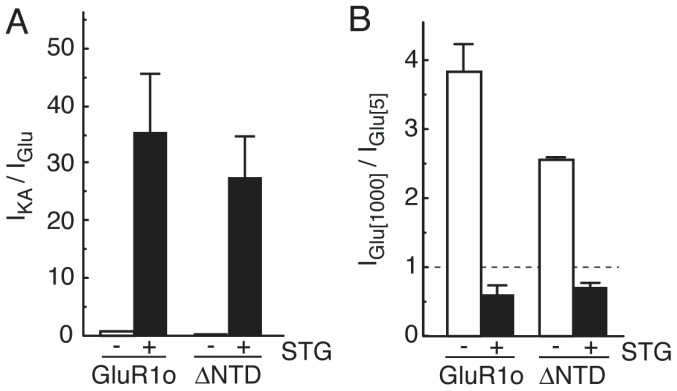
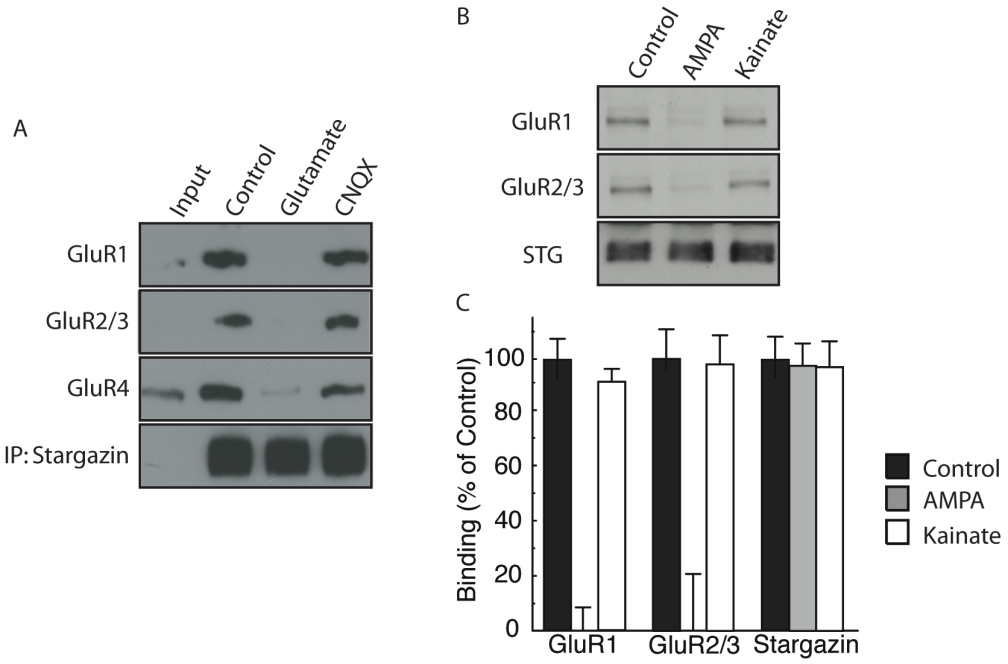
Neuron, Volume 61
Supplemental Data

Auto-inactivation of neuronal AMPA receptors via glutamate-regulated TARP interaction

