Neuron 50, 75–88, April 6, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.neuron.2006.02.027

Synaptic AMPA Receptor Exchange Maintains Bidirectional Plasticity

Stefanie G. McCormack,[1,3] Ruth L. Stornetta,[1,3] and J. Julius Zhu[1,2,3,*]
[1] Department of Pharmacology
[2] Department of Neuroscience
[3] Neuroscience Graduate Program
University of Virginia School of Medicine
Charlottesville, Virginia 22908

Summary

Activity-dependent synaptic delivery of GluR1-, GluR2L-, and GluR4-containing AMPA receptors (-Rs) and removal of GluR2-containing AMPA-Rs mediate synaptic potentiation and depression, respectively. The obvious puzzle is how synapses maintain the capacity for bidirectional plasticity if different AMPA-Rs are utilized for potentiation and depression. Here, we show that synaptic AMPA-R exchange is essential for maintaining the capacity for bidirectional plasticity. The exchange process consists of activity-independent synaptic removal of GluR1-, GluR2L-, or GluR4-containing AMPA-Rs and refilling with GluR2-containing AMPA-Rs at hippocampal and cortical synapses in vitro and in intact brains. In *GluR1* and *GluR2* knockout mice, initiation or completion of synaptic AMPA-R exchange is compromised, respectively. The complementary AMPA-R removal and refilling events in the exchange process ultimately maintain synaptic strength unchanged, but their long rate time constants (~15–18 hr) render transmission temporarily depressed in the middle of the exchange. These results suggest that the previously hypothesized "slot" proteins, rather than AMPA-Rs, code and maintain transmission efficacy at central synapses.

Introduction

A number of studies have shown that synaptic trafficking of AMPA-sensitive glutamate receptors (-Rs) plays a key role in synaptic transmission and plasticity (Bredt and Nicoll, 2003; Collingridge et al., 2004; Malenka and Bear, 2004; Malinow, 2003; Sheng and Kim, 2002; Thomas and Huganir, 2004). AMPA-Rs with long cytoplasmic termini (i.e., GluR1-, GluR2L-, and GluR4-containing AMPA-Rs) are driven into synapses during activity-induced synaptic enhancement (e.g., long-term potentiation or LTP), whereas AMPA-Rs with only short cytoplasmic termini (i.e., GluR2-containing AMPA-Rs) are removed from synapses during activity-induced synaptic depression (e.g., long-term depression or LTD). However, it is unclear how both plasticity processes are maintained given that synaptic potentiation and depression utilize different AMPA-Rs. One speculation is that synaptic exchange of GluR1-, GluR2L-, and GluR4-containing AMPA-Rs with GluR2-containing AMPA-Rs, an activity-independent trafficking process (Kolleker et al., 2003; Takahashi et al., 2003; Zhu et al., 2000), may restore the ability of synapses to undergo new potentiation and/or depression. However, the properties of this AMPA-R exchange are poorly understood, and whether and how synaptic AMPA-R exchange restores the capacity for bidirectional plasticity at synapses remains unknown.

In contrast to synaptic AMPA-R exchange (i.e., replacing synaptic GluR1-, GluR2L-, or GluR4-containing AMPA-Rs with nonsynaptic GluR2-containing AMPA-Rs), synaptic AMPA-R cycling (i.e., swapping synaptic and nonsynaptic GluR2-containing AMPA-Rs) has been well characterized (Lee et al., 2002; Luscher et al., 1999; Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998; Zhu et al., 2000). These studies have demonstrated that activity-independent synaptic cycling of GluR2-containing AMPA-Rs between synaptic and nonsynaptic sites has a rate time constant of ~20–25 min. In addition, synaptic AMPA-R cycling does not alter transmission efficacy, which is similar to synaptic AMPA-R exchange (Kolleker et al., 2003; Zhu et al., 2000). The ability of synapses to maintain transmission efficacy during AMPA-R cycling and exchange is consistent with the conventional view that AMPA-Rs may code synaptic strength; one-to-one AMPA-R replacement at synapses is ideal to maintain synaptic efficacy without being compromised by protein turnover. Recently, based on a published study (Zhu et al., 2000) as well as unpublished observations, Malinow and colleagues have proposed an alternative theory that "slot" proteins, which are delivered into synapses accompanying AMPA-Rs during synaptic potentiation, code transmission efficacy (Malinow, 2003). It is believed that "slot" proteins, instead of AMPA-Rs, maintain transmission efficacy because they can remain at synapses after AMPA-Rs are removed and then be refilled by other AMPA-Rs at a later time. However, data to support either theory are still missing.

To determine the functional role of synaptic AMPA-R exchange and find experimental evidence that may support the AMPA-R and/or "slot" protein coding theories, we examined synaptic AMPA-R exchange in vitro and in intact brains. We found that synaptic AMPA-R exchange maintained bidirectional plasticity by removing GluR1-, GluR2L-, or GluR4-containing AMPA-Rs from synapses and then refilling synapses with GluR2-containing AMPA-Rs. The two AMPA-R trafficking events had a slow rate time constant of ~15–18 hr, suggesting the existence of multiple trafficking pools of AMPA-Rs, including two activity-independent trafficking pools of GluR2-containing AMPA-Rs. The AMPA-R removal and refilling processes, although maintaining the ultimate transmission strength unchanged, caused a temporary depression of AMPA-R-mediated responses in the middle of the exchange. These results provide experimental evidence indicating that "slot" proteins, instead of AMPA-Rs, code and maintain transmission efficacy.

Results

Synaptic AMPA-R Exchange Restores Bidirectional Plasticity Capacity

To determine whether synaptic AMPA-R exchange plays a role in synaptic plasticity, we perturbed the

*Correspondence: jjzhu@virginia.edu

synaptic AMPA delivery process and then measured LTP before and after synaptic AMPA-R exchange (Figure 1A). We expressed Ras(wt)-GFP in CA1 pyramidal neurons for ~14 hr. Neurons expressing Ras(wt)-GFP had enhanced AMPA responses compared to nearby nonexpressing neurons (Figures 1B and 1C), consistent with the notion that Ras signaling induces synaptic potentiation or LTP by stimulating Erk1/2 activity and synaptic delivery of AMPA-Rs with long cytoplasmic termini (English and Sweatt, 1997; Zhu et al., 2002). We then applied an LTP-inducing stimuli and found that LTP was largely blocked in Ras(wt)-GFP-expressing neurons, although nearby nonexpressing neurons did show pathway-specific LTP (Figures 1B and 1C). After 14 hr of expression of Ras(wt)-GFP in normal media, we incubated slices in high Mg^{2+} media to block further activity-dependent synaptic AMPA-R trafficking but not synaptic AMPA-R exchange for the following 36 hr (Kolleker et al., 2003; Zhu et al., 2000, 2002). Reexamining LTP ~36 hr later showed that Ras(wt)-GFP expressing neurons generated LTP comparable to nearby nonexpressing neurons (Figures 1D and 1E). As controls, we examined effects of the high Mg^{2+} treatment on synaptic responses and membrane properties of Ras(wt)-GFP-expressing neurons. Although the high Mg^{2+} treatment resulted in the expected disappearance of enhanced rectification in Ras(wt)-RFP- and GluR2L-GFP-coexpressing neurons (Figure S1), it did not differentially affect NMDA responses and basic membrane properties in Ras(wt)-GFP-expressing and -nonexpressing neurons (Figures S2A and S2B; cf. Liao et al. [1999] and Rao and Craig [1997]). Together, these results suggest that synaptic AMPA-R exchange restores LTP capacity in Ras(wt)-GFP-expressing neurons.

To investigate the potential role of synaptic AMPA-R exchange in synaptic depression, we perturbed the synaptic AMPA removal process and measured LTD before and after synaptic AMPA-R exchange (Figure 2A). We expressed Rap1(wt)-GFP in CA1 neurons for ~14 hr and found that Rap1(wt)-GFP-expressing neurons had reduced AMPA responses compared to nearby nonexpressing neurons (Figures 2B and 2C). This result is consistent with the view that Rap1 signaling induces synaptic depression or LTD by stimulating p38 MAPK activity and synaptic removal of AMPA-Rs with only short cytoplasmic termini (Murray and O'Connor, 2003; Zhu et al., 2002) (for mGluR-dependent LTD, see also Bolshakov et al. [2000]). Applying an LTD-inducing stimuli in these neurons revealed that LTD was largely blocked in Rap1(wt)-GFP-expressing neurons (Figures 2B and 2C). As a control, nearby nonexpressing neurons did show pathway-specific LTD (Figures 2B and 2C). After 14 hr of expression of Rap1(wt)-GFP in normal media, we incubated slices in high Mg^{2+} media for the following 36 hr, which blocks further activity-dependent synaptic AMPA-R trafficking but spares synaptic AMPA-R exchange (Kolleker et al., 2003; Zhu et al., 2000, 2002). Reexamining LTD ~36 hr later showed that Rap1(wt)-GFP-expressing neurons generated pathway-specific LTD comparable to nearby nonexpressing neurons (Figures 2D and 2E). As controls, the high Mg^{2+} treatment was found not to differentially affect NMDA responses and basic membrane properties in Rap1(wt)-GFP-expressing and -nonexpressing neurons (Figures S2C and S2D). Together, these results suggest that synaptic AMPA-R exchange restores LTD capacity in Rap1(wt)-GFP-expressing neurons.

