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Supplementary Information for

Title: The NMDA receptor activation by D-serine and glycine is controlled by an astrocytic Phgdh-dependent serine shuttle

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Supplemental Methods

Materials- CBR-5884 was obtained from Tocris, dissolved at 40 mM in DMSO and stored at -20 ºC. Lu AE 00527 was provided by H. Lundbeck A/S (Denmark), dissolved at 10 mM in DMSO and stored at -20 ºC. D-[^{3}H(G)] serine (NET10922) and D-[1^{4}C(U)]glucose (NEC042X) were obtained from Perkin Elmer (Boston, MA). Unlabeled neutral amino acids, phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma. Basal Medium Eagle and fetal bovine serum were obtained from Biological Industries (Beit Haemek, Israel). Other reagents were of analytical grade.

[^{14}C]amino acid synthesis in acute cortical slices- Mice were anesthetized with isoflurane and killed by decapitation. The cortices were dissected, chopped into strips measuring 400 µm by 400 µm using a McIlwain tissue chopper. Slices were washed 3 times with reduced glucose aCSF buffer, composed of (in mM): NaCl 124, KCl 3.5, MgSO4 1.5, CaCl2 2.3, NaHCO3 26.2, NaH2PO4 1.2, glucose 2 and sucrose 9, pH 7.4) and continuously bubbled with 95% O_2/ 5% CO_2. Slices were divided into tubes; each containing 2.7 ml of oxygenated aCSF supplemented with D-[^{14}C]glucose and 1 mM kynurenic acid to prevent glutamate excitotoxicity. Aliquots of D-[^{14}C]glucose were dried in a speed-vacuum and dissolved in aCSF immediately before use. The slices were incubated for 2 h at 37 ºC with or without 40 µM CBR-5884. Then the slices were transferred to ice and washed 3 times with 10ml cold aCSF. After the aCSF was decanted, the free amino acids were released by adding 0.3 ml 10% trichloroacetic acid (TCA) followed by sonication and removal of precipitated protein by a 30 min centrifugation at 16,000 x g. TCA was removed by extraction with 5 volumes of water-saturated ether (repeated 6 times), and the samples were analyzed by HPLC as described elsewhere (1). The peaks corresponding to serine enantiomers and glycine were collected in vials, and the radioactivity was monitored by scintillation counting. Synthesis of [^{14}C]glycine was corrected for the loss of one carbon upon conversion of L-serine into glycine. The results were expressed as fractional synthesis from the total labeling before the HPLC separation.

Ex vivo electrophysiology in hippocampal slices- Experiments were carried out according to the European Council Directive (86/809/EEC) regarding the care and use of animals for experimental procedures, and approved by the local ethics committee. Transverse hippocampal slices (400 µm) were obtained from C57Bl6 wild-type (WT) or constitutive SR-KO mice. The animals were anesthetized with halothane before decapitation. Slices were prepared in ice-cold artificial cerebrospinal fluid (aCSF) and placed in a holding chamber for at least 1 h. The composition of aCSF was as follows (in mM): NaCl 124, KCl 3.5, $MgSO_4$ 1.5, $CaCl_2$ 2.3, $NaHCO_3$ 26.2, NaH_2PO_4 1.2, and glucose 11, pH 7.4. A single slice was transferred to the recording chamber at a time and continuously submerged with aCSF pre-gassed with 95% O_2/ 5% CO_2. Extracellular recordings were obtained at room temperature (RT) from the apical dendritic layer of the CA1 area using micropipettes filled with 2M NaCl. Presynaptic fiber volleys (PFVs) and AMPAR-mediated field excitatory postsynaptic potentials (fEPSPs) were evoked in control aCSF by electrical stimulation of Schaffer collaterals and commissural fibers located in the stratum radiatum. The averaged slope of three PFVs and fEPSPs was measured using Win LTP software (2), and the fEPSP/PFV ratio was plotted against stimulus intensity (300, 400, and 500 µA). Paired-

pulse facilitation (PPF) of basal synaptic transmission was induced by electrical stimulation of afferent fibers with paired-pulse (inter-stimulus interval of 30ms). PPF was calculated as the ratio of the slope of the second response over that of the first one. Specific NMDAR-mediated fEPSPs were isolated in slices perfused with aCSF containing low Mg^{+2} (0.1 mM) and supplemented with NBQX (10 µM). The effect of exogenous D-serine (100 µM) was assessed by determining the fEPSP/PFV ratio 15 min after the addition of the amino acid to the aCSF.

To investigate LTP of synaptic transmission, a test stimulus was applied every 10 sec in control medium and adjusted to get an fEPSP with a baseline slope of 0.1 V/sec. The averaged slope of 3 fEPSPs was measured for 15 min before the delivery of high-frequency stimulation (HFS), consisting of 1 train at 100 Hz pulses for 1 s at the test intensity. Testing with single pulses was then resumed for 60 min to determine the level of LTP.

In pharmacological experiments with CBR-5884, slices were incubated at 32-34°C in 150 ml aCSF supplemented with the Phgdh antagonist (40 µM). Lower temperatures were avoided to prevent precipitation of the drug. Recordings were then obtained between 2h 30 and 6 h after starting the incubation and done in CBR-contained aCSF throughout.

For LTP experiments testing the effects of L-serine in the inhibitory activity of CBR-5884, slices were incubated with highly pure L-serine (>99.9%) together with CBR-5884 or CBR-5884 alone for at least 2 h and transferred to the recording chamber perfused with the respective aCSF.

