Long-term potentiation depends on release of D-serine from astrocytes

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Long-term potentiation (LTP) of synaptic transmission provides an experimental model for studying mechanisms of memory1. The classical form of LTP relies on N-methyl-D-aspartate receptors (NMDARs), and it has been shown that astroglia can regulate their activation through Ca2+-dependent release of the NMDAR co-agonist D-serine2–4. Release of D-serine from glia enables LTP in cultures5 and explains a correlation between glial coverage of synapses and LTP in the supraoptic nucleus4. However, increases in Ca2+ concentration in astroglia can also release other signalling molecules, most prominently glutamate6–8, ATP9 and tumour necrosis factor-α10–11, whereas neurons themselves can synthesize and supply D-serine12,13. Furthermore, loading an astrocyte with exogenous Ca2+ buffers does not suppress LTP in hippocampal area CA1 (refs 14–16), and the physiological relevance of experiments in cultures or strong exogenous stimuli applied to astrocytes has been questioned17,18. The involvement of glia in LTP induction therefore remains controversial. Here we show that clamping internal Ca2+ in individual CA1 astrocytes blocks LTP induction at nearby excitatory synapses by decreasing the occupancy of the NMDAR co-agonist sites. This LTP blockade can be reversed by exogenous D-serine or glycine, whereas depletion of D-serine or disruption of exocytosis in an individual astrocyte blocks local LTP. We therefore demonstrate that Ca2+-dependent release of D-serine from an astrocyte controls NMDAR-dependent plasticity in many thousands of excitatory synapses nearby.

To investigate the role of astrocytes in NMDAR-dependent LTP, we focused on Schaffer collateral (SC)–CA1 pyramidal cell synapses, a classical subject of LTP studies. We patched passive astrocytes in the stratum radiatum and monitored SC-mediated field excitatory post-synaptic potentials (fEPSPs) in their immediate vicinity (Fig. 1a, b, and Supplementary Fig. 1). A standard high-frequency stimulation (HFS) protocol induced LTP, which was indistinguishable from that induced without patching an astrocyte (Fig. 1c and Methods). Because the intracellular solution routinely contained the high-affinity Ca2+ indicator Oregon Green bis-(α-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid-1 (OGB-1; 0.2 mM), these observations were in accord with studies documenting robust LTP near an astrocyte loaded with 10 mM EGTA14–16. However, exogenous Ca2+ buffers, although they suppress rapid Ca2+ transients, are unlikely to affect equilibrated (resting) free Ca2+ controlled by active homeostatic mechanisms. To constrain free astrocytic Ca2+ more efficiently, we ‘clamped’ its concentration at 50–80 nM, by adding 0.45 mM EGTA and 0.14 mM CaCl2 to the intracellular solution (Methods)19. Indeed, Ca2+ clamp abolished HFS-induced increases in Ca2+ concentration, which could be detected in astrocytes containing OGB-1 and EGTA only (Supplementary Fig. 2). Furthermore, biophysical simulations suggested that Ca2+ clamp could restrict Ca2+ nanodomains more efficiently than buffers alone (Supplementary Fig. 3).

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Figure 1 | Clamping astrocytic Ca2+ blocks LTP at nearby synapses in a D-serine-dependent manner. a, Experimental arrangement: IP, intracellular patch-pipette; EP, extracellular pipette; DG, dentate gyrus; green line, Schaffer collaterals. b, A typical patched astrocyte (Alexa Fluor 594, about 120 μm stack fusion). BV, blood vessel. Escape of Alexa to neighbouring cells can be seen. Inset: differential interference contrast at lower magnification. c, LTP of AMPAR fEPSPs in control conditions, with (green, n = 23) or without (grey, n = 28) astrocyte patched. Arrow indicates HFS onset; traces show characteristic AMPAR fEPSPs before (black) and after (grey) LTP induction; notations also apply in e–f. d, Clamping astrocytic Ca2+ (0.2 mM OGB-1, 0.45 mM EGTA, 0.14 mM CaCl2) abolishes local LTP (orange, n = 19) whereas 10 μM D-serine rescues it (green, n = 10). e, LTP in 10 μM D-serine (green, 163 ± 12%, n = 8; no astrocyte patched) is no different from that in the control (c), suggesting saturation of either the NMDAR co-agonist site or the downstream induction mechanism. APV (50 μM) completely blocks LTP (orange, n = 12). f, Summary for experiments in e–f, as indicated. Results are presented as means ± s.e.m. for fEPSPs measured 25–30 min after HFS relative to baseline. Three asterisks, P < 0.005 (one-population t-test); three plus signs, P < 0.002 (two-population t-test).