Exchange of Recombinant GluR4 and GluR2L with Endogenous GluR2 In Vitro

To learn how AMPA-Rs are exchanged at synapses, we first investigated the temporal properties of the exchange. We expressed GluR4-GFP in neonatal CA1 neurons and monitored the evoked AMPA-R- and NMDA-R-mediated synaptic responses over an ~5 day time course (Figures 3A–3C). GluR4-GFP expressed in CA1 pyramidal neurons mainly forms receptors devoid of GluR2 subunits; these receptors are thus rectified channels or electrophysiologically "tagged" (Zhu et al., 2000). Because AMPA responses in pyramidal neurons are largely nonrectifying, the synaptic presence of GluR4-GFP will be indicated by the enhancement of amplitude and rectification of AMPA responses. CA1 neurons expressing GluR4-GFP for 18 hr had ~85% increase in amplitude and ~60% increase in rectification of AMPA responses compared to nearby nonexpressing cells (Figures 2B and 2C). These results are consistent with our previous finding that spontaneous synaptic activity drives GluR4-GFP into synapses (Zhu et al., 2000).

After 18 hr of expression of GluR4-GFP in normal media, we incubated the slices in media containing 12 mM Mg^{2+}. The high Mg^{2+} treatment had little effect on GluR4-GFP expression (Figure S3) but was effective in blocking any subsequent delivery of GluR4-GFP by blocking synaptic activity (Zhu et al., 2000). Therefore, we could then monitor the long-term fate of the newly delivered, rectified GluR4-GFP by measuring and comparing AMPA responses in expressing and nonexpressing neurons in the next 96 hr (Figures 3B and 3C). We found that the rectification of AMPA responses in GluR4-GFP-expressing neurons gradually disappeared over the following days (Figure 3C), indicating that the rectified GluR4-GFP was slowly removed from synapses. Fitting an exponential to the rectification data points yielded a rate time constant of 17.3 hr (Figure 3C). The amplitude of AMPA responses in GluR4-GFP-expressing neurons decreased initially for ~20 hr and then slowly returned to the previously enhanced level or ~185% of that of nonexpressing neurons (Figure 3C), suggesting that endogenous nonrectified GluR2-containing AMPA-Rs were added back into synapses. Fitting an exponential to the amplitudes of AMPA responses 42 hr after adding high Mg^{2+}, during which GluR4-GFP was largely removed from synapses, gave a GluR2-containing AMPA-R addition rate time constant of 15.9 hr (Figure 3C). Together, the GluR4-GFP removal rate time constant of 17.3 hr and GluR2-containing AMPA-R addition rate time constant of 15.9 hr predicted a temporarily depressed AMPA transmission in the middle of the exchange, closely matching experimental data points (Figure 3C). These results together suggest that synaptic GluR4-GFP exchange is mediated by two activity-independent trafficking events: synaptic removal of GluR4-GFP and addition of endogenous GluR2-containing AMPA-Rs.

GluR2L mediates transmission in juvenile and adult CA1 neurons (Kolleker et al., 2003). To determine whether

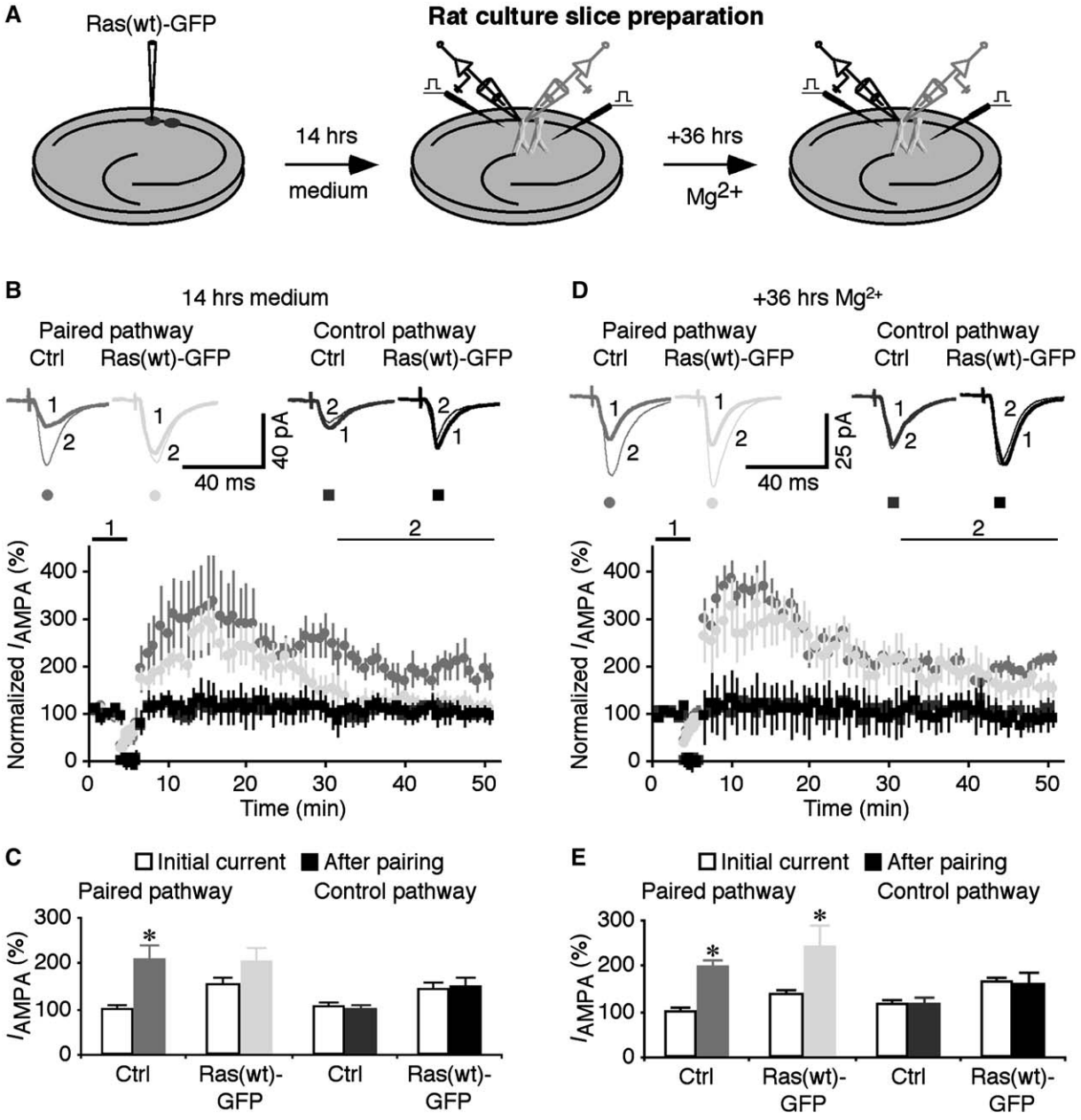

Figure 1. Synaptic AMPA-R Exchange Maintains Capacity for Synaptic Potentiation

(A) Schematic drawing showing the experimental design.

(B) Average AMPA-R-mediated synaptic responses obtained before (−60 mV, thick trace) and after (−60 mV, thin trace) LTP-inducing pairing in paired (upper left) and control pathway (upper right) from a pair of Ras(wt)-GFP-expressing and nearby -nonexpressing cells cultured in normal media for 14 hr. Lower plot, normalized simultaneously evoked responses recorded from all cell pairs expressing or nonexpressing Ras(wt)-GFP against time.

(C) Steady-state AMPA response amplitudes in paired (ctrl: 197.9% ± 20.3% from initial −27.4 ± 2.5 pA; exp: 122.8% ± 15.7% from initial −42.5 ± 3.4 pA; n = 11; $p < 0.005$) and control pathways (ctrl: 102.9% ± 10.0% from initial −28.3 ± 3.0 pA; exp: 102.9% ± 8.9% from initial −39.5 ± 4.0 pA; n = 11; $p = 0.93$) in Ras(wt)-GFP-expressing and -nonexpressing cells before and after pairing.

(D) Average AMPA-R-mediated synaptic responses obtained before (−60 mV, thick trace) and after (−60 mV, thin trace) LTP-inducing pairing in paired (upper left) and control pathway (upper right) from a pair of Ras(wt)-GFP-expressing and nearby -nonexpressing cells cultured in normal media for 14 hr followed by high Mg^{2+} media for 36 hr. Lower plot, normalized simultaneously evoked responses recorded from all cell pairs expressing or nonexpressing Ras(wt)-GFP against time.

(E) Steady state AMPA response amplitudes in paired (ctrl: 196.9% ± 13.7% from initial −26.4 ± 2.6 pA; exp: 176.9% ± 27.7% from initial −35.3 ± 3.2 pA; n = 11; $p = 0.33$) and control pathways (ctrl: 102.7% ± 15.8% from initial −30.4 ± 2.8 pA; exp: 97.8% ± 8.0% from initial −42.5 ± 4.2 pA; n = 11; $p = 0.79$) in Ras(wt)-GFP-expressing and -nonexpressing cells before and after pairing. AMPA-R-mediated current amplitudes and standard errors were normalized to average initial values from cells recorded in the normal bath solution. Asterisk indicates $p < 0.05$ (Wilcoxon test).

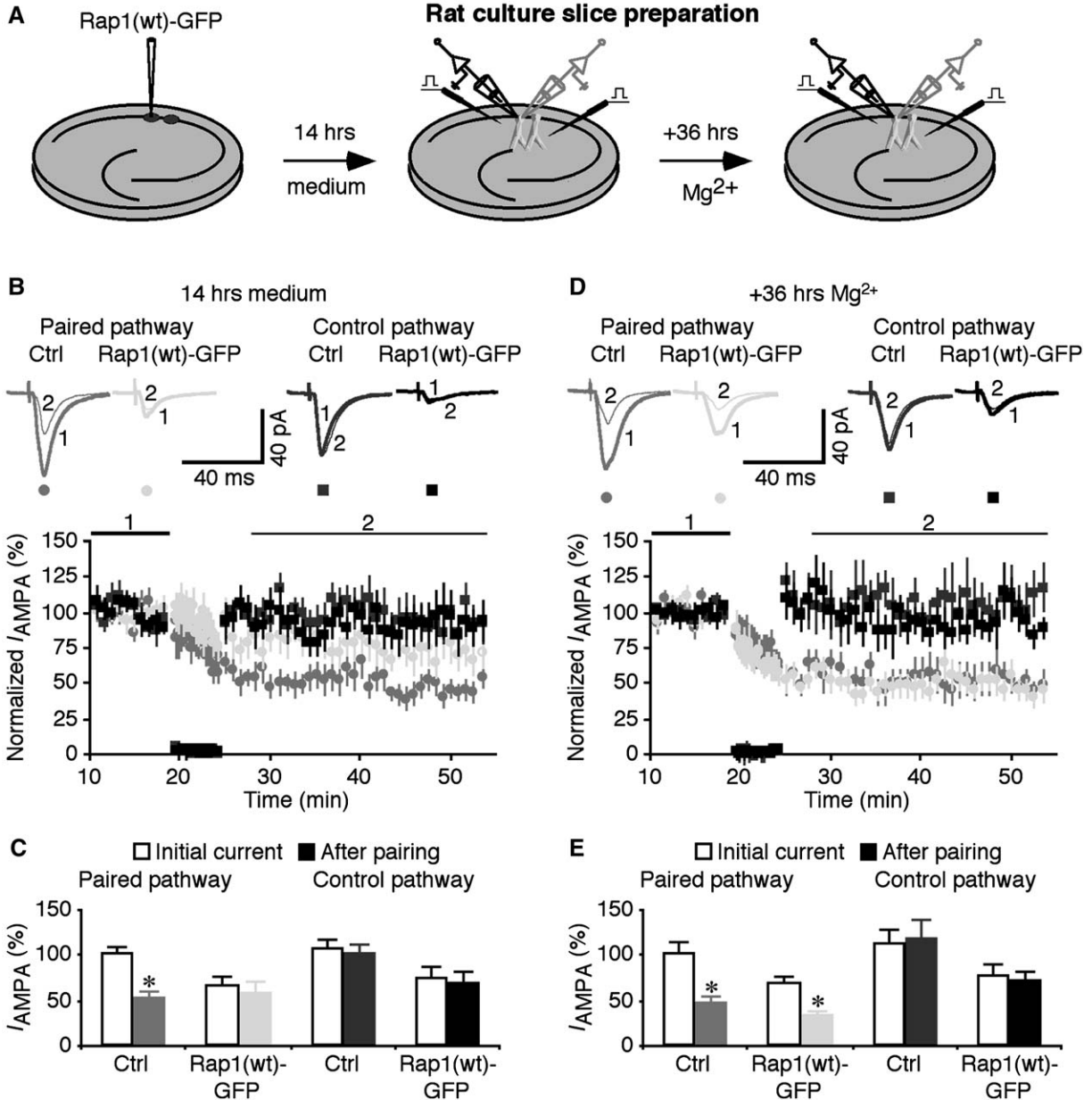

Figure 2. Synaptic AMPA-R Exchange Maintains Capacity for Synaptic Depression

(A) Schematic drawing showing the experimental design.

(B) Average AMPA-R-mediated synaptic responses obtained before (−60 mV, thick trace) and after (−60 mV, thin trace) LTD-inducing pairing in paired (upper left) and control pathway (upper right) from a pair of Rap1(wt)-GFP-expressing and nearby -nonexpressing cells cultured in normal media for 14 hr. Lower plot, normalized simultaneously evoked responses recorded from all cell pairs expressing or nonexpressing Rap1(wt)-GFP against time.

(C) Steady state AMPA response amplitudes in paired (ctrl: 50.3% ± 5.2% from initial −50.3 ± 4.9 pA; exp: 76.6% ± 9.0% from initial −33.0 ± 5.2 pA; n = 11; $p < 0.05$) and control pathways (ctrl: 97.6% ± 7.8% from initial −53.4 ± 6.2 pA; exp: 92.2% ± 8.0% from initial −37.5 ± 5.9 pA; n = 11; p = 0.66) in Rap1(wt)-GFP-expressing and -nonexpressing cells before and after pairing.

(D) Average AMPA-R-mediated synaptic responses obtained before (−60 mV, thick trace) and after (−60 mV, thin trace) LTD-inducing pairing in paired (upper left) and control pathway (upper right) from a pair of Rap1(wt)-GFP-expressing and nearby -nonexpressing cells cultured in normal media for 14 hr followed by high Mg^{2+} media for 36 hr. Lower plot, normalized simultaneously evoked responses recorded from all cell pairs expressing or nonexpressing Rap1(wt)-GFP against time.

(E) Steady state AMPA response amplitudes in paired (ctrl: 52.5% ± 9.0% from initial −49.3 ± 6.9 pA; exp: 49.4% ± 5.4% from initial −33.1 ± 4.9 pA; n = 11; p = 0.79) and control pathways (ctrl: 105.8% ± 11.2% from initial −55.1 ± 8.1 pA; exp: 94.7% ± 6.0% from initial −38.1 ± 6.1 pA; n = 11; p = 0.66) in Rap1(wt)-GFP-expressing and -nonexpressing cells before and after pairing. AMPA-R-mediated current amplitudes and standard errors were normalized to average initial values from cells recorded in the normal bath solution. Asterisk indicates $p < 0.05$ (Wilcoxon test).

synaptic exchange of GluR2L-containing AMPA-Rs uses the same mechanisms as GluR4, we expressed GluR2L(R→Q)-GFP in juvenile CA1 neurons. In this construct, an R→Q mutation is generated at its pore region to make it a rectified channel, or electrophysiologically "tagged" (Kolleker et al., 2003). We found that CA1

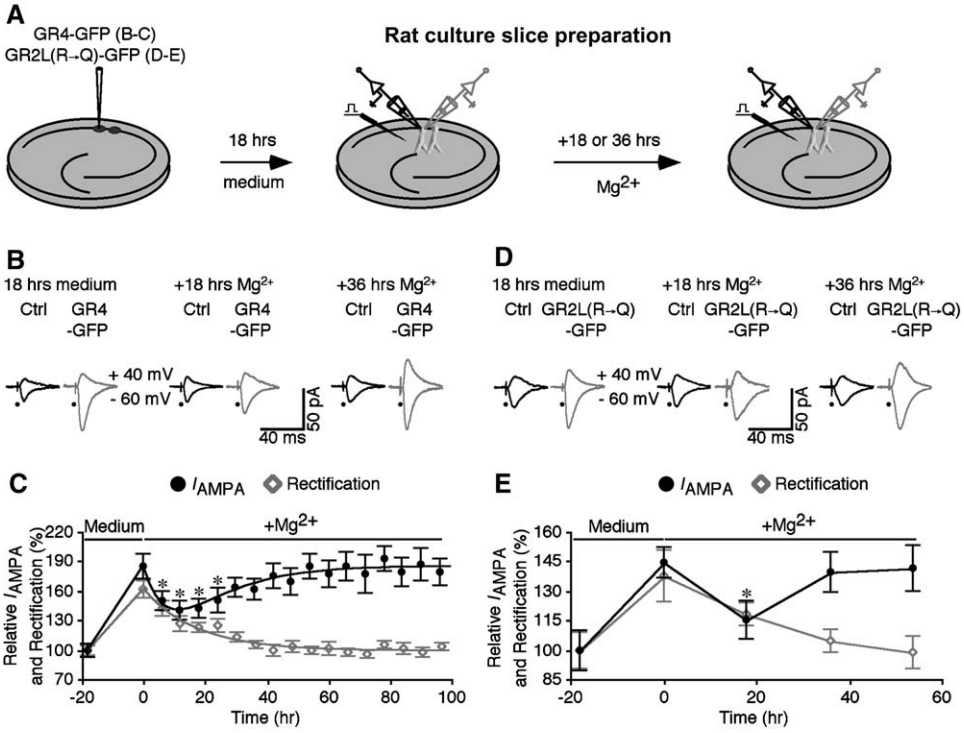

Figure 3. Synaptic Exchange of GluR4-GFP and GluR2L(R→Q)-GFP with GluR2 In Vitro

(A) Schematic drawing showing the experimental design.

(B) Evoked AMPA-R-mediated responses recorded from nonexpressing (Ctrl) and GluR4-GFP expressing CA1 cells cultured in normal media for 18 hr, normal media for 18 hr followed by high Mg^{2+} media for an additional 18 hr, or normal media for 18 hr followed by high Mg^{2+} media for an additional 36 hr.