For the experiments with serine dehydratase enzyme (SDH), slices were incubated in 150 ml of an aCSF solution containing 0.15 mg/ml (0.9 U/ml) of the enzyme. For NMDAR fEPSPs recordings, the slices were recorded between 3h 30 and 5 h after starting the incubation. The incubated slices were perfused with successive ringer solutions (0.1 Mg^{+2} and then 0.1 Mg^{+2} with 10 µM NBQX) to isolate the NMDAR component, which all contained SDH at the same concentration than the incubation. In LTP evaluation, slices were incubated in 50 ml of Ringer solution containing 0.44 mg/ml (2.64 U/ml) of SDH and were recorded between 3 h and 8 h after starting the incubation. When transferred to the recording chamber for LTP expression, slices were perfused with ringer containing 0.22 mg/ml (1.32 U/ml) SDH throughout the recording.

***Xenopus Oocytes Preparation, DNA constructs, and mRNA preparation*-** Experiments were approved by the Technion Institutional Animal Care and Use Committee (permit no. IL-129-09-17). *Xenopus laevis* oocytes were harvested, prepared, and injected with mRNA as previously described (3). Briefly, oocytes were obtained from anesthetized (0.4% tricaine solution) female frogs. Oocytes were extracted from ovaries, defolliculated by collagenase treatment in ND96 Ca^{+}-free solution (in mM: 96 NaCl, 2 KCL, 1 $MgCl_2$, 5 HEPES, pH 7.4) for about 45 minutes at RT. Cells were washed with ND96 Ca^{+}-free and transferred to NDE96 consisting of ND96 supplemented with 1.8 mM $CaCl_2$, 2.5 mM sodium pyruvate, 100 µg/ml streptomycin nd 62.75 µg/ml penicillin. Stage V oocytes were isolated, incubated overnight at 18 °C and injected with mRNA the next day. For mRNA preparation, rat GluN1a- and GluN2A-subunits were subcloned into pGEM-HE expression vector, linearized by NheI, followed by *in vitro* mRNA transcription using the mMessage-mMachine T7 kit (Thermo Scientific). mRNA concentrations were determined using a

spectrophotometer. Oocytes were injected with 5 ng/oocyte mRNA of each subunit at 1:1 ratio.

Electrophysiology in oocytes-Two electrode voltage clamp (TEVC) recordings were carried out in oocytes injected on the day before, as previously described (4). Recordings were performed using an Amplifier (Warner Instruments, USA) and Digitizer (Digidata-1550B; Molecular Devices, USA), controlled by pClamp10 software (Molecular Devices, USA). Electrodes consisted of pulled glass capillaries with chlorinated silver wire (Narishige, Japan), and filled with 3M KCl. Data were analyzed by Clampfit software (Molecular Devices, USA). Oocytes were clamped at -60 mV and perfused with Mg^{2+}-free bath recording solution (in mM: 100 NaCl, 0.3 $BaCl_2$, 5 HEPES, pH 7.3; adjusted with KOH). Receptors were activated using 10 μM of both D-serine and glutamate. CBR-5884 was dissolved in DMSO and applied at 40 μM. The solutions without CBR-5884 were supplemented with the same amount of vehicle (DMSO 0.1%). All solutions were warmed to 37 °C before the experiment to dissolve CBR-5884 completely. Solutions were continuously monitored for drug precipitation.

Recombinant serine dehydratase purification and assay- The cDNA for rat serine dehydratase (SDH) (NP_446414.3) was synthesized with codon optimization for bacterial expression (Genscript, USA) and cloned into pET28c(+) at sites NdeI/BamHI in frame with an N-terminal His-tag and transformed into BL21 codon plus bacteria. After inducing SDH expression for 4 h at 37 °C, the culture was transferred to 16°C for 18 hours. Then bacterial pellets were suspended and lysed by sonication in a buffer containing 50 mM sodium pyrophosphate (pH 7.4), 400 mM NaCl, 0.4 mM phenylmethylsulfonyl fluoride, 10 μM pyridoxal 5'-phosphate (PLP), 1 mM DTT and 10 mM imidazole. Triton X-100 was then added to 1% final concentration, and after a 20 min centrifugation at 30,000 x g, the recombinant N-terminal His-tagged SDH was batch purified by binding to Ni-NTA beads (Qiagen) for 2 h under rotation at 4°C. The beads were washed extensively in buffer containing 50 mM sodium pyrophosphate (pH 7.4), 400 mM NaCl, and 10 mM imidazole. SDH was eluted in buffer containing 10mM sodium pyrophosphate (pH 8.5), 400 mM imidazole and 10% glycerol and dialyzed overnight against 10 mM sodium pyrophosphate (pH 8.5), 10% glycerol and 2 μM PLP. The protein was concentrated to 30 mg/ml with using Centriprep (Merck) and stored at -80°C. One unit corresponded to the amount of the enzyme that degrades 1 μmol L-serine/min at 37 °C in PBS (pH 7.4) and 100 mM L-serine.

In vitro SR activity - N-terminal His-tagged human SR (5) was expressed and purified as described previously (6). SR racemization assays were carried out at 37 °C in 50 mM triethanolamine-HCl (pH 8.0), 1 or 5 mM L-serine, 2 mM ATP, 2 mM $MgCl_2$, 150 mM NaCl, 50 μM PLP and 5.1 μM SR, in the absence and presence of 1 mM glycine. The β-elimination assay was performed using 0.4 μM SR in the same buffered solutions, except for the presence of 5 mM DTT. For testing the effect of CBR-5884, we carried out SR racemization reaction for 2 h at 37 °C with and without 40 μM CBR-5884, in buffer containing 20 mM Tris-HCl (pH 7.4), 1 mM L-serine, 2 mM ATP, 2 mM $MgCl_2$, 50 mM NaCl, 50 μM PLP, 4 mM DTT and 1.7 μM mouse SR, and analyzed by HPLC as described (7).