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Strikingly, clamping the intra-astrocyte Ca\(^{2+}\) concentration completely suppressed LTP at nearby synapses, and the addition of 10 \(\mu\)M \(D\)-serine fully rescued it (Fig. 1d). \(D\)-Serine alone had no effect on LTP in control conditions (consistent with previous reports\(^{23}\)), whereas the NMDAR antagonist (2R)-amino-5-phosphonoplastic acid (APV) completely blocked it (Fig. 1e, f). These observations imply that Ca\(^{2+}\)--dependent astrocyte activity could indeed control the supply of an NMDAR co-agonist essential for LTP induction\(^{1,3,18,21}\). To understand the underlying mechanisms, we recorded SC-evoked NMDAR fEPSPs near the patched astrocyte (Fig. 1a, b and Methods) while switching from a cell-attached to a whole-cell configuration (Fig. 2a). The control pipette solution (Methods) had little influence on NMDAR responses (change 10\% relative to baseline, \(n = 6\); Fig. 2a, b), whereas Ca\(^{2+}\) clamp decreased them by 22\% (\(n = 13\), \(P < 0.0001\)). This reduction was reversed by the subsequent application of \(D\)-serine (10\% relative to baseline, \(n = 7\), \(P = 0.0012\); Fig. 2a, b and Supplementary Fig. 4), and the presence of \(D\)-serine from the start blocked the inhibitory effect of Ca\(^{2+}\) clamp (16\% \(\pm\) 12\%, \(n = 7\); Fig. 2a, b). Ca\(^{2+}\)--dependent astrocyte signalling could therefore regulate the occupancy of the NMDAR co-agonist site.

In resting conditions, 10 \(\mu\)M \(D\)-serine increased NMDAR-mediated excitatory postsynaptic currents (EPSCs) in CA1 pyramidal cells (by 29\% \(\pm\) 10\%; \(n = 5\), \(P = 0.039\); Fig. 2c) as well as local NMDAR EPSPs (by 25\% \(\pm\) 3\%, \(n = 8\), \(P < 0.001\); Supplementary Fig. 5), indicating that the NMDAR co-agonist sites were not saturated in baseline conditions, which is consistent with recent observations in hippocampal slices\(^{22}\) and in vivo\(^{23}\). Interestingly, LTP induction had no long-term effect on the NMDAR co-agonist site occupancy: \(D\)-serine applied 25 min after induction brought about equal increases in NMDAR EPSCs (by 23\% \(\pm\) 8\%, \(n = 6\), \(P = 0.034\); Fig. 2c) and fEPSPs (by 25\% \(\pm\) 9\%; \(n = 6\), \(P = 0.042\); Supplementary Fig. 5), which were indistinguishable from the pre-induction effects. In contrast, even mild repetitive stimulation of SCs (ten stimuli at 50 Hz) was sufficient