(C) Plots of amplitude and rectification of AMPA responses against time in culture in normal and high Mg^{2+} media. Black filled dots, AMPA responses in GluR4-GFP-expressing cells relative to nearby nonexpressing control cells cultured in normal media for 18 hr (ctrl: −30.7 ± 2.2 pA; exp: −56.9 ± 3.8 pA; n = 24; p < 0.001), followed by 6 hr high Mg^{2+} media (ctrl: −28.0 ± 2.3 pA; exp: −42.1 ± 2.8 pA; n = 23; p < 0.001), 12 hr high Mg^{2+} media (ctrl: −34.1 ± 2.3 pA; exp: −47.8 ± 3.1 pA; n = 24; p < 0.001), 18 hr high Mg^{2+} media (ctrl: −28.5 ± 2.6 pA; exp: −40.4 ± 2.8 pA; n = 22; p < 0.001), 24 hr high Mg^{2+} media (ctrl: −27.6 ± 2.6 pA; exp: −40.8 ± 3.6 pA; n = 24; p < 0.001), 30 hr high Mg^{2+} media (ctrl: −31.8 ± 2.4 pA; exp: −52.1 ± 4.1 pA; n = 24; p < 0.001), 36 hr high Mg^{2+} media (ctrl: −39.0 ± 2.9 pA; exp: −63.2 ± 4.1 pA; n = 24; p < 0.001), 42 hr high Mg^{2+} media (ctrl: −34.3 ± 3.2 pA; exp: −60.2 ± 4.3 pA; n = 24; p < 0.001), 48 hr high Mg^{2+} media (ctrl: −28.8 ± 2.6 pA; exp: −48.6 ± 3.9 pA; n = 23; p < 0.005), 54 hr high Mg^{2+} media (ctrl: −33.5 ± 2.7 pA; exp: −61.6 ± 4.2 pA; n = 24; p < 0.001), 60 hr high Mg^{2+} media (ctrl: −27.3 ± 2.9 pA; exp: −48.5 ± 3.7 pA; n = 22; p < 0.001), 66 hr high Mg^{2+} media (ctrl: −28.8 ± 2.5 pA; exp: −53.0 ± 4.4 pA; n = 24; p < 0.005), 72 hr high Mg^{2+} media (ctrl: −28.3 ± 2.5 pA; exp: −50.1 ± 5.4 pA; n = 24; p < 0.005), 78 hr high Mg^{2+} media (ctrl: −25.1 ± 2.4 pA; exp: −48.5 ± 3.0 pA; n = 24; p < 0.001), 84 hr high Mg^{2+} media (ctrl: −30.6 ± 2.2 pA; exp: −55.1 ± 4.6 pA; n = 24; p < 0.001), 90 hr high Mg^{2+} media (ctrl: −26.6 ± 2.5 pA; exp: −49.9 ± 4.6 pA; n = 24; p < 0.001), or 96 hr high Mg^{2+} media (ctrl: −32.3 ± 2.7 pA; exp: −57.8 ± 4.6 pA; n = 26; p < 0.005). Gray unfilled diamonds, rectification of AMPA responses in GluR4-GFP-expressing cells relative to nearby nonexpressing control cells cultured in normal media for 18 hr (ctrl: 1.91 ± 0.08; exp: 3.08 ± 0.17; n = 24; p < 0.001), followed by 6 hr high Mg^{2+} media (ctrl: 1.95 ± 0.08; exp: 2.76 ± 0.15; n = 23; p < 0.005), 12 hr high Mg^{2+} media (ctrl: 1.86 ± 0.06; exp: 2.35 ± 0.13; n = 24; p < 0.005), 18 hr high Mg^{2+} media (ctrl: 1.92 ± 0.11; exp: 2.37 ± 0.10; n = 22; p < 0.01), 24 hr high Mg^{2+} media (ctrl: 1.79 ± 0.08; exp: 2.23 ± 0.12; n = 24; p < 0.01), 30 hr high Mg^{2+} media (ctrl: 1.77 ± 0.06; exp: 1.99 ± 0.09; n = 24; p = 0.056), 36 hr high Mg^{2+} media (ctrl: 1.78 ± 0.09; exp: 1.87 ± 0.08; n = 24; p = 0.46), 42 hr high Mg^{2+} media (ctrl: 1.76 ± 0.11; exp: 1.76 ± 0.10; n = 24; p = 0.89), 48 hr high Mg^{2+} media (ctrl: 1.83 ± 0.10; exp: 1.89 ± 0.10; n = 23; p = 0.67), 54 hr high Mg^{2+} media (ctrl: 1.80 ± 0.05; exp: 1.78 ± 0.07; n = 24; p = 0.57), 60 hr high Mg^{2+} media (ctrl: 1.78 ± 0.07; exp: 1.79 ± 0.13; n = 22; p = 0.45), 66 hr high Mg^{2+} media (ctrl: 1.79 ± 0.09; exp: 1.73 ± 0.07; n = 24; p = 0.59), 72 hr high Mg^{2+} media (ctrl: 1.87 ± 0.09; exp: 1.78 ± 0.07; n = 24; p = 0.41), 78 hr high Mg^{2+} media (ctrl: 1.76 ± 0.10; exp: 1.86 ± 0.07; n = 24; p = 0.39), 84 hr high Mg^{2+} media (ctrl: 1.72 ± 0.08; exp: 1.75 ± 0.09; n = 24; p = 0.67), 90 hr high Mg^{2+} media (ctrl: 1.72 ± 0.10; exp: 1.68 ± 0.08; n = 24; p = 0.98), or 96 hr high Mg^{2+} media (ctrl: 1.73 ± 0.08; exp: 1.79 ± 0.08; n = 26; p = 0.71). Gray line (t > 0) fits for rectification with synaptic removal of GluR4-GFP at a rate time constant of 17.3 hr during exchange. Black line (t > 0) fits for amplitude with synaptic removal of GluR4-GFP at the same rate time constant and synaptic addition of GluR2-containing AMPA-Rs at a rate time constant of 15.9 hr during exchange.

(D) Evoked AMPA-R-mediated responses recorded from nonexpressing (Ctrl) and GluR2L(R→Q)-GFP-expressing CA1 cells cultured in normal media for 18 hr, normal media for 18 hr followed by high Mg^{2+} media for an additional 18 hr, or normal media for 18 hr followed by high Mg^{2+} media for an additional 36 hr.

(E) Plots of amplitude and rectification of AMPA responses against time in culture in normal and high Mg^{2+} media. Black filled dots, AMPA responses in GluR2L(R→Q)-GFP-expressing cells relative to nearby -nonexpressing control cells cultured in normal media for 18 hr (ctrl: −31.4 ± 3.3 pA; exp: −45.4 ± 2.4 pA; n = 24; p < 0.001), followed by 18 hr high Mg^{2+} media (ctrl: −30.7 ± 2.6 pA; exp: −35.2 ± 3.0 pA; n = 26; p < 0.005), 36 hr high Mg^{2+} media (ctrl: −30.9 ± 2.9 pA; exp: −43.1 ± 3.2 pA; n = 24; p < 0.005), or 54 hr high Mg^{2+} media (ctrl: −32.9 ± 3.0 pA; exp: −46.5 ± 3.7 pA; n = 22; p < 0.005). Gray unfilled diamonds, rectification of AMPA responses in GluR2L(R→Q)-GFP-expressing cells relative to nearby -nonexpressing control cells cultured in normal media for 18 hr (ctrl: 1.85 ± 0.17; exp: 2.55 ± 0.24; n = 24; p < 0.001), followed by 18 hr high Mg^{2+} media (ctrl: 1.77 ± 0.07; exp: 2.08 ± 0.10; n = 26; p < 0.01), 36 hr high Mg^{2+} media (ctrl: 1.75 ± 0.17; exp: 1.84 ± 0.10; n = 24; p = 0.75), or 54 hr high Mg^{2+} media (ctrl: 1.77 ± 0.14; exp: 1.74 ± 0.14; n = 22; p = 0.94). Asterisk indicates the points with relative amplitudes significantly different from GluR4 and GluR2L(R→Q)-GFP-expressing cells cultured in normal media for 18 hr (p < 0.05; Mann-Whitney Rank Sum nonparametric test). AMPA-R-mediated current amplitude and rectification were normalized to average values from control cells.

neurons expressing GluR2L(R→Q)-GFP for 18 hr in normal media had ~45% increase in amplitude and ~35% increase in rectification of AMPA responses compared to nearby nonexpressing neurons (Figures 3D and 3E), consistent with the idea that spontaneous synaptic activity delivers GluR2L(R→Q)-GFP into synapses (Kolleker et al., 2003). We then incubated the slices in media containing 12 mM Mg^{2+}, which blocks any subsequent delivery of GluR2L(R→Q)-GFP (Kolleker et al., 2003), and monitored the changes in AMPA responses in the next ~2 days. During this period, the rectification of AMPA responses in GluR2L(R→Q)-GFP-expressing neurons gradually disappeared (Figures 3D and 3E), indicating that the rectified GluR2L(R→Q)-GFP was removed from synapses. During the same period, the amplitude of AMPA responses was initially depressed before regaining the previous enhanced level (Figures 3D and 3E), suggesting that endogenous nonrectified GluR2-containing AMPA-Rs were slowly added back into synapses. These results together suggest that synaptic GluR2L(R→Q)-GFP exchange is mediated by activity-independent synaptic removal of GluR2L(R→Q)-GFP and addition of endogenous GluR2-containing AMPA-Rs.

Synaptic Delivery of AMPA-Rs with Long Cytoplasmic Termini Initiates AMPA-R Exchange

Strong synaptic activity (e.g., LTP-inducing stimuli), but not spontaneous activity, drives GluR1 into synapses in culture slices (Hayashi et al., 2000; Zhu et al., 2002). To study whether newly delivered GluR1 exchanges with GluR2-containing AMPA-Rs, we first drove recombinant GluR1-GFP and/or endogenous GluR1 into synapses by using a classic LTP-inducing paradigm (Figure 4A; cf. Worley et al. [1993]). In one set of experiments, we expressed GluR1-GFP in CA1 neurons of *GluR1* knockout mice in order to monitor the change of AMPA response amplitude in expressing neurons. We found that ~2 hr after LTP-inducing stimuli, GluR1-GFP-expressing neurons had ~40% increase in both amplitude and rectification of AMPA responses compared to nearby nonexpressing neurons (Figures 4B and 4C). We then incubated the slices in media containing high Mg^{2+}, which blocks any subsequent activity-dependent synaptic delivery or removal of AMPA-Rs (Zhu et al., 2002), and monitored the changes in AMPA responses in the next ~2 days. During this period, the rectification of AMPA responses in GluR1-GFP-expressing neurons gradually disappeared (Figures 4B and 4C), indicating that the rectified GluR1-GFP was removed from synapses. During the same period, the amplitude of AMPA responses was initially depressed before returning to the previous enhanced level (Figures 4B and 4C), indicating that endogenous nonrectified GluR2-containing AMPA-Rs were slowly added back into synapses. These results together indicate that synaptic GluR1-GFP exchange is mediated by activity-independent synaptic removal of GluR1-GFP and addition of endogenous GluR2-containing AMPA-Rs.

To determine whether endogenous GluR1 is exchanged with GluR2-containing AMPA-Rs in the same manner as recombinant GluR1-GFP, we expressed GluR1-GFP in CA1 neurons of wild-type mice in another set of experiments. About 2 hr after LTP-inducing stimuli, GluR1-GFP-expressing neurons had ~40% increase in rectification of AMPA responses compared to nearby nonexpressing neurons (Figures 4D and 4E), indicating the synaptic delivery of rectified GluR1-GFP in expressing neurons. The amplitude of AMPA responses was the same in GluR1-GFP-expressing and nearby -nonexpressing neurons (Figures 4D and 4E). These results suggest that LTP-inducing stimuli drives synaptic insertion of GluR1 and GluR1-GFP in nonexpressing neurons and expressing neurons to a saturated level (cf. Qin et al. [2005]). Incubating the slices in high Mg^{2+} media resulted in a gradual disappearance of the rectification of AMPA responses in GluR1-GFP-expressing neurons in the following ~2 days (Figures 4D and 4E). The amplitude of AMPA responses remained the same in GluR1-GFP-expressing and nearby -nonexpressing neurons during the same period (Figures 4D and 4E), suggesting that endogenous GluR1 behaved the same as recombinant GluR1-GFP, presumably exchanging with GluR2-containing AMPA-Rs at the same rate time constant. These results, together with those from *GluR1* knockout mice (Figures 4B and 4C), also indicate that blocking synaptic delivery of endogenous GluR1 compromises the initiation of synaptic AMPA-R exchange.

Synaptic Refilling of GluR2 Completes AMPA-R Exchange

A number of studies have demonstrated that synaptic trafficking of AMPA-Rs with only short cytoplasmic termini (i.e., GluR2-containing AMPA-Rs) is dominated by GluR2 subunits (Chung et al., 2003; Lee et al., 2004; Meng et al., 2003; Shi et al., 2001; Steinberg et al., 2004). To confirm that synaptic refilling of GluR2-containing AMPA-Rs plays a key role in synaptic AMPA-R exchange, we examined this process in *GluR2* knockout mice (Figure 5A). We first expressed GluR4-GFP in neonatal CA1 neurons prepared from *GluR2* knockout mice. CA1 neurons expressing GluR4-GFP for 18 hr had ~80% increase in amplitude of AMPA responses compared to nearby nonexpressing cells (Figures 5B and 5C). We then incubated the slices in media containing high Mg^{2+} in the next ~2 days and found that the enhanced AMPA responses gradually disappeared and never returned to the previous enhanced level (Figures 5B and 5C). Similarly, expressing GluR2L(R→Q)-GFP initially resulted in an enhancement of AMPA responses in juvenile CA1 neurons prepared from *GluR2* knockout mice (Figures 5D and 5E). Again, the enhanced AMPA responses slowly diminished in the following ~2 days in the presence of high Mg^{2+} in culture media and never returned to the previous enhanced level (Figures 5D and 5E). These results suggest that GluR3 alone (i.e., without GluR2) is not sufficient for refilling synapses during synaptic AMPA-R exchange. Collectively, these results indicate that blocking synaptic refilling of GluR2-containing AMPA-Rs blocks the completion of synaptic AMPA-R exchange.

Exchange of Recombinant GluR1 and GluR4 with Endogenous GluR2 In Vivo

We wished to know whether and how AMPA-Rs with long cytoplasmic termini exchange with GluR2 at synapses in vivo. Recent studies have shown that experience-dependent synaptic activity drives GluR1 into synapses in the intact brain (Qin et al., 2005; Rumpel et al.,

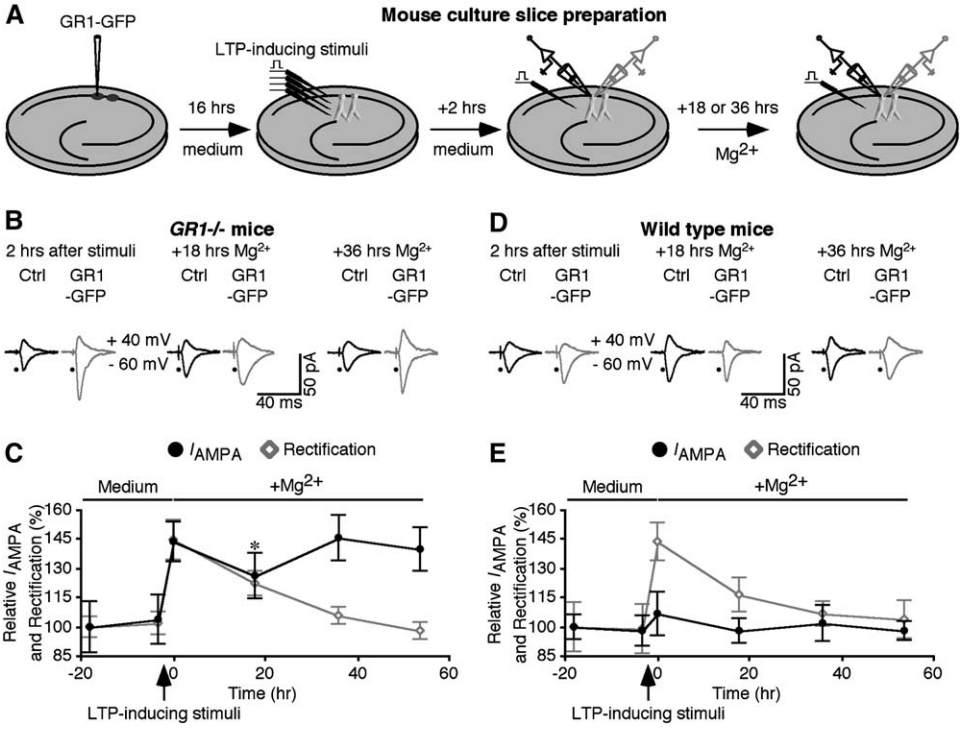

Figure 4. Synaptic AMPA-R Exchange in *GluR1* Knockout and Wild-Type Mice

(A) Schematic drawing showing the experimental design.

(B) Evoked AMPA-R-mediated responses recorded from nonexpressing (Ctrl) and GluR1-GFP-expressing CA1 cells from *GluR1* knockout mice cultured in normal media for 18 hr (LTP-inducing stimuli was delivered 16 hr after expression), normal media for 18 hr followed by high Mg^{2+} media for an additional 18 hr, or normal media for 18 hr followed by high Mg^{2+} media for an additional 36 hr.

(C) Plots of amplitude and rectification of AMPA responses against time of expression. Black filled dots, AMPA responses in GluR1-GFP-expressing cells relative to nearby -nonexpressing control cells after 15 hr expression (ctrl: −31.1 ± 4.2 pA; exp: −34.3 ± 4.1 pA; n = 18; p = 0.78), 18 hr expression (ctrl: −32.3 ± 2.9 pA; exp: −46.0 ± 3.2 pA; n = 20; p < 0.001), followed by additional 18 hr expression (ctrl: −30.2 ± 2.3 pA; exp: −38.0 ± 3.6 pA; n = 22; p < 0.001), additional 36 hr expression (ctrl: −29.5 ± 2.5 pA; exp: −42.9 ± 3.6 pA; n = 20; p < 0.001), or additional 54 hr expression with high Mg^{2+} media (ctrl: −29.6 ± 2.6 pA; exp: −41.1 ± 3.4 pA; n = 20; p < 0.001). Gray unfilled diamonds, rectification of AMPA responses in GluR1-GFP-expressing cells relative to nearby -nonexpressing control cells after 15 hr expression (ctrl: 1.90 ± 0.10; exp: 1.93 ± 0.12; n = 18; p = 0.95), 18 hr expression (ctrl: 1.83 ± 0.11; exp: 2.64 ± 0.19; n = 20; p < 0.001), followed by additional 18 hr expression (ctrl: 1.91 ± 0.07; exp: 2.34 ± 0.11; n = 22; p < 0.005), additional 36 hr expression (ctrl: 1.79 ± 0.09; exp: 1.89 ± 0.08; n = 20; p = 0.39), or additional 54 hr expression with high Mg^{2+} media (ctrl: 1.86 ± 0.09; exp: 1.81 ± 0.08; n = 20; p = 0.50).

(D) Evoked AMPA-R-mediated responses recorded from nonexpressing (Ctrl) and GluR1-GFP-expressing CA1 cells from wild-type mice cultured in normal media for 18 hr (LTP-inducing stimuli was delivered 16 hr after expression), normal media for 18 hr followed by high Mg^{2+} media for an additional 18 hr, or normal media for 18 hr followed by high Mg^{2+} media for an additional 36 hr.

(E) Plots of amplitude and rectification of AMPA responses against time of expression. Black filled dots, AMPA responses in GluR1-GFP expressing cells relative to nearby nonexpressing control cells after 15 hr expression (ctrl: −32.7 ± 4.1 pA; exp: −31.8 ± 4.0 pA; n = 20; p = 0.94), 18 hr expression (ctrl: −31.6 ± 2.8 pA; exp: −33.5 ± 3.0 pA; n = 22; p = 0.26), followed by additional 18 hr expression (ctrl: −30.5 ± 2.5 pA; exp: −29.8 ± 2.7 pA; n = 22; p = 0.71), additional 36 hr expression (ctrl: −29.1 ± 2.6 pA; exp: −29.5 ± 2.7 pA; n = 22; p = 0.81), or additional 54 hr expression with high Mg^{2+} media (ctrl: −35.6 ± 3.6 pA; exp: −34.8 ± 3.4 pA; n = 22; p = 0.69). Gray unfilled diamonds, rectification of AMPA responses in GluR1-GFP-expressing cells relative to nearby -nonexpressing control cells after 15 hr expression (ctrl: 1.93 ± 0.12; exp: 1.91 ± 0.15; n = 20; p = 0.91), 18 hr expression (ctrl: 1.81 ± 0.07; exp: 2.59 ± 0.20; n = 22; p < 0.001), followed by additional 18 hr expression (ctrl: 1.75 ± 0.07; exp: 2.03 ± 0.11; n = 22; p < 0.05), additional 36 hr expression (ctrl: 1.85 ± 0.10; exp: 1.97 ± 0.11; n = 22; p = 0.16), or additional 54 hr expression with high Mg^{2+} media (ctrl: 1.81 ± 0.08; exp: 1.87 ± 0.09; n = 22; p = 0.73). Asterisk indicates the points with relative amplitudes of AMPA responses significantly different from expressing cells after 18 hr expression of GluR1-GFP (p < 0.05; Mann-Whitney Rank Sum nonparametric test). AMPA-R-mediated current amplitude and rectification were normalized to average values from control cells.

2005; Takahashi et al., 2003). In particular, experience-dependent synaptic activity drives synaptic delivery of recombinant GluR1-GFP and endogenous GluR1 in GluR1-GFP-expressing and -nonexpressing neurons to the same extent (Figures 4D and 4E) (see also Qin et al. [2005]). To monitor the change of AMPA response amplitude in expressing neurons, we expressed GluR1-GFP in layer 2/3 pyramidal neurons of the barrel cortex in *GluR1* knockout mice (Figure 6A) in which no endogenous GluR1 would be available for delivery or exchange (Qin et al., 2005). Monosynaptic AMPA-R-mediated responses in expressing and nonexpressing layer 2/3 pyramidal neurons were then evoked by stimulating cortical layer 3 about 300 μm away from recorded cell pairs (Gil et al., 1999). We found that cortical neurons expressing GluR1-GFP for 18 hr had ~45% increase in amplitude and ~35% increase in rectification of AMPA responses compared to nearby nonexpressing neurons (Figures 6B and 6C). In the following ~2 days, we trimmed all large whiskers of the knockout mice that expressed GluR1-GFP to manipulate the experience-dependent synaptic inputs. Layer 2/3 pyramidal neurons

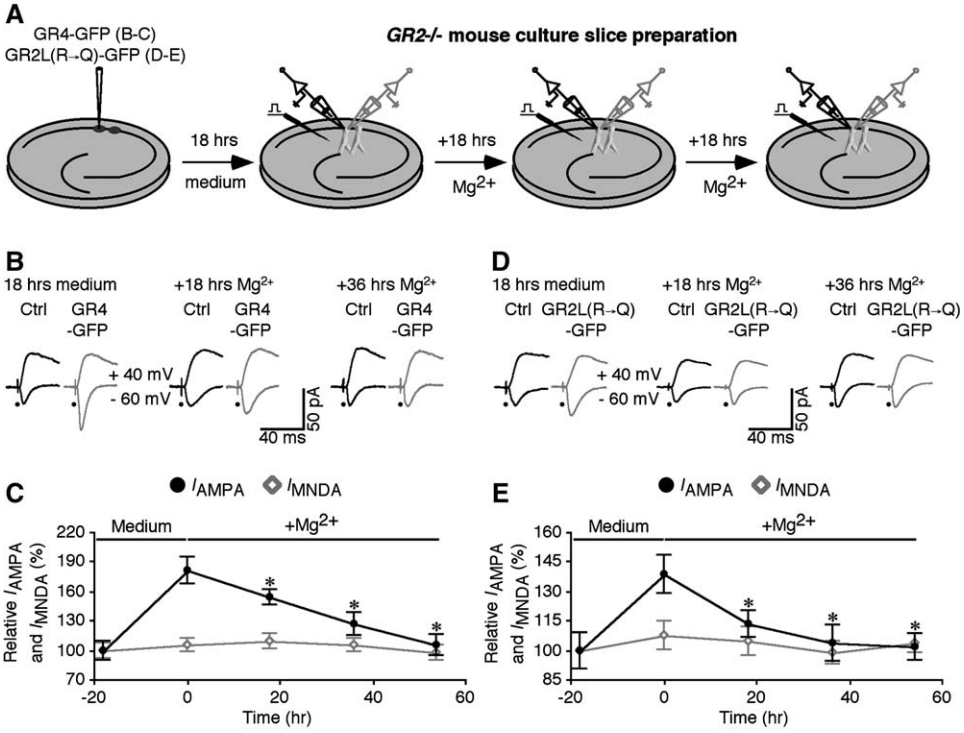

Figure 5. Synaptic AMPA-R Exchange in *GluR2* Knockout Mice

(A) Schematic drawing showing the experimental design.

(B) Evoked AMPA-R-mediated responses recorded from nonexpressing (Ctrl) and GluR4-GFP-expressing CA1 cells from *GluR2* knockout mice cultured in normal media for 18 hr, normal media for 18 hr followed by high Mg^{2+} media for an additional 18 hr, or normal media for 18 hr followed by high Mg^{2+} media for an additional 36 hr.

(C) Plots of amplitude and rectification of AMPA responses against time of expression. Black filled dots, AMPA responses in GluR4-GFP-expressing cells relative to nearby nonexpressing control cells after 18 hr expression (ctrl: −26.1 ± 2.6 pA; exp: −47.4 ± 3.7 pA; n = 20; $p < 0.001$), followed by additional 18 hr expression (ctrl: −29.1 ± 2.6 pA; exp: −44.7 ± 3.9 pA; n = 20; $p < 0.005$), additional 36 hr expression (ctrl: −29.2 ± 3.0 pA; exp: −36.8 ± 3.4 pA; n = 20; p = 0.22), or additional 54 hr expression with high Mg^{2+} media (ctrl: −30.7 ± 2.9 pA; exp: −32.2 ± 3.4 pA; n = 20; p = 0.85). Gray unfilled diamonds, NMDA responses in GluR1-GFP-expressing cells relative to nearby nonexpressing control cells after 18 hr expression (ctrl: 44.4 ± 3.5 pA; exp: 47.0 ± 3.0 pA; n = 20; p = 0.13), followed by additional 18 hr expression (ctrl: 53.8 ± 4.2 pA; exp: 59.1 ± 3.8 pA; n = 20; p = 0.10), additional 36 hr expression (ctrl: 50.2 ± 3.8 pA; exp: 52.3 ± 3.1 pA; n = 20; p = 0.28), or additional 54 hr expression with high Mg^{2+} media (ctrl: 52.1 ± 4.4 pA; exp: 50.9 ± 3.9 pA; n = 20; p = 0.97).

(D) Evoked AMPA-R-mediated responses recorded from nonexpressing (Ctrl) and GluR2L(R→Q)-GFP-expressing CA1 cells from *GluR2* knockout mice cultured in normal media for 18 hr, normal media for 18 hr followed by high Mg^{2+} media for an additional 18 hr, or normal media for 18 hr followed by high Mg^{2+} media for an additional 36 hr.

(E) Plots of amplitude and rectification of AMPA responses against time of expression. Black filled dots, AMPA responses in GluR2L(R→Q)-GFP-expressing cells relative to nearby nonexpressing control cells after 18 hr expression (ctrl: −32.5 ± 2.9 pA; exp: −45.1 ± 3.3 pA; n = 20; $p < 0.005$), followed by additional 18 hr expression (ctrl: −27.0 ± 2.2 pA; exp: −30.6 ± 1.9 pA; n = 20; p = 0.05), additional 36 hr expression (ctrl: −28.2 ± 2.4 pA; exp: −29.3 ± 2.7 pA; n = 20; p = 0.88), or additional 54 hr expression with high Mg^{2+} media (ctrl: −28.1 ± 2.2 pA; exp: −28.5 ± 2.0 pA; n = 20; p = 0.91). Gray unfilled diamonds, NMDA responses in GluR2L(R→Q)-GFP-expressing cells relative to nearby -nonexpressing control cells after 18 hr expression (ctrl: 58.4 ± 5.1 pA; exp: 62.5 ± 4.5 pA; n = 20; p = 0.10), followed by additional 18 hr expression (ctrl: 47.2 ± 2.7 pA; exp: 49.1 ± 3.3 pA; n = 20; p = 0.37), additional 36 hr expression (ctrl: 53.6 ± 4.0 pA; exp: 52.8 ± 3.2 pA; n = 20; p = 0.91), or additional 54 hr expression with high Mg^{2+} media (ctrl: 46.3 ± 2.4 pA; exp: 47.7 ± 2.3 pA; n = 20; p = 0.32). Asterisk indicates the points with relative amplitudes of AMPA responses significantly different from expressing cells after 18 hr expression of GluR4-GFP or GluR2L(R→Q)-GFP ($p < 0.05$; Mann-Whitney Rank Sum nonparametric test). AMPA-R- and NMDA-R-mediated current amplitude and rectification were normalized to average values from control cells.

in the intact barrel cortex constantly receive numerous synaptic inputs (Zhu and Connors, 1999; Zhu et al., 2004). Whisker trimming, which prevents subsequent delivery of GluR1-GFP (Takahashi et al., 2003), did not block spontaneous synaptic activity in these pyramidal neurons (n = 11 in knockout mice and n = 8 for rats) (Figure S4). Without sensory experience-dependent synaptic activity, the rectification of AMPA responses in GluR1-GFP expressing neurons decreased slowly to the level of nearby nonexpressing neurons, whereas the amplitude of AMPA responses was initially decreased before returning to the previous enhanced level in the following ~2 days (Figures 6B and 6C). These results indicate that synaptic GluR1-GFP exchange is mediated by synaptic removal of GluR1-GFP and addition of endogenous GluR2-containing AMPA-Rs in vivo.

GluR4 subunits are expressed in juvenile and adult cortical neurons (Ong et al., 1996; Petralia and Wenthold, 1992). We wanted to know whether GluR4 exchanges with GluR2-containing AMPA-Rs in the same manner as GluR1 in vivo. We expressed GluR4-GFP in layer 2/3 pyramidal neurons of the intact rat brain. Cortical neurons expressing GluR4-GFP for 18 hr had

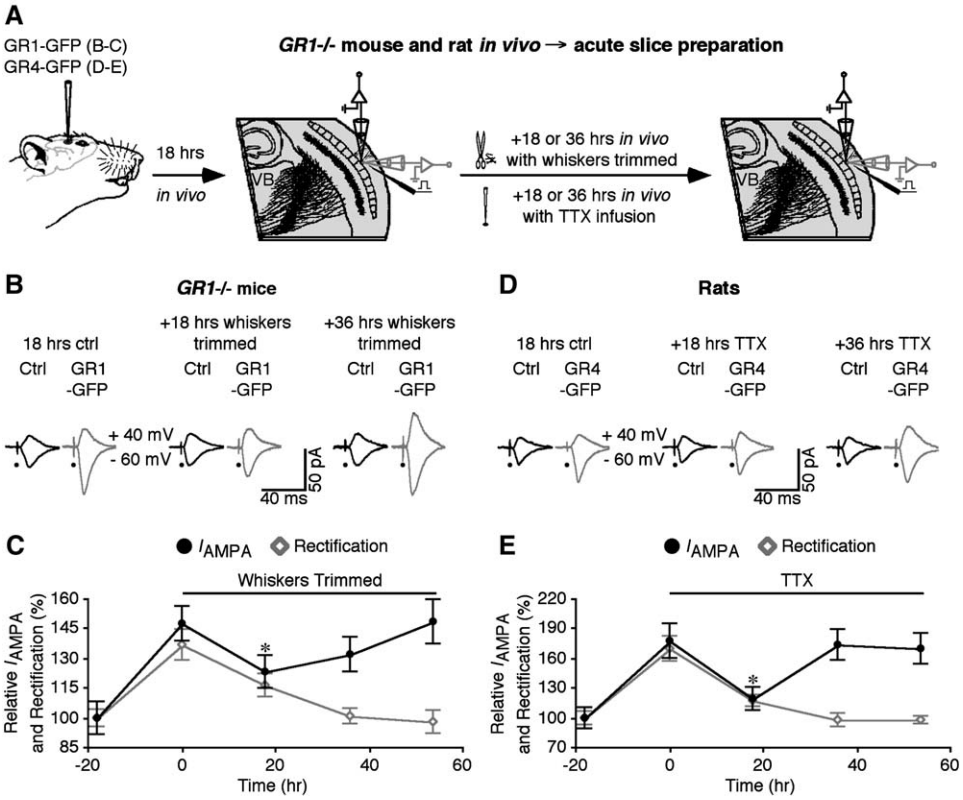

Figure 6. Synaptic Exchange of GluR1-GFP and GluR4-GFP with GluR2 In Vivo

(A) Schematic drawing showing the experimental design.

(B) Evoked AMPA-R-mediated responses recorded from nonexpressing (Ctrl) and GluR1-GFP-expressing layer 2/3 cortical cells from *GluR1* knockout mice after 18 hr expression, after 18 hr expression followed by an additional 18 hr expression with whiskers trimmed, or after 18 hr expression followed by an additional 36 hr expression with whiskers trimmed.

(C) Plots of amplitude and rectification of AMPA responses against time of expression. Black filled dots, AMPA responses in GluR1-GFP-expressing cells relative to nearby -nonexpressing control cells after 18 hr expression (ctrl: −19.1 ± 1.6 pA; exp: −28.1 ± 1.6 pA; n = 24; $p < 0.001$), followed by additional 18 hr expression with whiskers trimmed (ctrl: −20.2 ± 1.8 pA; exp: −24.8 ± 1.7 pA; n = 24; $p < 0.005$), additional 36 hr expression with whiskers trimmed (ctrl: −19.9 ± 1.4 pA; exp: −26.3 ± 1.7 pA; n = 24; $p < 0.001$), or additional 54 hr expression with whiskers trimmed (ctrl: −18.2 ± 1.5 pA; exp: −26.9 ± 2.0 pA; n = 24; $p < 0.001$). Gray unfilled diamonds, rectification of AMPA responses in GluR1-GFP-expressing cells relative to nearby -nonexpressing control cells after 18 hr expression (ctrl: 1.45 ± 0.06; exp: 1.98 ± 0.11; n = 24; $p < 0.001$), followed by additional 18 hr expression with whiskers trimmed (ctrl: 1.43 ± 0.06; exp: 1.67 ± 0.08; n = 24; $p = 0.052$), additional 36 hr expression with whiskers trimmed (ctrl: 1.37 ± 0.05; exp: 1.38 ± 0.05; n = 24; $p = 0.98$), or additional 54 hr expression with whiskers trimmed (ctrl: 1.44 ± 0.08; exp: 1.40 ± 0.09; n = 24; $p = 0.73$).

(D) Evoked AMPA-R-mediated responses recorded from nonexpressing (Ctrl) and GluR4-GFP-expressing layer 2/3 cortical cells from rats after 18 hr expression, after 18 hr expression followed by an additional 18 hr expression with cortical infusion of TTX, or after 18 hr expression followed by an additional 36 hr expression with cortical infusion of TTX.

(E) Plots of amplitude and rectification of AMPA responses against time of expression. Black filled dots, AMPA responses in GluR4-GFP-expressing cells relative to nearby -nonexpressing control cells after 18 hr expression (ctrl: −18.5 ± 2.1 pA; exp: −32.8 ± 3.3 pA; n = 18; $p < 0.001$), followed by additional 18 hr expression with infusion of TTX (ctrl: −19.2 ± 1.6 pA; exp: −22.8 ± 2.2 pA; n = 21; $p < 0.05$), additional 36 hr expression with infusion of TTX (ctrl: −22.2 ± 2.2 pA; exp: −38.4 ± 3.3 pA; n = 20; $p < 0.005$), or additional 54 hr expression with infusion of TTX (ctrl: −20.0 ± 1.6 pA; exp: −34.0 ± 3.1 pA; n = 20; $p < 0.001$). Gray unfilled diamonds, rectification of AMPA responses in GluR4-GFP-expressing cells relative to nearby -nonexpressing control cells after 18 hr expression (ctrl: 1.39 ± 0.10; exp: 2.36 ± 0.18; n = 18; $p < 0.001$), followed by additional 18 hr expression with infusion of TTX (ctrl: 1.41 ± 0.07; exp: 1.64 ± 0.09; n = 21; $p < 0.05$), additional 36 hr expression with infusion of TTX (ctrl: 1.38 ± 0.07; exp: 1.35 ± 0.09; n = 20; $p = 0.74$), or additional 54 hr expression with infusion of TTX (ctrl: 1.41 ± 0.07; exp: 1.37 ± 0.05; n = 20; $p = 0.68$). Asterisk indicates the points with relative amplitudes significantly different from expressing cells after 18 hr expression of GluR1-GFP or GluR4-GFP ($p < 0.05$; Mann-Whitney Rank Sum nonparametric test). AMPA-R-mediated current amplitude and rectification were normalized to average values from control cells.

~80% increase in amplitude and ~70% increase in rectification of AMPA responses compared to nearby nonexpressing cells (Figures 6D and 6E). In the following ~2 days, we infused TTX in several places close to the virus injection site. Cortical infusion of TTX blocked synaptic activity in rat layer 2/3 pyramidal neurons (n = 9) (Figure S4). During this period, the rectification of AMPA responses in GluR4-GFP-expressing neurons was gradually reduced to the level of nonexpressing neurons, whereas the amplitude of AMPA responses was initially decreased before returning to the previous enhanced level (Figures 6D and 6E). These results indicate that synaptic GluR4-GFP exchange is mediated by synaptic removal of GluR4-GFP and addition of endogenous GluR2-containing AMPA-Rs in vivo in a synaptic activity-independent manner.

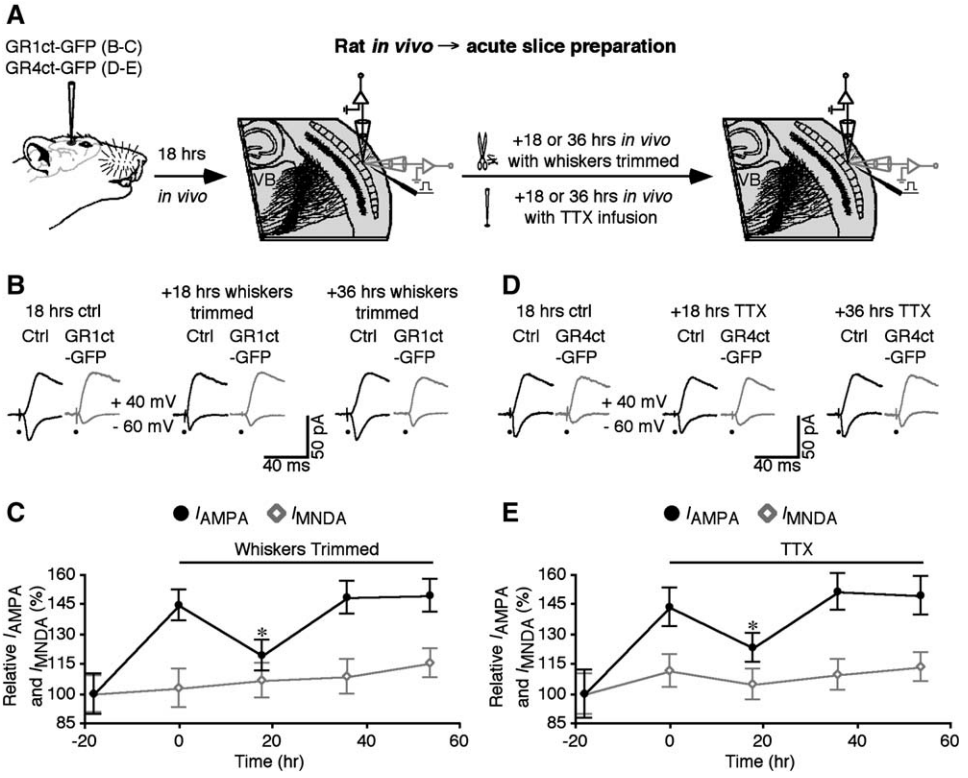

Figure 7. Synaptic Exchange of Endogenous GluR1 and GluR4 with GluR2 In Vivo

(A) Schematic drawing showing the experimental design.

(B) Evoked AMPA-R- and NMDA-R-mediated responses recorded from nonexpressing (Ctrl) and GluR1ct-GFP-expressing layer 2/3 cortical cells after 18 hr expression, after 18 hr expression followed by an additional 18 hr expression with whiskers trimmed, or after 18 hr expression followed by an additional 36 hr expression with whiskers trimmed.

(C) Plots of amplitude of AMPA and NMDA responses against time of expression. Black filled dots, AMPA responses in nonexpressing control cells relative to nearby GluR1ct-GFP-expressing cells after 18 hr expression (ctrl: −23.7 ± 1.7 pA; exp: −16.4 ± 1.3 pA; n = 24; $p < 0.001$), followed by additional 18 hr expression with whiskers trimmed (ctrl: −20.3 ± 1.5 pA; exp: −17.0 ± 1.3 pA; n = 25; $p < 0.05$), additional 36 hr expression with whiskers trimmed (ctrl: −21.7 ± 1.4 pA; exp: −14.2 ± 1.2 pA; n = 24; $p < 0.005$), or additional 54 hr expression with whiskers trimmed (ctrl: −18.7 ± 1.0 pA; exp: −12.5 ± 1.0 pA; n = 24; $p < 0.001$). Gray unfilled diamonds, NMDA responses in nonexpressing control cells relative to nearby GluR1ct-GFP-expressing cells after 18 hr expression (ctrl: 37.2 ± 3.3 pA; exp: 36.3 ± 3.4 pA; n = 24; $p = 0.78$), followed by additional 18 hr expression with whiskers trimmed (ctrl: 33.0 ± 2.5 pA; exp: 30.9 ± 2.7 pA; n = 25; $p = 0.41$), additional 36 hr expression with whiskers trimmed (ctrl: 33.7 ± 2.5 pA; exp: 31.2 ± 2.8; n = 24; $p = 0.60$), or additional 54 hr expression with whiskers trimmed (ctrl: 30.9 ± 2.2; exp: 26.7 ± 1.8; n = 24; $p = 0.13$).

(D) Evoked AMPA-R- and NMDA-R-mediated responses recorded from nonexpressing (Ctrl) and GluR4ct-GFP-expressing layer 2/3 cortical cells after 18 hr expression, after 18 hr expression followed by an additional 18 hr expression with cortical infusion of TTX, or after 18 hr expression followed by an additional 36 hr expression with cortical infusion of TTX.

(E) Plots of amplitude of AMPA and NMDA responses against time of expression. Black filled dots, AMPA responses in nonexpressing control cells relative to nearby GluR4ct-GFP-expressing cells after 18 hr expression (ctrl: −23.3 ± 1.9 pA; exp: −16.3 ± 1.6 pA; n = 24; $p < 0.001$), followed by additional 18 hr expression with infusion of TTX (ctrl: −21.0 ± 1.5 pA; exp: −17.0 ± 1.3 pA; n = 24; $p < 0.005$), additional 36 hr expression with infusion of TTX (ctrl: −23.3 ± 2.0 pA; exp: −15.3 ± 1.5 pA; n = 20; $p < 0.001$), or additional 54 hr expression with infusion of TTX (ctrl: −21.0 ± 2.1 pA; exp: −14.1 ± 1.4 pA; n = 24; $p < 0.005$). Gray unfilled diamonds, NMDA responses in nonexpressing control cells relative to nearby GluR4ct-GFP-expressing cells after 18 hr expression (ctrl: 35.6 ± 3.8 pA; exp: 32.0 ± 2.6 pA; n = 24; $p = 0.39$), followed by additional 18 hr expression with infusion of TTX (ctrl: 33.9 ± 2.1 pA; exp: 32.3 ± 2.5 pA; n = 24; $p = 0.43$), additional 36 hr expression with infusion of TTX (ctrl: 37.8 ± 2.9 pA; exp: 34.6 ± 2.7 pA; n = 24; $p = 0.22$), or additional 54 hr expression with infusion of TTX (ctrl: 35.6 ± 3.0 pA; exp: 31.5 ± 2.2; n = 24; $p = 0.08$). Asterisk indicates the points with relative amplitudes of AMPA responses significantly different from expressing cells after 18 hr expression of GluR1ct-GFP or GluR4ct-GFP ($p < 0.05$; Mann-Whitney Rank Sum nonparametric test). AMPA-R- and NMDA-R-mediated current amplitudes were normalized to average values from expressing cells.

Exchange of Endogenous GluR1 and GluR4 with Endogenous GluR2 In Vivo

It is important to know whether endogenous AMPA-Rs exchange with GluR2-containing AMPA-Rs in the same manner as recombinant AMPA-Rs. To this end, we expressed the GFP-tagged cytoplasmic terminus of GluR1, GluR1ct-GFP, in the intact rat barrel cortex (Figure 7A). This construct is known to selectively block synaptic delivery of endogenous GluR1 (Kolleker et al., 2003; Qin et al., 2005; