Supporting Information

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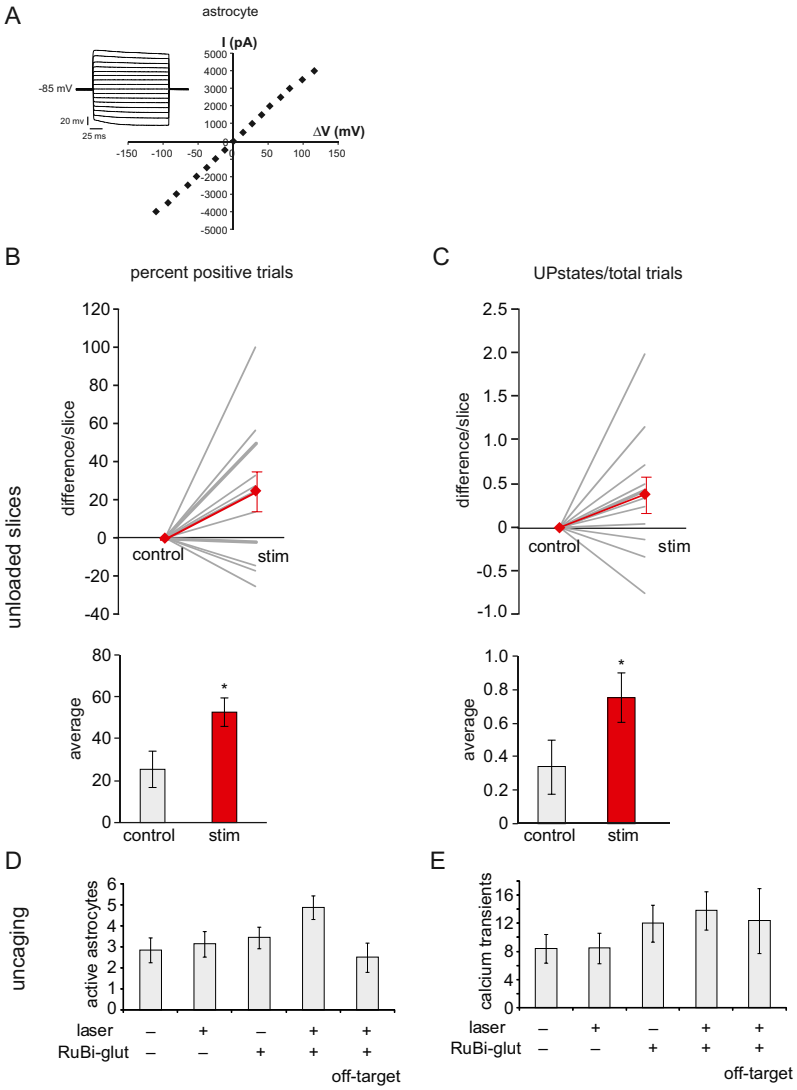

Fig. S1. Sulforhodamine 101 (SR101) and Fluo-4 do not affect stimulation-induced UP states by astrocytes. (*A*) Current-voltage (IV) plot of a patched passive astrocyte. (*B* and *C*) Stimulation (red)/control (gray) protocol as carried out in Fig. 2, with data analyzed by slice as in Fig. 2 *F* and *G*. Slices were not loaded with any dyes. (*B*) Percent trials with at least one UP state (positive trials). Significant difference between control (24.2 ± 7.5%) and stimulation (49.1 ± 6.9%) trials. (*C*) Number of UP states in all trials. Significant differences between control (0.32 ± 0.11) and stimulation (0.71 ± 0.16) trials ($n = 12$ slices, 96 trials; $^*P < 0.05$). (*D* and *E*) Calcium data from glutamate uncaging experiments, showing active astrocytes (*D*) and total calcium transients (*E*) per trial under all conditions tested.