SR activity in HEK293 transfected cells- Cells were cultured in DMEM and 10% fetal calf serum and transfected in 24 well plates (1.7×10^5 cells/well) either with untagged SR-WT or SR-K56G in pRK5 vector using Lipofectamine 2000 according to the manufacturer's protocol (Invitrogen). Forty-eight hours after transfection, the cells were washed with serum-free BME medium, which lacks L-serine and glycine, and supplemented with 0.4 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Then the cells were incubated for 4 hours with medium containing 2 mM L-serine, which was rendered free of contaminant D-serine (8), in the presence of either vehicle (DMSO 0.1 %) or 40 µM CBR-5884. Synthesis of D-serine was determined by adding TCA 5%, and total D-serine (cells and medium) produced was monitored by HPLC analysis, and normalized by the amount of protein/well.

Primary cell cultures- Pregnant Sprague-Dawley rats were anesthetized with isoflurane and killed by decapitation with the approval of the Committee for Supervision of Animal Experiments (Technion-Israel Institute of Technology). Serum-free neuronal cultures from the cerebral cortex were prepared from E16–17 as described previously (9) with the following modifications. Cells were plated on the day of isolation in Neurobasal medium supplemented with 2% B27 and 100 U/ml penicillin and 100 µg/ml streptomycin. On DIV1, the culture medium was changed to in-house made Neurobasal medium containing four times less L-serine (0.1 mM) and no glycine. Afterward, half of the culture media was replaced every 2-3 days with the in-house neurobasal medium. Biochemical experiments were carried out at DIV8–12. Primary astrocyte cultures were obtained from the cerebral cortex of P0-P1 Sprague-Dawley rat pups. The cells were seeded in 12 well plates (Nunc) but without poly-D-lysine and cultured in Basal Medium Eagle (BME) medium supplemented with 10% FBS, 0.4 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. At the second day in culture, the wells were vigorously washed with sterile PBS to dislodge poorly adherent neurons and microglia. This procedure was repeated twice every two days.

L-Serine synthesis in astrocyte cultures- Primary astrocyte cultures were prepared as above and used at DIV 14. The cells were washed twice with BME made without serum and therefore lacking any L-serine. Then, fresh BME supplemented with 2% B27 was added and supplemented either with 40 µM CBR-5884 or DMSO vehicle (0.1%). The cells were incubated at 37 ºC for 48 h, and an aliquot of the culture media was analyzed by HPLC. The values of L-serine synthesis were normalized by the amount of protein in the well.

D-Serine synthesis in neuronal cultures- For endogenous D-serine synthesis, cortical neurons were plated in 24-well plates at 0.42×10^6 cells/well using modified Neurobasal-B27 with low L-serine and no glycine as described above. At DIV10, half of the medium was collected and completed with fresh Neurobasal-B27 containing 1 mM L-serine and no glycine. L-Serine was rendered free of contaminant D-serine by treatment with D-serine dehydratase as described previously (8). Forty-eight hours after L-serine addition, an aliquot of the medium was analyzed by HPLC. To monitor the intracellular D-serine, cells were quickly washed 3 times with ice-cold Hepes-buffered saline (HBSS, in mM: 137

NaCl, 5.4 KCl, 0.34 K_2HPO_4, 0.44 KH_2PO_4, 0.41 $MgSO_4$, 0.49 $MgCl_2$, 1.07 $CaCl_2$, 5.6 D-glucose, 10 Hepes pH 7.4) and the free amino acids were released by adding 5% TCA. After removal of denatured proteins by a 10 min centrifugation at 16,000 x g, TCA was removed by extraction with water-saturated diethyl ether and samples analyzed by HPLC. The results were normalized by the protein concentration/well. Blanks consisted of samples treated with D-serine dehydratase before the HPLC analysis as previously described (8) and their values subtracted from the samples.

Determination of brain amino acid levels- C57Bl/6 mice were anesthetized with isoflurane and killed by decapitation. Cerebral cortices and hippocampi were dissected at P0, P4, P8, P12 and P90, The samples were homogenized with 10 volumes of 5% TCA and subsequently processed for HPLC analysis as previously described (1), except that α-aminoadipic acid was substituted for L-homocysteic acid as the internal standard.

Western-blots- Cerebral cortices and hippocampi from P3, P10, and P18 old C57BL/6 WT mice were dissected and homogenized with 20 strokes of a glass homogenizer in 10 volumes of a buffer consisting of 20 mM Tris-HCl (pH 7.4), 50 mM NaCl and protease inhibitor cocktail (Mini-complete, Roche). Samples were cleared by centrifugation at 1500 x g at 4ºC, and 40 µg of extracts were analyzed by SDS-PAGE and Western-blot. Expression levels were quantified by monitoring the densitometry of the chemiluminescent signal in each lane (Image Quant, GE Healthcare) and normalized by their respective β-actin levels.

Antibodies- All antibodies were used at 1:1000 dilution and included anti-AMT (Santa Cruz Biotechnology, sc-99267), anti-β-actin (MP Biomedicals, Clone C4), anti-SR (Santa Cruz Biotechnology, sc-365217), anti-GCSH (Sigma-Aldrich, HPA041368), anti-GLDC (Abcam, ab97625), SHMT1 (Cell Signaling, 12612), SHMT2 (Cell Signaling, 12762).

