Supplemental Data

Glia-Derived D-Serine Controls NMDA

Receptor Activity and Synaptic Memory

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Supplemental Experimental Procedures

Immunohistochemistry

The SON is composed only of OT- and VP-secreting neurons, and to label their somata and dendrites we used specific monoclonal antibodies raised against the carrier proteins of each of the neuropeptides, OT-associated- (OT-NP) or VP-associated-neurophysins (VP-NP) (kindly provided by H. Gainer, NIH). Double immunofluorescence for D-serine and OT-NP or VP-NP was carried out on freely floating hypothalamic sections (50 µm), cut on a vibratome that contained the SON from virgin and lactating rats, fixed as described in experimental procedures. The D-serine rabbit serum was used at a dilution of 1/2000, the OT- and VP-NP mouse monoclonal antibodies were diluted 1/100 and 1/400, respectively. Sections were incubated in a mixture of these antibodies for 3 days (at 4° C) and immunoreactivities revealed with a mixture of FITC-conjugated anti-rabbit (diluted 1/1000) and Texas Red-conjugated anti-mouse (diluted 1/1000) antibodies (3h, room temp). Observations were made with epifluorescence on a Leica DMR microscope or with a Leica confocal scanning microscope with appropriate filters. Controls included omission of primary antibodies and incubation of sections in each of the primary antibodies followed by inappropriate immunolabels. No specific reactions were visible in these sections.

Acutely Isolated Neurons

SON neurons were isolated as previously described (Oliet and Bourque, 1992; Hussy et al., 1997). Briefly, female Wistar rats were anaesthetized with isoflurane and decapitated. Brains were removed from the cranial vault and tissue blocks containing the SON were dissected out using iridectomy scissors. The blocks were incubated for 35 min (room temperature) in oxygenated ACSF containing deoxyribonuclease I (2 mg/ml; Sigma) and proteases X and XIV (4 mg/ml Sigma). The pieces of tissue were then transferred to an enzyme-free solution containing (in mM): NaCl 126.6, NaH_2PO_4 1.25, ascorbic acid 0.4, KCl 2.5, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 20 and glucose 22.2 (pH 7.4; 295 mosmol/kg). The blocks were triturated mechanically and the resulting cell suspensions were plated on the glass slide of the recording chamber. Neurons were perfused with ACSF and identified by their morphology and size (Fig. S2A) were recorded within 3 h after dissociation. Whole-cell patch-clamp recordings were obtained with pipettes filled with regular CsCl-based solution. NMDA (500 µM) was applied through a patch pipette connected to a picospritzer and positioned in the vicinity (50 to 100 µm) of magnocellular somata (Fig. S2A). The application lasted 1 s and was repeated every 60 s. NMDA outward currents were recorded at + 40 mV. Earlier experiments (Hussy et al., 1997) carried out on isolated SON neurons established that glycine potentiated NMDA responses in a dose-dependent manner with an EC50 of 2 µM and with maximal responses obtained with 10 µM. In our recordings, therefore, we used glycine at these two concentrations.