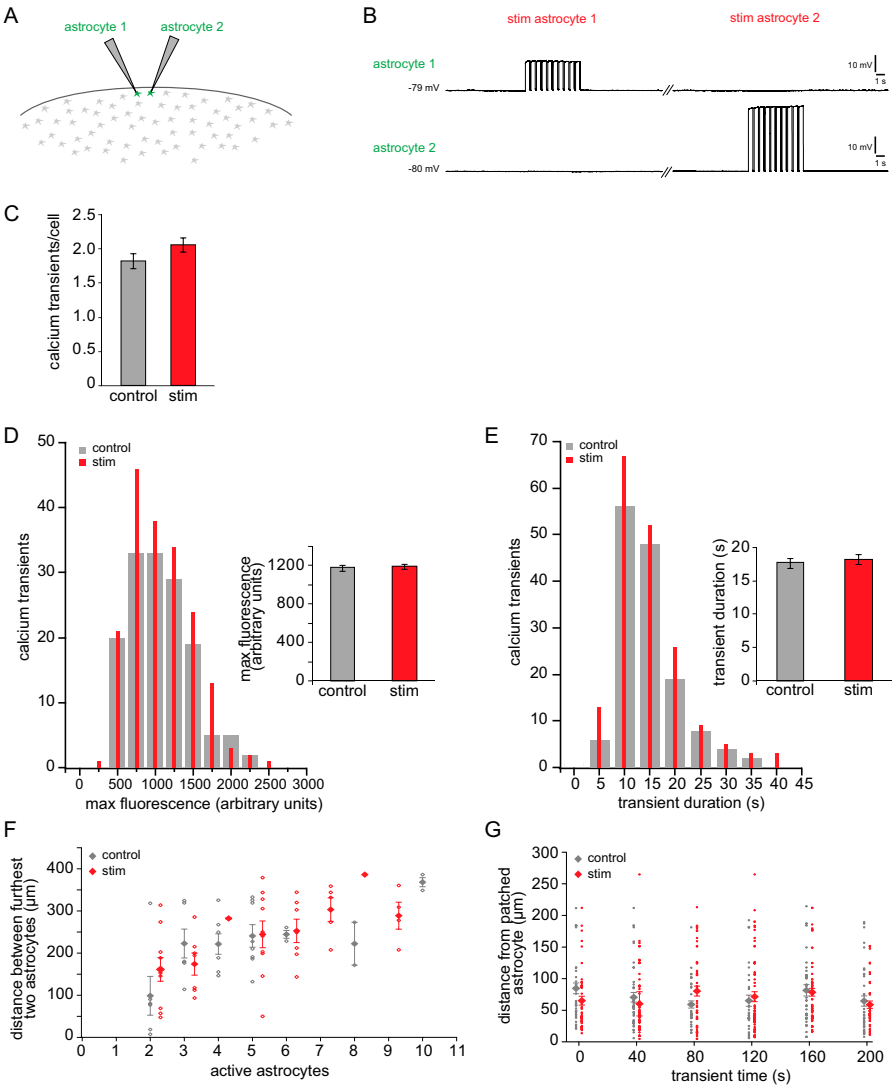

Fig. S2. Astrocyte calcium imaging during single-cell stimulation. (*A*) Schematic of dual-patch astrocyte experiment. Two neighboring astrocytes are whole-cell patch-clamped, and each one is individually depolarized as in Fig. 2*A* at the trial start. (*B*) Depolarization in an astrocyte does not spread to the neighboring astrocyte soma as measured electrophysiologically. (*C*) Average calcium transients in each active astrocyte for the two trial types, control (gray) and stimulated (red), are not significantly different, indicating that the differences displayed in Fig. 3 are due to the increase in active astrocytes. (*D*) Maximum fluorescence measurements for all calcium transients described in Fig. 3 (706 total transients). (*Left*) Distribution of values of transients from both control (gray) and stimulation (red) trials. (*Right*) Average values in two trial types. (*E*) Same transients as analyzed in *D*, showing the duration of calcium transients. (*Left*) Distribution histogram. (*Right*) Average values. No significant differences in averages were observed in either measure. (*F*) Spatial spread of active astrocytes, as measured by the distance between the farthest two active astrocytes in an individual trial. Data are grouped by trial type, and there is no significant difference between conditions. (*G*) Time of calcium transients from every active astrocyte in the two conditions, binned and plotted as a function of distance from the patched astrocyte. Similar averages across the time trial indicate an absence of calcium waves.