In vivo microdialysis- Ten week-old C57Bl/6 mice were anesthetized with 100 mg/Kg ketamine and 10 mg/Kg xylazine and positioned in the stereotaxic instrument (Lab Standard with mouse adaptor, Stoelting, Wood Dale, IL). A CMA7 cannula (Harvard Apparatus, Holliston, MA) was implanted in the caudate putamen at the following coordinates with reference to bregma: + 0.8 mm anterior, ± 1.3 mm lateral, and -2.5 mm ventral, and the mice were allowed to recover for 18 h. Then the probe (CMA7, 6KDa, 0.24 mm membrane) was slowly inserted and perfused for 12-18 hours with solution containing (in mM) NaCl 147, KCl 2.7, $CaCl_2$ 1.2, $MgCl_2$ 0.85 at 0.3µl/min. The samples were then collected, and glycine was given i.p. 1 hour after the baseline was established. During perfusion of the probe and collection of the samples, the mice were freely moving and had to access to food and water. The samples were immediately frozen at -80 degrees and analyzed by HPLC. The positioning of the probe was verified by histological analysis of the probe tract.

D-[^{3}H]serine uptake and release from cells- Release of D-serine from HEK293 cells co-transfected with Asc-1 (SLC7a10) and 4F2hc (SLC3A2) was carried out as previously

described (10), except that D-[^{3}H]serine substrate was present at 5 µM in the HBSS buffer used for the transport assays. For release experiments, Asc-1/4F2hc transfected HEK293 cells or primary cortical neuronal cultures were first preloaded with 5 µM D-[^{3}H]serine. Then the wells were washed 4 times with HBSS buffer, and release was initiated by adding new HBSS buffer at RT supplemented with glycine, Lu AE00527 or DMSO 0.1% vehicle for 1-2 min. The released D-[^{3}H]serine (out) was monitored in the release medium by scintillation counting. The remaining intracellular D-[^{3}H]serine (in) was determined after cells lysis with 100 µl of water per well and the fractional release was calculated using the formula: D-[^{3}H]serine out x 100/(D-[^{3}H]serine out + D-[^{3}H]serine in).

D-[^{3}H]serine release from slices- Acute cortical slices from mice were prepared using a McIlwain tissue chopper as previously described (10). After washing in oxygenated Krebs-HEPES buffer (KB) (in mM: 127 NaCl, 1.3 NaH_2PO_4, 15 HEPES, 10 D-glucose, 1 $MgCl_2$, 5 KCl, and 2.5 $CaCl_2$, pH 7.4), the slices were loaded with 10 µM D-[^{3}H]serine for 30 min in oxygenated KB and then transferred to the Suprafusion 1000 (SF-6) apparatus (Brandel). Subsequently, the slices were equilibrated for 30-45 min by perfusion with oxygenated KB in 0.3 ml chambers at a flow rate of 0.6 ml/min at 37 °C. After equilibration, samples were collected at 1.6 min intervals before and after stimulation with glycine. Total D-[^{3}H]serine loading was estimated after the slices were incubated for 20 min with 0.1M HCl to release all intracellular free amino acids. D-[^{3}H]serine was monitored by liquid scintillation counting, and the values expressed as the fractional release (% total).

Endogenous D-serine release from acute cortical slices- Four to five-month-old CAMKIIα-Cre SR-KO or SR fl/fl littermates were anesthetized with isoflurane and killed by decapitation; the cerebral cortices were dissected and treated as described above for D-[^{3}H]serine release, except that there was no preloading step. Endogenous D-serine peak was monitored by HPLC and normalized by the protein content of the slices harvested from the perfusion chambers at the end of the experiment. The samples were spiked with the internal standard aminoadipic acid and quantified by HPLC analysis, as previously described (10).

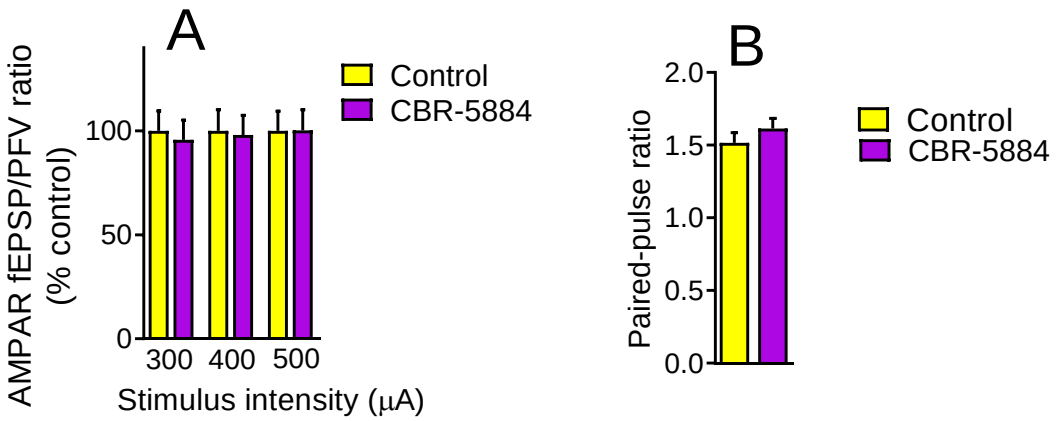

Fig. S1. CBR 5884 does not affect the basal synaptic transmission and paired-pulse ratio- A, the basal synaptic transmission at the Schaffer collaterals-CA1 synapses determined by the AMPAR fEPSP/PFV ratio was unaffected by 40 μM CBR-5884 (n=21 slices). B, CBR-5884 did not affect the paired-pulse facilitation in slices (n=22). All values are average ± SEM.

