

Reyes-Haro et al., <http://www.jgp.org/cgi/content/full/jgp.200910354/DC1>

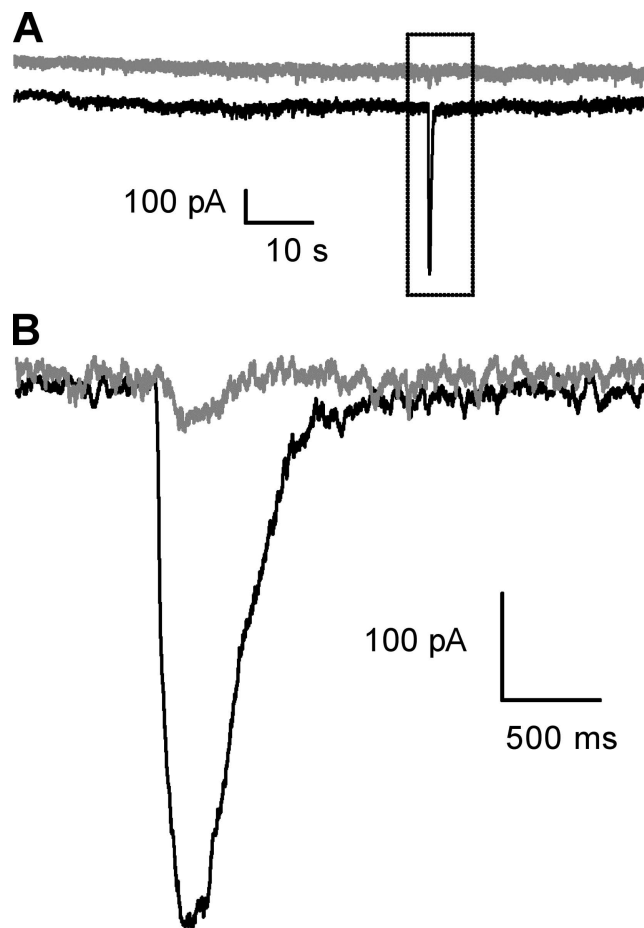


Figure S1. Representative paired recording showing synchronized nSICs. The paired recording was obtained in aCSF with low Ca^{2+} and Mg^{2+} + TTX (1 μM) + strychnine (1 μM) + gabazine (10 μM). This is one of the two paired recordings where synchrony of nSICs was observed (4 out of 50 nSICs; $n = 12$ paired recordings). (B) Synchronized nSICs are shown in higher time resolution.

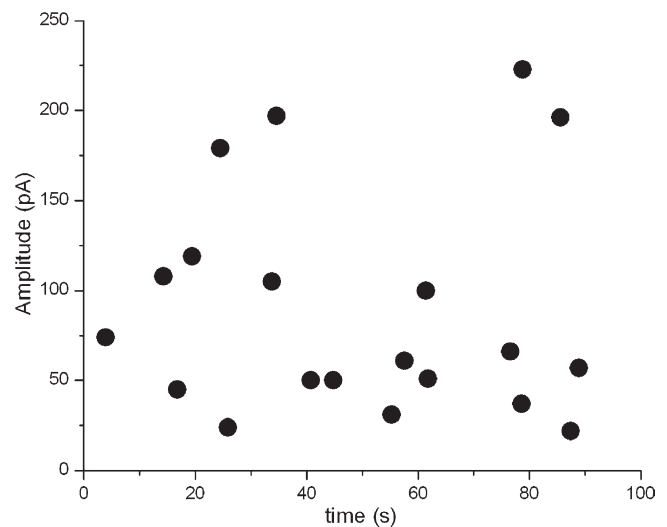


Figure S2. Raster plot showing the amplitudes and time occurrence of nSICs recorded after calcium wave evoked by electrical stimulation. The nSICs ($n = 20$ nSICs from 24 neurons) were obtained in Mg^{2+} -free aCSF + TTX (1 μM) + strychnine (1 μM) + gabazine (10 μM).