Brain Stem Slices

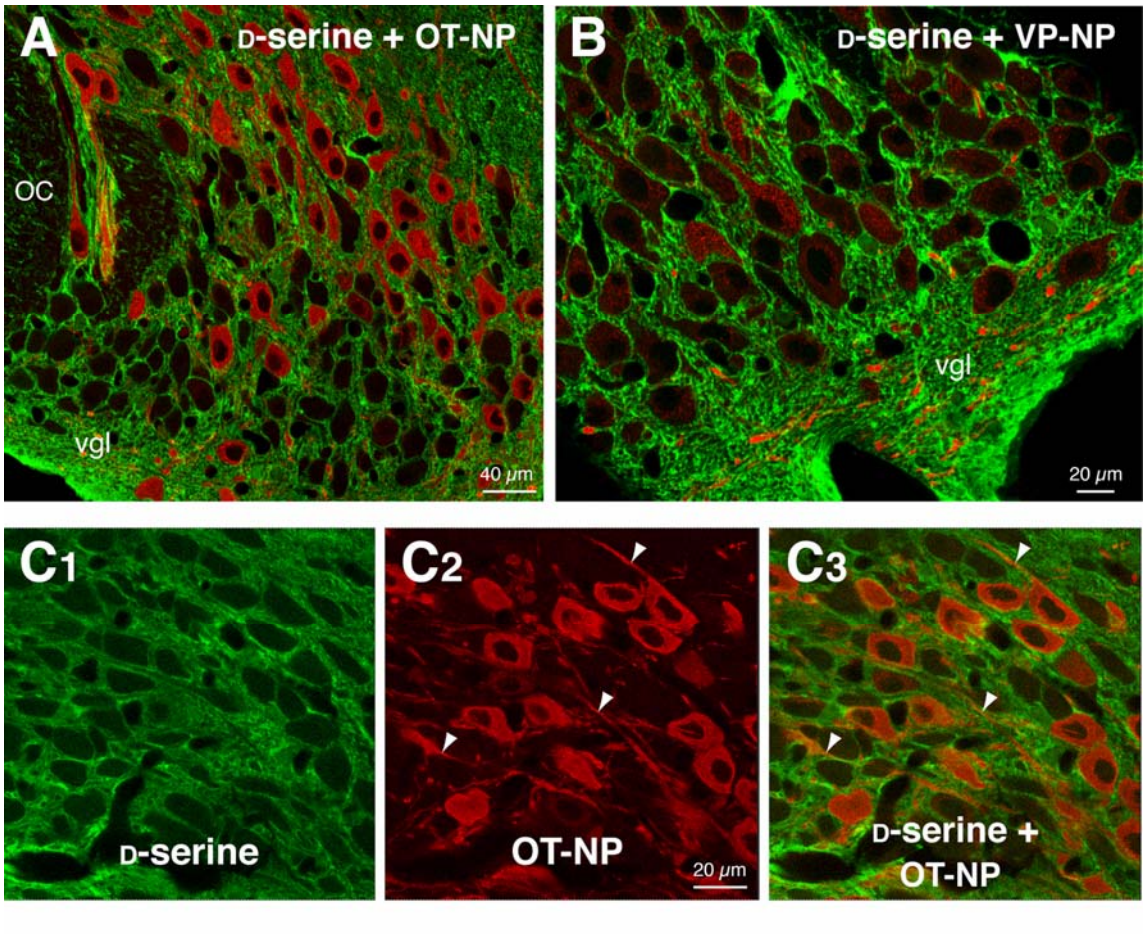
Coronal slices (300 µM) of the brain stem containing the area postrema were prepared from female Wistar rats (1 to 3 months), using the same procedure as described for hypothalamic slices (see Experimental Procedures). Synaptic responses were evoked in NTS neurons by placing a stimulating electrode in the solitary tract. Recordings were obtained in the presence of 1 µM strychnine to block glycinergic receptors.

Supplemental References

Hussy, N., Boissin-Agasse, L., Richard, P. and Desarménien, M.G. (1997). NMDA receptor properties in rat supraoptic magnocellular neurons: characterization and postnatal development. Eur. J. Neurosci. 9, 1439-1449.

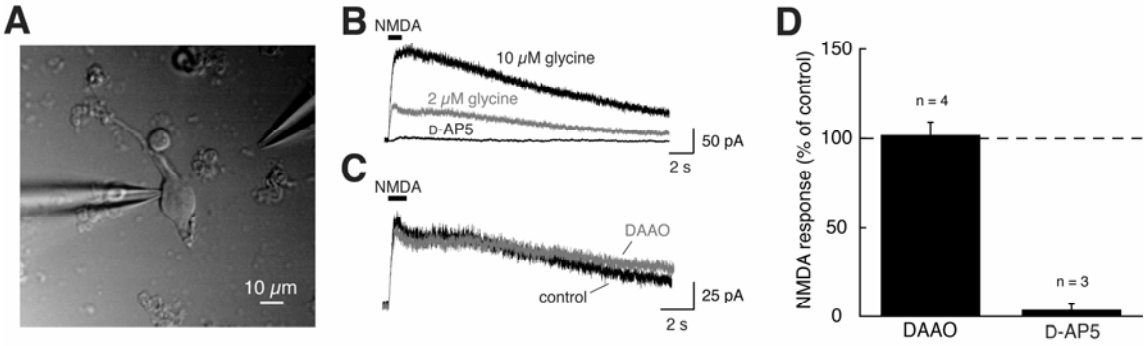
Oliet, S.H.R. and Bourque, C.W. (1992) Properties of supraoptic magnocellular neurones isolated from the adult rat. J. Physiol. 455, 291-306.