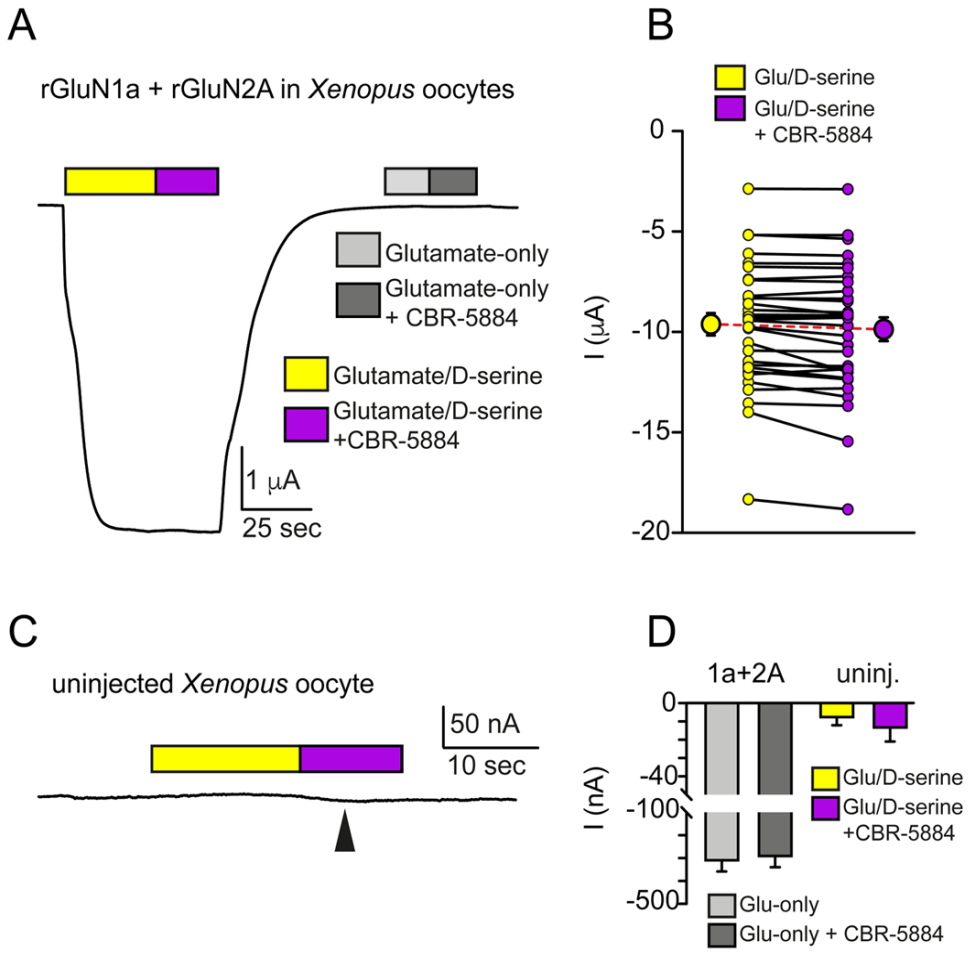

Fig. S2. CBR-5884 does not affect NMDAR currents in oocytes- A, representative recording of GluNR-current obtained from *Xenopus* oocytes co-expressing rat GluN1a and GluN2A subunits. Receptor activation (inward current) was elicited by applying saturating D-serine and glutamate concentrations (10 μM; yellow bar). Co-application of 40 μM CBR-5884 with both ligands (magenta bar) did not inhibit NMDAR currents. Application of glutamate-only (without D-serine; grey bar) did not yield any GluNR-current, nor did co-application of CBR-5884 (dark grey). B, summary of inward currents in the absence and in the presence of CBR-5448 (N=2 batches, n= 31 oocytes). CBR-5448 produced only a negligible 2% increase in the total current, (p=0.002; paired t-test). C, a representative recording from an uninjected oocytes shows lack of response to D-serine and glutamate and only a tiny increase in leak current following CBR-5884 co-administration (arrowhead). D, summary of the effect of CBR-5884 on the leak current in the absence of D-serine (glutamate-only, grey; glutamate with CBR-5884, dark grey) in GluN1a and GluN2A injected-oocytes (denoted 1a+2A, N=2, n=31, p=0.011) and uninjected oocytes (D-serine and glutamate, yellow; D-serine, glutamate and CBR-5884, magenta, N=2, n=9, p=0.07).

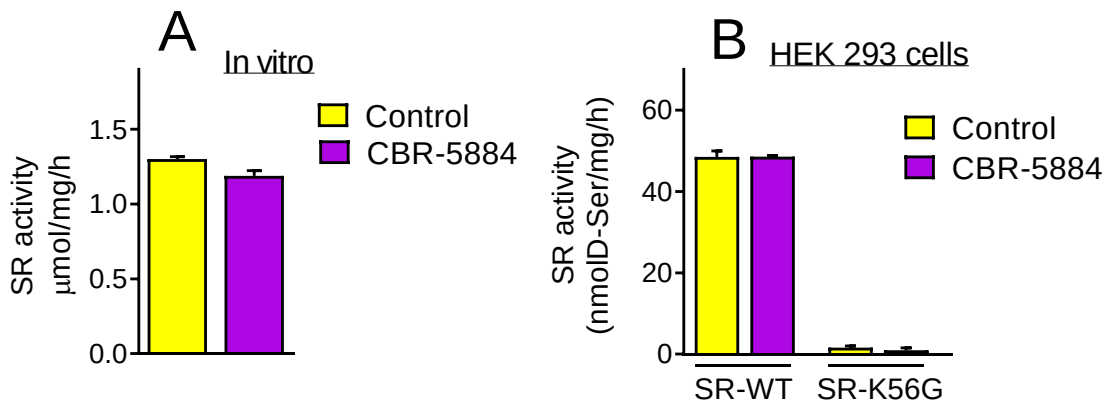

Fig. S3. CBR-5884 does not directly affects SR activity - A, *in vitro* racemization by mouse recombinant SR is virtually unaffected by 40 µM CBR-5884. B, CBR-5884 did not affect the SR activity in HEK293 cells transfected with mouse SR. Blanks consisted of cells transfected with the catalytically inactive SR mutant K56G. The values represent the average ± SEM of three experiments.