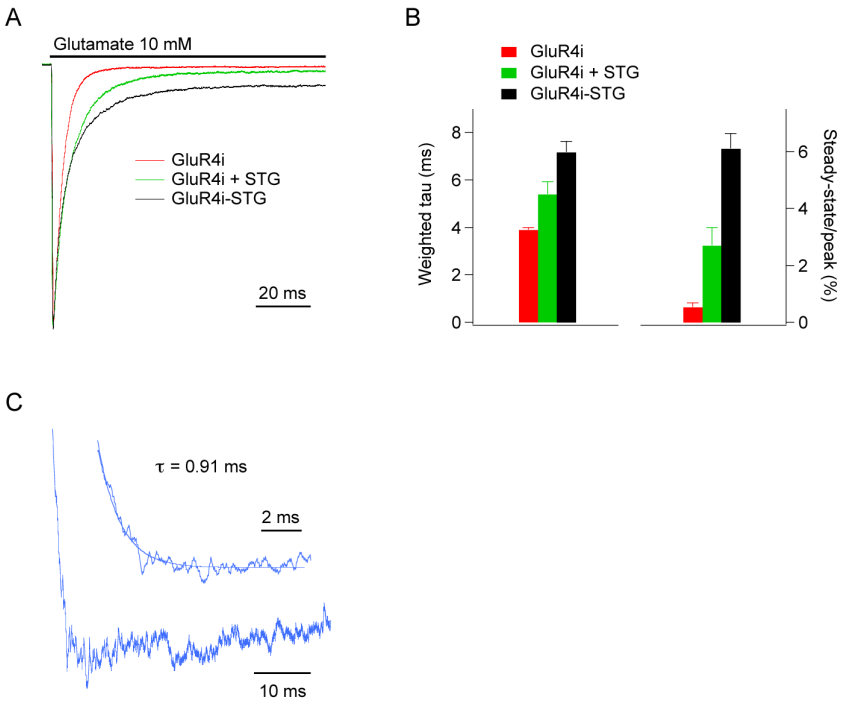
Megumi Morimoto-Tomita, Wei Zhang, Christoph Straub, Chang-Hoon Cho, Kwang S. Kim, James R. Howe, and Susumu Tomita



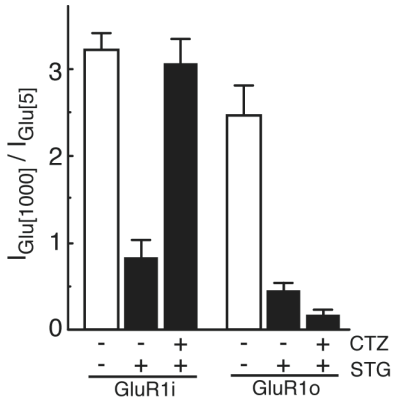
Supplementary figure 1. The N-terminal domain of the AMPA receptor is not involved in the concentration dependent modulation of AMPA receptors by stargazin. Glutamate (5 μM and 1000 μM) and kainate (10 μM)-evoked currents were recorded in oocytes injected with GluR1 flop (GluR1o, 20 ng)) or GluR1o lacking its N-terminal domain (ΔNTD, 20 ng)). Stargazin (STG) was also injected where indicated (0.1 ng ea.). (A) Stargazin modulates kainate efficacy in oocytes injected with either GluR1 flop (GluR1o) or ΔNTD transcripts. (B) The N-terminal domain of GluR1o is not required for concentration-dependent modulation of AMPA receptors mediated by stargazin. Data represent the mean ± s.e.m. (*n*=5-6).



Supplementary figure 2. AMPA receptors were dissociated from stargazin by the application of an agonist, but not an antagonist. (A-C) Solubilized membranes from rat whole brain were immunoprecipitated with an anti-stargazin antibody. Beads were then washed with glutamate (100 μM) and bound proteins were detected by Western blotting. (A) Glutamate induced the dissociation of stargazin from all AMPA receptor isoforms (GluR1-4), whereas the antagonist CNQX had no effect. (B, C) AMPA, but not kainate, induced the dissociation of stargazin from AMPA receptors. Data represent the mean ± s.e.m. (n=3).