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**Figure 2 | Activation of the NMDAR co-agonist site is astrocyte-dependent and use-dependent.** **a**, NMDAR fEPSP slope (Fig. 1a arrangement) monitored during the transition from cell-attached to whole-cell mode, as indicated. Grey circles, control (\(n = 6\); Methods); orange, Ca\(^{2+}\) clamp (\(n = 13\) throughout, \(n = 13\) before \(D\)-serine application); green, Ca\(^{2+}\) clamp with 10 \(\mu\)M \(D\)-serine in bath (\(n = 7\)). Segments are averaging epochs; traces show examples (see also Supplementary Fig. 4). **b**, Summary of experiments shown in **a**. Results are presented as means \(\pm\) s.e.m. (applies throughout); numerals indicate epochs in **a**. Three asterisks, \(P < 0.0001\) (one-population \(t\)-test; three plus signs, \(P < 0.005\) (two-population \(t\)-tests). **c**, Effect of \(D\)-serine on NMDAR EPSCs before and after LTP induction. Green circles, amplitude of AMPAR EPSCs (\(n = 6\); left panel; \(V_m = -70\) mV) and, subsequently, NMDAR EPSCs in the same cell after LTP (right panel; 10 \(\mu\)M 2,3-dihydroxy-6-nitro-7-sulphamoylbenzo(f)quinoxaline (NBQX), \(V_m = -10\) mV); grey segment, \(D\)-serine application. Open circles (\(n = 5\); top axis, right panel), NMDAR EPSC amplitudes in no-LTP conditions. Traces: upper left, characteristic AMPAR EPSCs in control (black) and after LTP (grey); lower right, NMDAR EPSCs before (black) and after (grey) application of \(D\)-serine, in control and after LTP, as indicated. **d**, A train of ten pulses at 50 Hz transiently potentiates synaptic NMDAR responses in a glycine-dependent and glia-dependent manner. Traces show examples of single-stimulus EPSCs including a prominent NMDAR-mediated component (\(V_m = +40\) mV; no AMPAR blockade was used to ensure pharmacological continuity with LTP induction protocols) in baseline conditions (left; grey, individual traces; black, average), 20 s (middle) and 2 min (right) after the stimulus train, in control (upper row) and in 0.1 mM glycine (lower row). Bar graph: average ratio of after-train EPSC potentiation in glycine to after-train EPSC potentiation in control, for test experiments illustrated by traces (green; average change in the NMDAR-mediated response expressed as the area under the EPSC curve over the 100–300 ms after-peak interval: \(\Delta 23\% \pm 8\%\), \(P = 0.032\), \(n = 6\)), and also for control experiments (orange) including AMPAR EPSCs (amplitude change \(5\% \pm 9\%\); \(P = 0.41\), \(n = 4\)) and NMDAR EPSCs in FAC (15\% \(\pm\) 14\%; \(P = 0.32\), \(n = 6\), as indicated (examples in Supplementary Fig. 6); circles, individual experiments. 

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to boost the co-agonist site occupancy transiently: short-term potentiation of the NMDAR-mediated EPSC component 20 s after the train was decreased by 20 ± 7% \((n = 6, P = 0.043)\) when the co-agonist site was saturated by 0.1 mM glycine, before returning to the baseline level 2 min later (Fig. 2d). This was not due to changes in cell excitability or release probability because similar potentiation of \(a\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) responses showed no sensitivity to glycine (Fig. 2d and Supplementary Fig. 6). Furthermore, the glial metabolic poison fluoracetate (FAC, 5 mM) made the NMDAR EPSC potentiation also insensitive to glycine, thus implicating glia (Fig. 2d and Supplementary Fig. 6). In concord with these observations, HFS induced transient increases in \(\text{Ca}^{2+}\) concentration in 54% of astrocytes (consistent with earlier reports\(^6\)) but no long-term changes in spontaneous \(\text{Ca}^{2+}\) activity (Supplementary Fig. 7).

If astrocytes enable LTP induction by increasing activation of the NMDAR co-agonist site, then decreasing the site availability should prevent LTP\(^6\). To replicate the roughly 25% decrease of NMDAR responses under astrocytic \(\text{Ca}^{2+}\) clamp (Fig. 2a, b), we used the selective NMDAR glycine-site blocker 5,7-dichlorokynurenic acid (DCKA) at 750 nM (Fig. 3a and Supplementary Fig. 8). Notably, 750 nM DCKA abolished LTP, similarly to 50 \(\mu\)M APV (Fig. 3a). Consistent both with these observations and with the \(\text{Ca}^{2+}\) clamp effects, FAC also decreased NMDAR fEPSPs by 23 ± 4% \((n = 16, P = 0.00017;\) Supplementary Fig. 9a, b) and blocked LTP in a \(\alpha\)-serine-sensitive manner (Fig. 3b, c). Although the mechanisms and specificity of FAC actions are incompletely understood, we confirmed that the effect of FAC on NMDAR fEPSPs paralleled that on LTP (Supplementary Fig. 9c, d), was absent in the presence of glycine, and did not involve changes in release probability or axonal excitability (Supplementary Fig. 10).

However, such experiments provide no direct evidence for \(\alpha\)-serine release from astroglia: an alternative possibility is that an NMDAR co-agonist is released from elsewhere, for example, neurons\(^1\(^9\),\(^10\), in response to an unknown astrocytic signal. To investigate this, we blocked \(\alpha\)-serine synthesis inside an astrocyte by using the relatively specific serine racemase inhibitor \(1\)-erythro-3-hydroxyaspartate (HOAsp)\(^26\) loaded at 400 \(\mu\)M (to suppress competition from \(\alpha\)-serine) but found no effect on local LTP (Fig. 3d). However, HOAsp does not influence \(\alpha\)-serine itself and therefore cannot prevent release of the stored agonist. We therefore attempted to deplete the putative astrocytic pool of \(\alpha\)-serine by applying HFS in the presence of APV. When APV was washed out and HFS was applied again, LTP induction was indeed suppressed, whereas the same protocol with no HOAsp loaded yielded robust potentiation (Fig. 3e, f). This result demonstrates causality between astrocytic synthesis of \(\alpha\)-serine and local LTP.