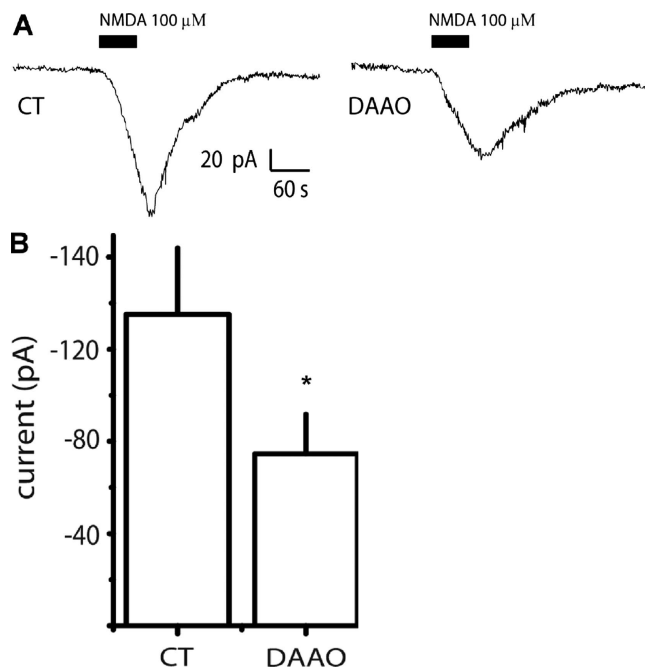
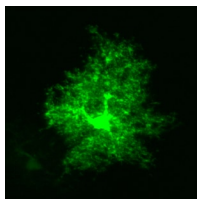


Figure S3. nSICs are reduced by degrading D-serine. (A) Representative current recordings of a PPN clamped at -70 mV. Bath application of NMDA ($100\text{ }\mu\text{M}$) induced an inward current. The trace on the left is an example of a control current, the trace on the right after preincubation with DAAO (0.17 U/ml). (B) Summary of the effect of DAAO on NMDA currents. NMDA elicited an inward current of $135.15 \pm 28.77\text{ pA}$ in control slices ($n = 10$). The NMDA-elicited inward current was $74.56 \pm 17.22\text{ pA}$ ($n = 9$) when DAAO was preincubated. Data are the mean \pm SEM. Asterisk represents significant difference (*, $P = 0.04$).



Video 1. 3D reconstruction of an astrocyte in an MNTB slice, a series of 35 two-photon images were taken in steps of $1\text{ }\mu\text{m}$. The fluorescence is due to the expression of eGFP in the GFAP-eGFP transgenic mouse line. The reconstruction displays 51 frames at a speed of 6 frames per second, turning 360° in 7° steps. The video shows that the PPN, visible as a hollow area in the center, is completely covered by astrocytic processes and that the astrocyte soma is in close contact to the PPN. The diameter of the displayed frame is $90\text{ }\mu\text{m}$. Note that the astrocyte is located on the PPN like a hat on a head.

TABLE S1.
Calcium response in astrocytes increases the frequency of nSICs

| | Before astrocytic calcium response | After astrocytic calcium response | n | p |
|--|---------------------------------------|--------------------------------------|----|--------|
| Control (normal aCSF) | 0.015 ± 0.010 | 0.058 ± 0.037 | 16 | 0.27 |
| aCSF + TTX (1 µM) | 0.011 ± 0.011 | 0.097 ± 0.037 | 16 | 0.036 |
| aCSF/Mg ²⁺ free + TTX (1 µM) | 0.015 ± 0.010 | 0.013 ± 0.050 | 17 | 0.13 |
| aCSF/Mg ²⁺ free + TTX (1 µM) + strychnine (1 µM) + gabazine (10 µM) | 0.022 ± 0.012 | 0.3 ± 0.1 | 20 | 0.0089 |
| aCSF/Mg ²⁺ free + TTX (1 µM) + strychnine (1 µM) + gabazine (10 µM) + CNQX (25 µM) | 0 | 0.179 ± 0.066 | 24 | 0.0094 |
| aCSF/Mg ²⁺ free + TTX (1 µM) + strychnine (1 µM) + gabazine (10 µM) + CNQX (25 µM) + ifenprodil (100 µM) | 0 | 0.006 ± 0.004 | 17 | 0.15 |
| aCSF/Mg ²⁺ free + TTX (1 µM) + strychnine (1 µM) + gabazine (10 µM) + DAAO (0.17 U/ml) | 0.04 ± 0.018 | 0.0096 ± 0.056 | 15 | 0.71 |
| aCSF/Mg ²⁺ free + TTX (1 µM) + strychnine (1 µM) + gabazine (10 µM) + BAPTA (40 mM) injected in an astrocyte previously | 0 | 0.015 ± 0.015 | 8 | 0.33 |

Table shows the values of experiments shown in Fig. 7 C.