Figure S1. Absence of D-Serine Labeling in SON Neurons



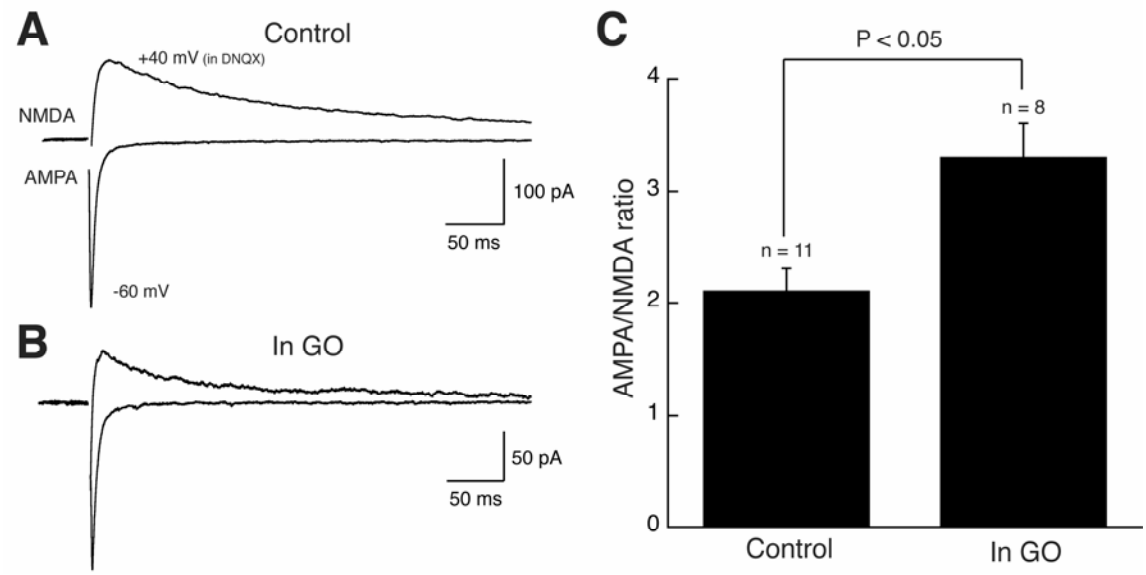
Confocal microscopy of 50 µM vibratome sections of the SON that underwent simultaneous immunfluorescence for D-serine (green) and either OT- or VP-NP (red). D-serine immunoreactivity was represented by a fine, fibrillar reaction in the neuropile, presumably in astrocytic processes (Fig. 1C,D) surrounding neuronal profiles; the reaction was particularly strong in the ventral limits of the nucleus (vgl) where astrocytic somata and large fibers accumulate. The neuronal reaction, due to immunoreactivity for either of the neurophysins filled SON neuron somata and dendrites (arrowheads in C). Note the complete lack of co-localization of D-serine with either OT-NP or VP-NP. In A and B, each image represents projections of 17 (1 µm/step) and 22 (0.5 µm/step) sections, respectively; the two series of projections were then merged to give the serial section reconstructions for visualization of the amino acid and either neuropeptides. In C, each image represents a single optical section showing either D-serine (C1) or OT-NP (C2) immunoreactivities; C3 represents an overlay of the two.