How far do individual CA1 astrocytes extend their influence on LTP? Although astrocytes occupy separate domains\(^27\), they can communicate through gap junctions\(^2\), which were left intact in the present study. To determine whether these cells could act independently, we monitored LTP simultaneously in two neighbouring areas associated with two astrocytes. First, we confirmed that field potentials recorded through an astrocytic patch-pipette reliably represent local fEPSPs, with no bias from glutamate transporter or \(K^+\) currents\(^4\) (Supplementary Fig. 11). We next patched two neighbouring astrocytes and monitored SC-evoked ‘astrocytic’ fEPSPs (a-fEPSPs) representing the two respective areas (Fig. 4a–c). Clamping \(\text{Ca}^{2+}\) in one astrocyte HOAsp (400 \(\mu\)M) does not suppress LTP induction (potentiation is 50 ± 15%, \(n = 6, P = 0.021\); arrangement as in Fig. 1a); traces show average fEPSPs before (black) and after (grey) application of HOAsp, one-cell example; time scale applies to \(d\) and \(e\). Intra-astrocyte HOAsp blocks induction of LTP (orange; fEPSP change +12 ± 11%, \(n = 7, P = 0.32\)) after depletion of \(\alpha\)-serine with HFS in APV; arrows indicate HFS onset. Omitting HOAsp robustly induces LTP (green; 52 ± 11%, \(n = 6, P = 0.0052\)). HOAsp was unlikely to affect glutamate metabolism because no rundown of glutamatergic responses was observed. \(f\), Summary of experiments shown in \(d\) and \(e\). Asterisk (left), \(P = 0.021\); two asterisks, \(P = 0.0052\) (one-population \(t\)-test); asterisk (right), \(P = 0.024\) (two-population \(t\)-test).
astrocyte suppressed LTP at nearby synapses but not at synapses near the neighbouring, control cell (Fig. 4d, e and Supplementary Fig. 12). A qualitatively identical result was obtained when putative vesicular astrocyte suppressed LTP at nearby synapses but not at synapses near the extracellular recording pipette and the patched astrocyte. In Ca\(^{2+}\)-clamp conditions, LTP partly recovered at 70–100 μm from the patched soma, reaching its full strength at approximately 200 μm, which is consistent with the profile of Alexa escaping to neighbouring cells (Fig. 4h and Supplementary Fig. 13). With a LC-TT-loaded astrocyte, LTP recovery occurred at markedly shorter distances (Fig. 4h), which is consistent with the toxin’s inability to cross gap junctions.

Our findings demonstrate that induction of NMDAR-dependent LTP at excitatory hippocampal synapses depends on the availability of NMDARs provided by the Ca\(^{2+}\)-dependent release of D-serine from a local astrocyte. Repetitive synaptic activity transiently enhances D-serine supply by glia, paralleled by a short-term increase in astrocytic free Ca\(^{2+}\) concentration. Neighbouring astrocytes may have distinct effects on local synapses, but they also extend their influence beyond their morphological boundaries. This could potentially give rise to a Hebbian mechanism regulating hetero-synaptic NMDAR-dependent plasticity across a neuronal domain affected by an activated astrocyte. The importance of D-serine in LTP does not exclude the potential role of astroglial glutamate release\(^5,6\), and the precise mechanisms acting on the microscopic scale require further investigation. Individual astrocytes occupy about 9 × 10\(^4\) μm\(^2\) of CA1 neuropil (while overlapping by only 3–10\%)\(^7\), and the numeric synaptic density in this area is about 2 μm\(^{-3}\) (ref. 30), thus suggesting that a single astrocyte could affect synaptic input plasticity on hundreds of principal cells.