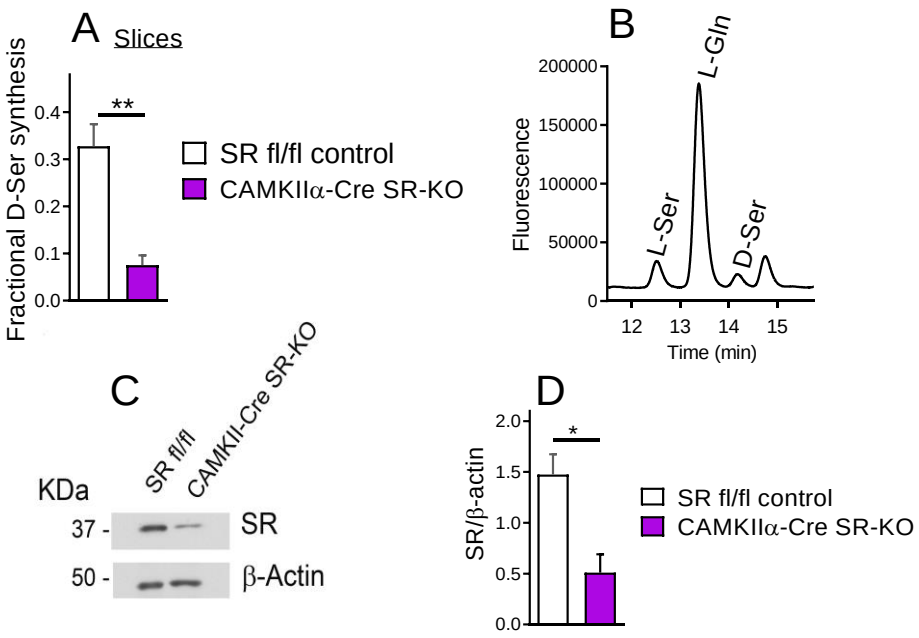

Fig. S4. De novo D-serine synthesis in glutamatergic neurons- A, synthesis of D-[^{14}C]serine synthesis from [^{14}C]glucose in acute cortical slices of 5-month-old SR fl/fl controls or CAMKIIα-Cre SR-KO mice. Values are expressed as D-[^{14}C]-serine normalized by the L-[^{14}C]serine labeling in each sample. Unpaired two-tailed Student's t-test (p=0.0097). B, representative HPLC chromatogram of acute cortical slices depicting the separation of L-serine, L-glutamine, and D-serine used for collecting the radioactive peaks in synthesis experiments. C, representative Western-blot is showing SR expression in 5-month-old SR fl/fl controls and CAMKIIα-Cre SR-KO mice. The lower panel depicts β-actin loading control. D, densitometric analysis of SR expression in SR fl/fl controls and CAMKIIα-Cre SR-KO mice. Unpaired two-tailed Student's t-test (p=0.011). Values are average ± SEM of 3-7 (A) or 5 (D) experiments.

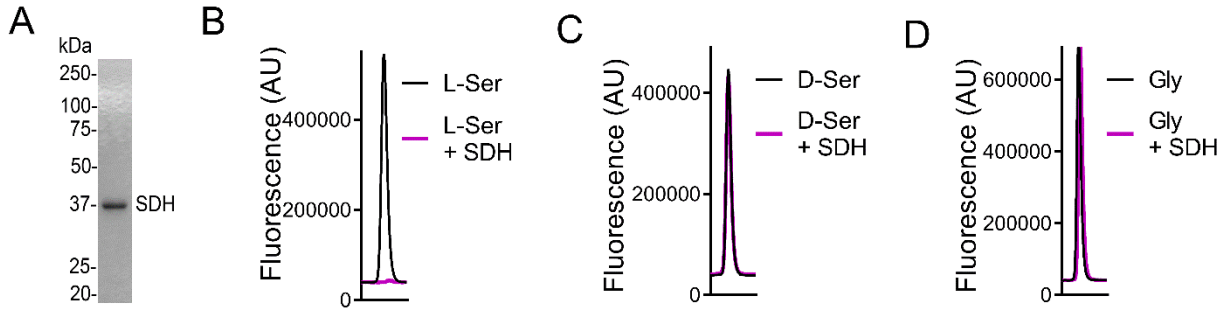

Fig. S5. Recombinant SDH preparation- A, SDS-Page analysis of 2 µg purified recombinant SDH enzyme. B, HPLC analysis of 1 mM L-serine incubated for 30 min in the absence (black) or presence (magenta) of 1 mg/mL SDH. C, HPLC analysis of 1 mM D-Serine incubated for 16 h in the absence or presence of 5 mg/mL SDH. D, same as C, but with 1 mM glycine.