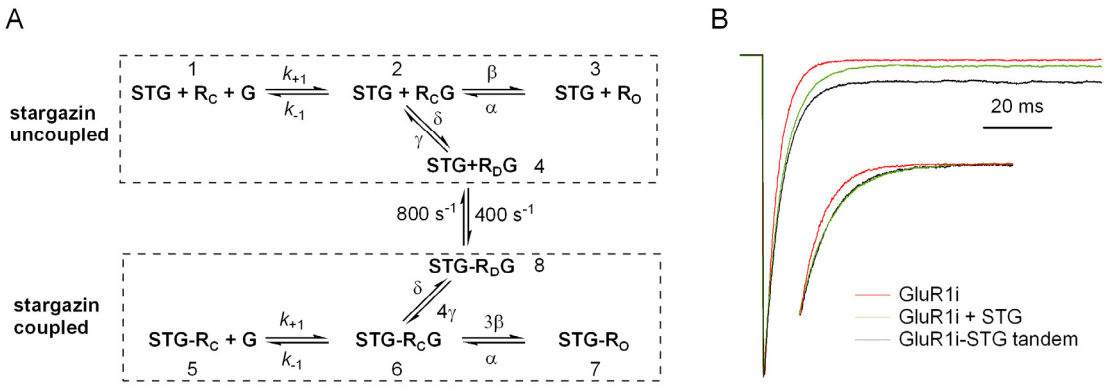


Supplementary figure 3. Glutamate-induced dissociation of stargazin from GluR4 receptors occurs rapidly after receptor desensitization. (A) Responses to 200 ms applications of 10 mM glutamate in outside-out patches from tsA201 cells transfected with GluR4i alone, GluR4i with stargazin, or tandem GluR4i-stargazin. Each trace is the mean of 10 to 20 trials from individual patches. The currents were scaled so the peak amplitudes were the same. (B) Mean (± s.e.m.) values for weighted time constants (left) and steady-state currents (right) obtained from bi-exponential fits to the decays of currents evoked by 200 ms applications of 10 mM glutamate (n = 5-10). Current obtained by subtracting the mean response for GluR4i with stargazin from the mean response for the GluR4i-stargazin tandem (data from all patches were averaged and the peak amplitudes were scaled as in panel A). The inset shows the first 10 ms of the difference current, which decayed mono-exponentially (tau = 0.91 ms).



Supplementary figure 4. The concentration-dependent modulation of AMPA receptors mediated by stargazin was blocked by AMPA receptor potentiators.

Glutamate-evoked currents (5 μM and 1000 μM) were measured with and without 50 μM cyclothiazide (CTZ) in oocytes injected with GluR1i cRNA alone (20 ng) or with STG cRNA (0.1 ng each) (*n*=5, 6). Cyclothiazide, which blocks the desensitization of AMPA receptor flip isoforms, blocked the concentration-dependent modulation of GluR1i. Cyclothiazide enhanced the concentration-dependent modulation of GluR1 flop (GluR1o), which is less sensitive to cyclothiazide (*n*=6). Data represent the mean ± s.e.m. from the indicated number of experiments.



Supplementary figure 5. A simple kinetic mechanism for stargazin dissociation. (A) Gating mechanism in which receptors in resting states (R_C) can bind glutamate (G) and transition to either open (R_O) or desensitized (R_D) states. Receptor states 1-4 are not effectively coupled with stargazin, whereas states 5-8 are and this coupling results in a 3-fold increase in the rate constant for channel opening and a 4-fold increase in the rate constant for recovery from desensitization. In stargazin co-expression experiments, the vast majority of resting receptors are initially associated with stargazin. Desensitization promotes the dissociation of stargazin, and at equilibrium desensitized receptors that are uncoupled from stargazin outnumber stargazin-coupled receptors by a factor of 2 (as indicated by the rate constants governing transitions between the two forms of desensitized receptor). (B) Current traces from Monte Carlo simulations using the gating mechanism in panel A and a virtual 100 ms application of 10 mM glutamate. The red trace was obtained using only stargazin uncoupled states (1-4) and corresponds to expression of GluR1i alone. The black trace was obtained using only stargazin coupled states (5-8) and corresponds to the GluR1i-stargazin tandem. The green trace was obtained using states 1-8 and corresponds to co-expression of GluR1i with stargazin. Despite the simplicity of the mechanism, the simulations reproduce the slowing of desensitization seen for GluR1i with stargazin and the GluR1i-stargazin tandem, as well as the relative amplitudes of the steady-state currents for the three experimental conditions illustrated in Fig. 6A. Difference currents obtained by subtracting the

GluR1i with stargazin currents from the GluR1i-stargazin tandem current reached steady-state levels in 12 to 15 ms. The values for rate constants were: $k_{+1} = 1 \times 10^7\ M^{-1}s^{-1}$, $k_{-1} = 3000\ s^{-1}$, $\beta = 5000\ s^{-1}$, $\alpha = 3000\ s^{-1}$, $\delta = 1500\ s^{-1}$, $\gamma = 5\ s^{-1}$. The initial probabilities were set equal to 1 for state 1 in the GluR1i alone simulations and equal to 1 for state 5 in the GluR1i with stargazin and GluR1i-stargazin tandem simulations.