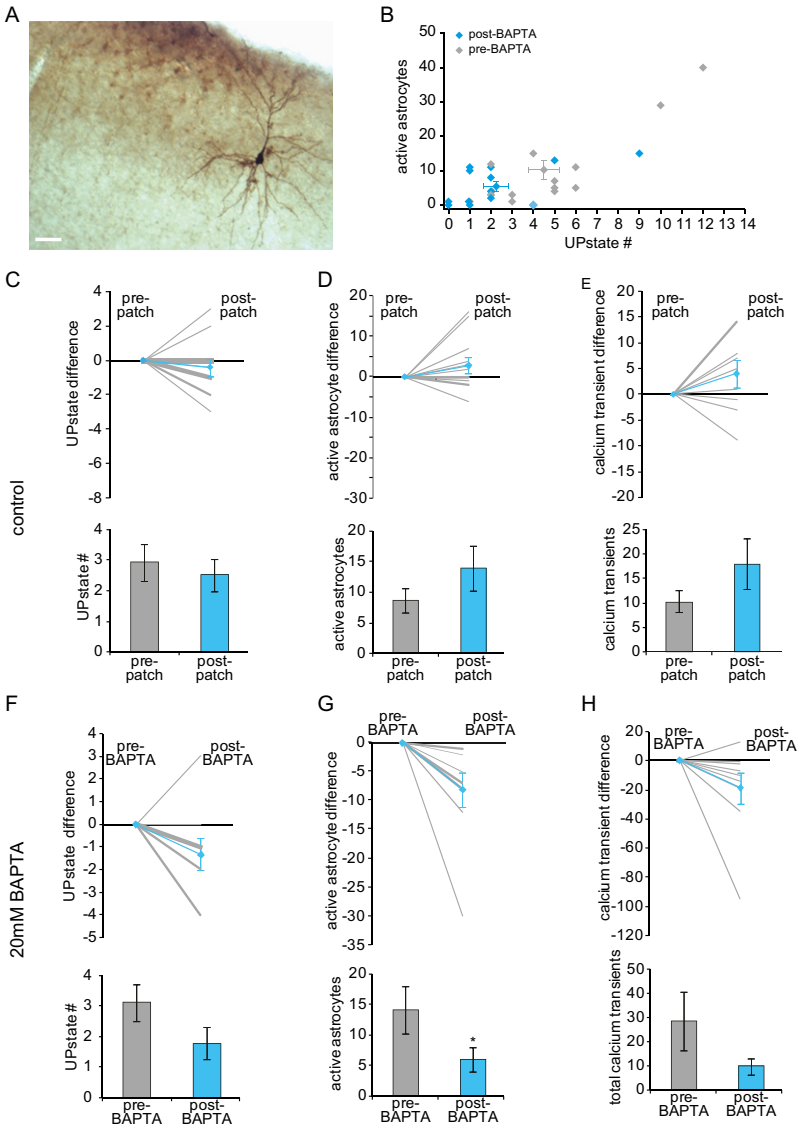

Fig. S3. BAPTA [1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid] control experiments. (*A*) Example of biocytin-labeled astrocytes and a neuron after a single astrocyte had been patched with 50 mM BAPTA in the patch pipette (compare with Fig. 1*E*). Both the neuron and the astrocyte patch pipettes contained biocytin. (Scale bar, 50 μm.) (*B*) Correlation between total UP states and the number of active astrocytes before and after astrocytic BAPTA addition. Averages and SEM are shown for each condition on the same graph (points with error bars). (*C*–*E*) Results of BAPTA control experiment (Fig. 4). Sum of four trials pre- and postpatching of astrocyte with no BAPTA in the pipette (n = 12 slices). Difference (*Upper*) and actual (*Lower*) graphs for UP states (*C*), active astrocytes (*D*), and calcium transients (*E*). No significant differences were observed. (*F*–*H*) Results of the same BAPTA protocol shown in Fig. 4, but with 20 mM BAPTA in the patch pipette (n = 9 slices). Difference (*Upper*) and actual (*Lower*) graphs for UP states (*F*), active astrocytes (*G*), and calcium transients (*H*). A significant decrease is observed in the number of active astrocytes (*G*), while the other two measures do not reach significance. *$P < 0.05$.

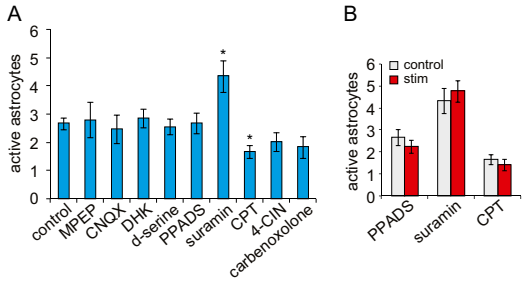

Fig. S4. Pharmacology. (*A*) Active astrocyte data from the same pharmacological experiments as shown in Fig. 5 *A* and *B*. (*B*) Active astrocyte data from the same pharmacological experiments as shown in Fig. 5 *C* and *D*. In all pharmacological experiments, active astrocyte numbers showed similar changes as the measure of total calcium transients. *$P < 0.05$. MPEP, 2-Methyl-6-(phenylethynyl)pyridine; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DHK, dihydrokainate; PPADS, Pyridoxalphosphate-6-azephenyl-2′,4′-disulfonic acid; CPT, cyclopentyltheophylline; 4-CIN, alpha-cyano-4-hydoxycinnamate.