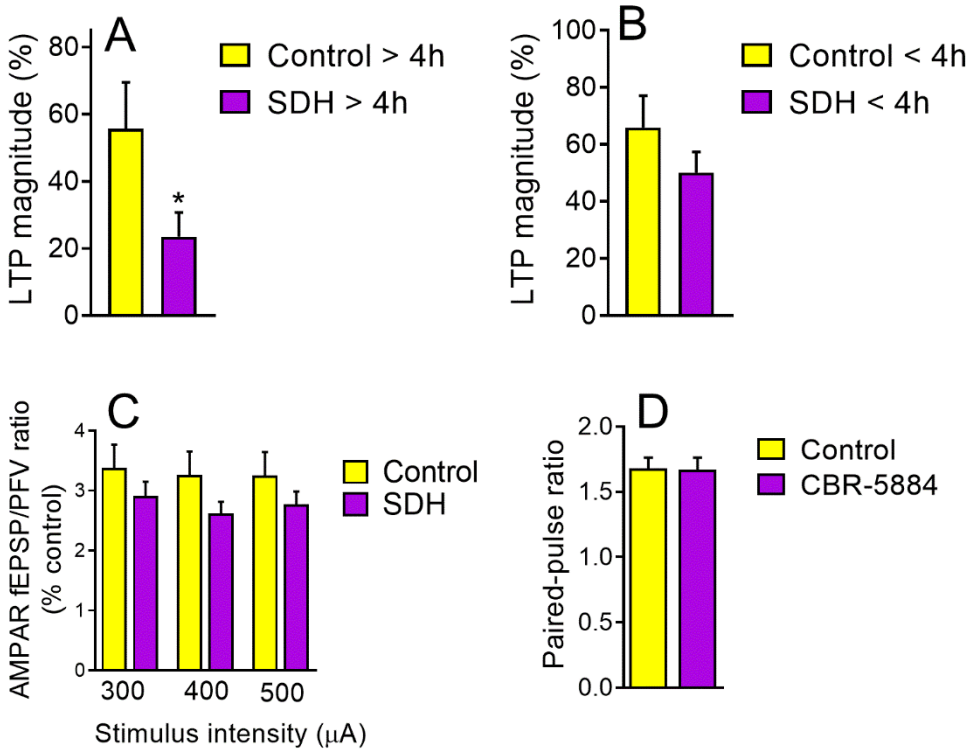

Fig. S6. Time-dependent effect of SDH incubation on the LTP magnitude and impact on basal neurotransmission and paired-pulse facilitation- A, LTP magnitude of the last 15 min recording of Fig. 2A depicting only the pool of slices incubated over 4 h without or with SDH (n=5 and 8 slices, respectively). Unpaired two-tailed Student's t-test (p=0.045). B, LTP magnitude of the last 15 min recording of Fig. 2A including only the pool of slices incubated less than 4 h without or with SDH (n=7 and 5 slices, respectively). Unpaired two-tailed Student's t-test (p=0.30). C, the basal synaptic transmission determined by the AMPAR fEPSP/PFV ratio was unaffected by SDH (n=19 slices). D, SDH did not affect the paired-pulse facilitation (n=19 slices). Values are average ± SEM.

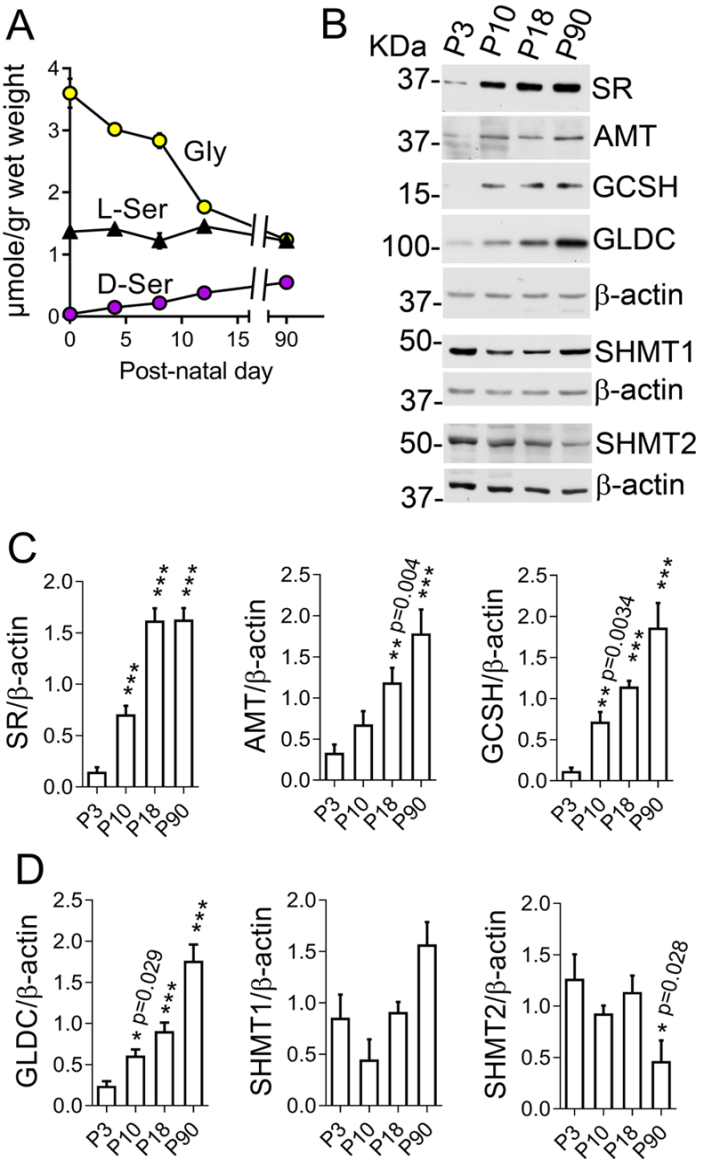

Fig. S7. Postnatal changes in glycine, L-serine and D-serine levels and their metabolic enzymes in the neocortex. A, cortical levels of amino acids in mice. Values are average ± SEM of 5 (P0, P4, P8 and P12) and 10 (P90) animals. B, representative Western-blot analysis of SR, aminomethyltransferase (AMT), glycine cleavage system protein H (GCSH), glycine decarboxylase (GLDC), serine hydroxymethyltransferase 1 (SHMT1) and 2 (SHMT2) and β-actin loading controls. C and D, densitometric analysis of Western-blots like in B (4-7 mice). One-way ANOVA and Dunnet's post hoc test of P10, P18 and P90 values compared to P3. Values are average ± SEM.