**METHODS SUMMARY**

Whole-cell recordings from passive astrocytes (n = 146) were made in stratum radiatum, area CA1 in acute transverse hippocampal slices prepared from adult rats. Cells (30–100 μm deep inside the slice) were loaded with a bright morphological tracer Alexa Fluor 594 and the high-affinity Ca\(^{2+}\)-indicator OGB-1 and imaged in two-photon excitation mode (λ\(_{ex}\) = 800 nm). Field EPSPs were recorded with either an extracellular recording electrode placed in the immediate vicinity of the astrocyte soma under study or through the astrocytic patch pipette, as described. Whole-cell EPSCs were recorded from CA1 pyramidal cells. Electric stimuli were applied to Schaffer collateral fibres. LTP was induced by a standard HFS protocol (three 100-pulse trains at 100 Hz, 60 or 20 s apart). Inside the recorded astrocyte, conditions of Ca\(^{2+}\) homeostasis were altered by using intracellular solutions containing EGTA, OGB-1 and CaCl\(_2\); the excytosis machinery was suppressed with light-chain tetanus toxin; synthesis of D-serine was inhibited with the serine racemase inhibitor HOAsp.

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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METHODS

Preparation. Acute hippocampal slices 350 μm thick were obtained from 4–8-week-old male Sprague–Dawley or Wistar rats, in full compliance with national guidelines on animal experimentation. Slices were prepared in an ice-cold slicing solution containing (in mM): NaCl 75, sucrose 250, KCl 2.5, MgCl2 7, NaH2PO4 1.25, CaCl2 0.5, and glucose 6 (osmolality 300–310 mOsM), stored in the slicing solution at 34 °C for 15 min before being transferred to an interface chamber for storage in an extracellular solution containing (in mM) NaCl 119, KCl 2.5, MgSO4 1.3, NaH2PO4 1, NaHCO3 26, CaCl2 2, glucose 12 (pH 7.4; osmolarity 295–305 mOsM).

Preparation of brain slices. Acute hippocampal slices 350–400 μm thick were prepared from 3–4–6-week-old male Sprague–Dawley or Wistar rats, in full compliance with national guidelines on animal experimentation. Slices were prepared in an ice-cold slicing solution containing (in mM): NaCl 75, sucrose 250, KCl 2.5, MgCl2 7, NaH2PO4 1.25, CaCl2 0.5, and glucose 6 (osmolality 300–310 mOsM), stored in the slicing solution at 34 °C for 15 min before being transferred to an interface chamber for storage in an extracellular solution containing (in mM) NaCl 119, KCl 2.5, MgSO4 1.3, NaH2PO4 1, NaHCO3 26, CaCl2 2, glucose 12 (pH 7.4; osmolarity 295–305 mOsM). All solutions were continuously bubbled with 100% O2/5% CO2. Slices were allowed to rest for at least 60 min before recordings started. For recordings, slices were transferred to the submersion-type recording chamber and superfused, at 23–26 °C, with artificial cerebrospinal fluid saturated with 95% O2/5% CO2 (in mM): NaCl 125, KCl 2.5, NaH2PO4 1.25, NaHCO3 26, glucose 12 (pH 7.4; osmolarity 295–305 mOsM) in the presence of 1.3 mM Mg2+ and either 2.0 or 2.5 mM Ca2+ (where necessary), 50 μM picrotoxin was added to block GABA receptors and a cut between CA3 and CA1 was made to suppress epileptiform activity.

Electrophysiology. Whole-cell recordings in astrocytes were obtained with standard patch pipettes (3–4 MΩ) filled with a ‘control’ intracellular solution containing (in mM) KCH3O3S 135, HEPES 10, disodium phosphocreatine 10, MgCl2 2, Na2ATP 4, NaGTP 0.4, and either a bipolar or coaxial stimulus barrel electrode placed to a distance of ≥200 μm from the recording electrodes. Field EPSPs (fEPSPs) were recorded with a standard patch pipette filled with the extracellular solution. Predominantly AMPAR-mediated fEPSPs (with no NMDAR blockers added) were denoted as AMPAR fEPSPs, while NMDAR fEPSPs were isolated with 10 μM 2,3-dihydroxy-6-nitro-7-sulphamoylbenzo(f)quinoxaline (NBQX) and 0.2 mM Mg2+ in the bath medium (except for post-LTP recordings performed in 1.3 mM Mg2+ to maintain unchanged cell excitability).

