Pathological Relevance

In the prefrontal cortex, NMDARs dynamically control neuronal circuitry and subserve cognitive functions such as working memory, decision making, and experience-dependent plasticity (Malenka and Bear 2004; Feldman 2009). Therefore, the control of D-ser and its metabolic enzymes may be crucial for physiological processes depending on NMDARs in the mature cerebral cortex (Malenka and Bear 2004; Feldman 2009). Moreover, alterations of D-ser levels and function should have important consequences for the activity of neuronal circuitry in the PFC. Accumulating experimental evidence shows that cognitive symptoms of schizophrenia are related to a hypofunction of NMDARs in the PFC (Millan 2005; Ross et al. 2006). Interestingly, convergent lines of evidence suggest an involvement of perturbed D-ser transmission in schizophrenia (Ross et al. 2006; Bendikov et al. 2007; Verrall et al. 2007; Sacchi et al. 2008; Labrie et al. 2009). Indeed, single polymorphisms for SR and DAAO have been linked to schizophrenia (Schumacher et al. 2004; Detera-Wadleigh and McMahon 2006; Pollegioni and Sacchi 2010). In rodents, genetic loss of DAAO activity reverses schizophrenia-like phenotypes (Almond et al. 2006; Labrie et al. 2010). Coadministration in patients of D-ser with conventional neuroleptics partially ameliorated the negative and cognitive symptoms of schizophrenia in certain though not all studies (Tsai et al. 1998; Heresco-Levy et al. 2005). Noteworthy, Kantrowitz et al. (2010) have reported recently that administration of high doses of D-ser alone ($\geq$60 mg/kg/day) in a 4-week trial displays effectiveness in treatment of both persistent symptoms and neurocognitive functions in antipsychotics-stabilized patients with schizophrenia or schizoaffective disorder. Although yet to be confirmed, the results already opened novel perspectives for treatment of refractory symptoms in schizophrenia by D-serine-based therapy. The demonstration that D-ser controls the activity of NMDARs and long-term synaptic plasticity supports therapeutic strategies targeting D-ser in the treatment of schizophrenia and other brain disorders involving either under or over activity of cortical populations of NMDARs.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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