Figure S2. Absence of a Competitive Antagonist Contaminant in DAAO-Containing Solution



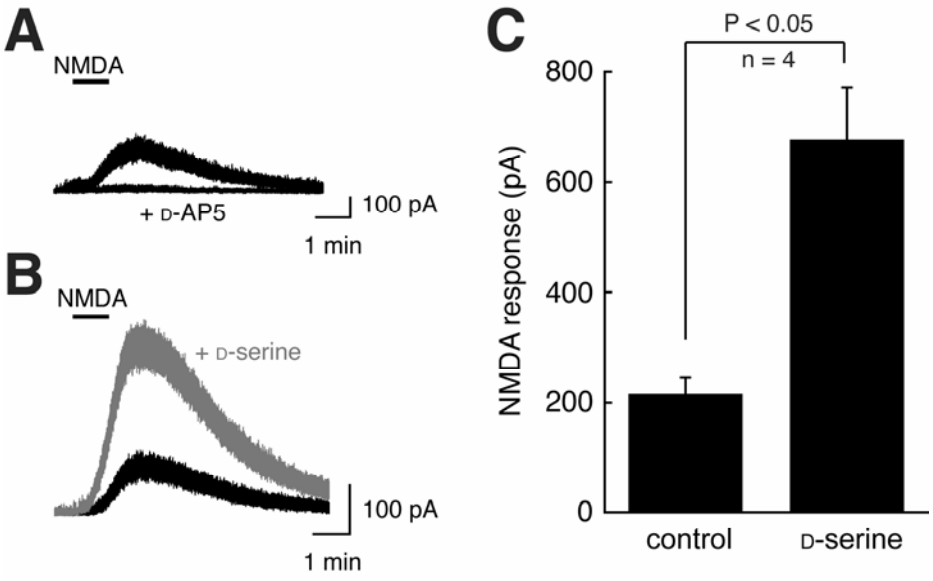
(A) Example of an acutely isolated SON neuron during a patch-clamp recording (pipette on the left). NMDA responses were elicited with a pipette containing 500 µM NMDA positioned to the right.
(B) Representative examples of NMDA responses obtained at +40 mV. Traces in grey and black were elicited in the presence of 2 and 10 µM glycine in the external solution, respectively. Note that the response obtained with 2 µM glycine was 50 % less than that obtained with 10 µM glycine. These responses were largely abolished in the presence of 50 µM D-AP5.
(C) NMDA responses elicited in the presence of 2 µM glycine, a sub-saturating concentration for the glycine site, were not affected by applications of a solution containing D-amino acid oxidase (DAAO), which rules out a competitive action of an antagonist contaminant in the enzyme-containing solution.
(D) Histogram summarizing of the effect of DAAO (n=4) and D-AP5 (n=3) on NMDA responses obtained in the presence of 2 µM glycine. Data are reported as means ± S.E.M.

Figure S3. GO Inhibits NMDAR-Mediated EPSCs in NTS Neurons



(A) Examples of evoked synaptic currents obtained in NTS neurons in response to solitary tract stimulation. The AMPA component of EPSCs was obtained by holding the neurons at -60 mV whereas the NMDA component was measured at +40 mV in the presence of DNQX (10µM).
(B) Example of evoked glutamatergic EPSCs obtained in NTS neurons in a brain stem slice treated with glycine oxidase (GO), for 45 min.
(C) Summary histogram of the AMPA/NMDA ratio measured in NTS neurons in the presence (n=8) and absence (n=11) of GO. The ratio was significantly increased (P=0.008) in the presence of GO, thereby revealing a significant contribution of endogenous glycine in modulating NMDARs in this part of the brain. Data are reported as means ± S.E.M.

Figure S4. Effect of D-Serine on Bath-Applied NMDA-Mediated Responses



(A) A response obtained in a SON neuron when 50 µM NMDA was bath-applied for 1 min that was largely inhibited by 50 µM D-AP5.
(B) The same application of NMDA induced a much larger response in the presence of D-serine (100 µM).
(C) Histogram summarizing the facilitating action of D-serine on responses elicited by bath-application of NMDA (n=4). Data are reported as means ± S.E.M.