Supplemental Material

Diffusional Trapping of GluR1 AMPA Receptors by Input-Specific Synaptic Activity

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Supplementary Figure Legends

Supplementary Figure S1. Synapse-Specific Visualization of Presynaptic Boutons Expressing Tetanus Toxin Light Chain.
Hippocampal neurons were infected with lentivirus expressing synaptophysin-GFP:IRES:TetTx on DIV7 and visualized on DIV15. Presynaptic terminals were labeled using a rhodamine derivative of a mitochondrial marker (Mitotracker Red) as described (Tardin et al., 2003). Individual axons expressing synaptophysin-GFP:IRES:TetTx and their associated presynaptic boutons contacting postsynaptic dendrites (green, arrows) were readily distinguished for intermingled presynaptic boutons of nontransfected neurons (red, arrowheads). Scale bar, 2 μm.

Supplementary Figure S2. Synapse-Specific Inactivation of Vesicle Cycling.
Hippocampal neurons were infected with lentivirus expressing synaptophysin-GFP:IRES:TetTx on DIV7 and visualized on DIV15. Prior to visualization, neurons were incubated with 15 μM FM4-64 in the presence of 60 mM KCl for 1 min to label functional presynaptic boutons capable of recycling synaptic vesicles. Note that the presynaptic terminals of neurons expressing TetTx (green) exhibited no detectable FM4-64 uptake (red). Scale bar, 1 μm.

Supplementary Movie Legends

Supplementary Movie S1. GluR1 Receptors Move Rapidly Through Inactive Synapses but are Stabilized At Active Synapses
Time lapse images of GluR1 receptors labeled with semiconductor quantum dot (QD)-conjugated surface antibodies (red blinking circles) moving on the dendrites of cultured hippocampal neurons (DIV16). Silent presynaptic boutons of neurons expressing synaptophysin-GFP:IRES:TetTx are indicated in green. Untransfected active boutons are

indicated in blue. Note the two GluR1-QDs labeled in Figure 1D. The timelapse is 52 s in duration and is run 4x real time. The image is 10 μm by 10 μm.

Supplementary Movie S2. An Individual GluR1 Receptor Moves Through an Inactive Synapse Before Becoming Stabilized at a Nearby Active Synapse
Time lapse images of GluR1 receptors labeled with semiconductor quantum dot (QD)-conjugated surface antibodies (red blinking circles) moving on the dendrites of cultured hippocampal neurons (DIV16). Silenced presynaptic boutons of neurons expressing synaptophysin-GFP:IRES:TetTx are indicated in green. Untransfected active boutons are indicated in blue. Note the GluR1-QD labeled in Figure 1E which moves through a silent synapse (green) before approaching and remaining at a small nearby active synapse (blue). The time lapse is 45 s in duration and is run at 5x real time. The image is 6 μm by 6 μm.

Supplementary Movie S3. GluR1 Mobility is Highly Confined Within Active Synapses.
Time lapse images of a GluR1-QD (red blinking circles) immobilized at an active synapse (green spot in this movie). Note that the color label of the active synapse in the movie (green) is different than in Figure 4A. The stationary GluR1-QD explores only a small portion of the synapse. The time lapse is 45 s in duration and is 5x real time. The image is 3.5 μm by 3.5 μm.

Supplementary Movie S4. GluR1 is Less Confined at Silenced Synapses.
Time lapse images of a GluR1-QD (red blinking circles) moving within and around an inactive synapse (green). The GluR1-QD enters and exits the synapse and ultimately explores most of the synaptic domain. The time lapse is 50 s in duration and is 2x real time. The image is 1.5 μm by 3 μm.

References

Tardin, C., Cognet, L., Bats, C., Lounis, B., and Choquet, D. (2003). Direct imaging of lateral movements of AMPA receptors inside synapses. EMBO J *22*, 4656-4665.

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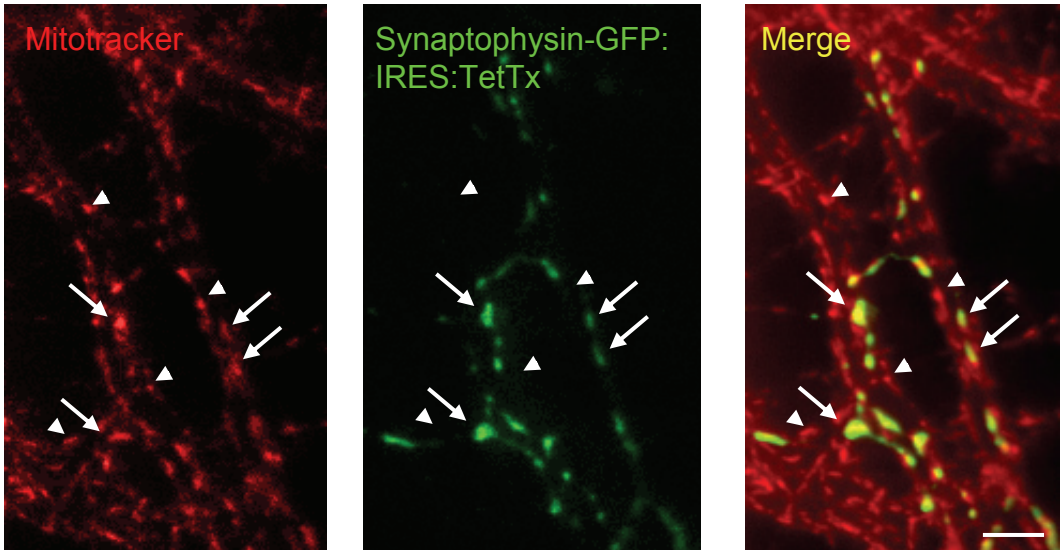
Supplementary Figure S1



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Supplementary Figure S2