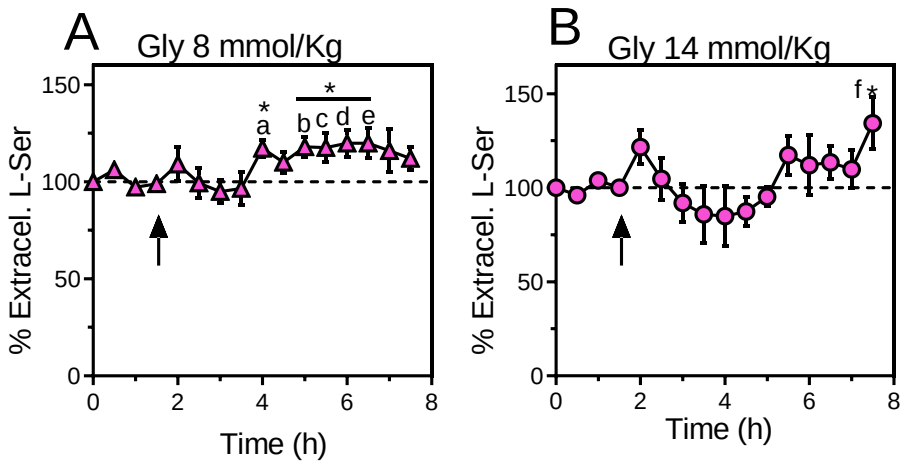

Fig. S8. ***In vivo*** **microdialysis of L-serine levels upon glycine administration**- A, microdialysis of the striatum of 10-week-old mice for L-serine. Injection of 8 mmol/Kg glycine (i.p.) (A) or 14 mmol/Kg glycine (i.p.) (B) was carried out 90 min after the baseline was established (arrow). Values are average ± SEM of 5 (A) or 4 (B) independent experiments. Data were analyzed by repeated-measures one-way ANOVA and Dunnett's post hoc test by comparing the samples collected at different times with the baseline value determined 30 min before glycine injection. a, $p=0.041$; b, $p=0.030$; c, $p=0.036$; d, $p=0.015$; e, $p=0.015$; f, $p=0.048$. Unmarked data points had $p>0.05$.

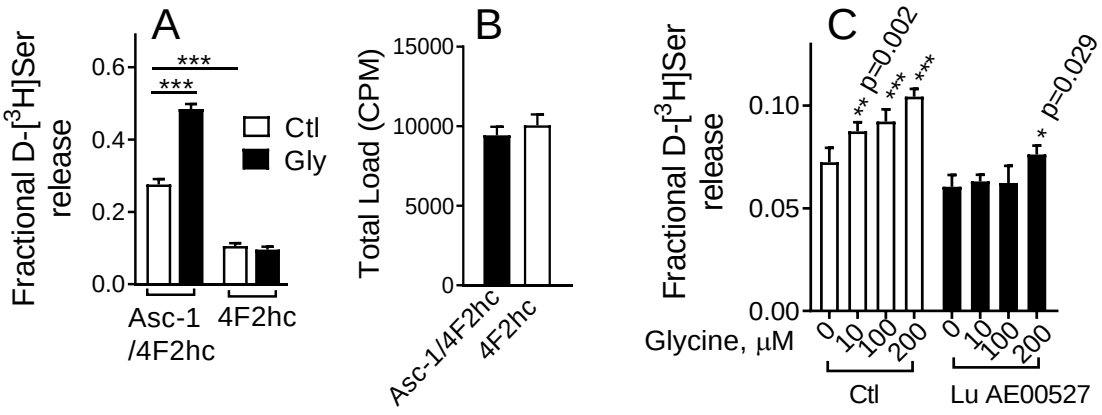

Fig. S9. Glycine releases D-serine from Asc-1 transfected cells and primary neuronal cultures- A, HEK293 cells were transfected with rat Asc-1/4F2hc or with rat 4F2hc alone and release of preloaded D-[^{3}H]-serine elicited by 200 μM glycine. To attain the same level of D-serine loading, Asc-1/4F2hc and 4F2hc cells were preloaded with 10 μM D-[^{3}H]-serine for 10 and 20 min, respectively. Values are average ± SEM of 4 experiments. Repeated measures one-way ANOVA and Bonferroni's post hoc test. B, levels of D-[^{3}H]-serine preloaded in the cells used in A. C, primary neuronal cultures (DIV 8-14) were preloaded for 20 min with 10 μM D-[^{3}H]-serine, and D-serine release was induced by 10, 100 or 200 μM glycine in the absence (open bars) or presence (closed bars) of the selective Asc-1 inhibitor Lu AE00527 (10 μM). Values are average ± SEM of 6 experiments. ***, different from the control (0) glycine at $p<0.001$. Repeated measures one-way ANOVA and Dunnett's post hoc test.

References for supplemental methods

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