Whole-cell recordings in CA1 pyramidal neurons were performed with an intracellular solution containing (in mM): potassium glutamate 130, KCl 5, HEPES 10, disodium phosphocreatine 10, MgCl2 2, Na2ATP 4, NaGTP 0.4, or, alternatively, calcium methanesulphonate 150, MgCl2 1.3, EGTA 1, HEPES 10, CaCl2 0.1. AMPAR fEPSCs in CA1 pyramidal cells were recorded at Vm = −70 mV, and NMDAR fEPSCs were recorded in at Vm = −10 mV in the presence of 10 μM NBQX, unless indicated otherwise.

Statistics. Group data are routinely reported as means ± s.e.m. indicated otherwise, and the statistical difference between the population averages was estimated with the t-test (for paired or independent samples). Two-tailed tests were routinely used, and sample pairing was used where appropriate, for example when monitoring real-time changes in a parameter against its baseline value or when comparing cells in paired recordings.

Where required, slices were preincubated in the glial metabolism inhibitor FACAC for at least 50 min, unless indicated otherwise; t-serine was added 10–15 min before LTP induction. Recordings were performed with a Multiclamp 700A or 700B (Molecular Devices). Signals were filtered at 3–6 kHz, digitized and sampled through an analogue-to-digital converter (National Instruments) at 10 kHz, and stored for offline analysis with either LabView (National Instruments) routines or pClamp9 software (Molecular Devices). Receptor blockers were purchased from Tocris, and FAC was purchased from Sigma-Aldrich.

Two-photon excitation imaging. We used a Radiance 2100 imaging system (Zeiss–Bio-Rad) optically linked to a femtosecond pulse laser MaiTai (Spectra-Physics–Newport) and integrated with patch-clamp electrophysiology. Once in whole-cell mode, Alexa normally equilibrated across the astrocyte tree within 10–15 min. Astrocytes loaded with fluorescein indicators (see above) were imaged as three-dimensional stacks of 60–100 optical sections in the Alexa emission channel (540LP/700SP filter; fP = 800 nm), collected in image frame mode (512 pixels × 512 pixels, eight-bit) at 0.5–1 μm steps.

For illustration purposes, the stacks were 2-axis averaged with ImageJ routines (Rasband WS, ImageJ); National Institutes of Health; http://rsb.info.nih.gov/ij/ (1997–2008)). Transient Ca2+ signals evoked in patched astrocytes by HFS (Supplementary Fig. 2) were imaged in the OGB-1 (green) channel (515/30 filter) in line-scan mode (500 Hz), and corrected for focus fluctuations in the Alexa (red) channel, as detailed elsewhere30–37 and illustrated in Supplementary Fig. 2.

To image Ca2+ activity simultaneously in a population of astrocytes (Supplementary Fig. 7), slices were incubated with the glia-specific dye sulforhodamine 101 (SR101, 5 μM; Invitrogen) and the Ca2+ indicator Fluo–4 acetyloxymethyl ester or OGB-1 acetyloxymethyl ester (5 μM; Invitrogen) in the presence of 0.04% Pluronic (Invitrogen), for 40–50 min at 37 °C and allowed to rest for at least 20 min. The viability of loaded slices was verified by monitoring EPSPs in response to Schaffer collateral stimulation, as described earlier. Time-lapse imaging was performed by acquiring 800–20,000 pairs of SR101 and Fluoro-4/OGB-1 fluorescence images frames at a rate of 2 Hz (256 pixels × 256 pixels) or 7 Hz (64 pixels × 64 pixels). To evoke a Ca2+ response, HFS (100 pulses at 100 Hz) was delivered halfway through the experiment. In these experiments, time-dependent fluorescent transients were expressed as T(x,y,t) where T denotes the averaged, background-corrected Fluoro-4/OGB-1 fluorescence of a SR101 positive (astrocytic) soma.

Visualization of astrocytic pairs and separating images of individual astrocytes in dual-patch experiments (Fig. 4) was performed with the following routine: first, cell 1 was patched, the dye was allowed to equilibrate and the resulting ‘cell 1 image’ (three-dimensional stack) was stored; second, cell 2 was patched, the dye was equilibrated and the resulting ‘cell 1 + cell 2 image’ was stored; third, ‘cell 1 image’ was subtracted from ‘cell 1 + cell 2 image’, yielding the fluorescent ‘cell 2 image’. Comparing ‘cell 1 image’ and ‘cell 2 image’ could be used to reveal staining (diffusion) overlap between the two cells, which could be revealed with false-colour tables applied to the two images. Image analyses were performed offline with ImageJ.

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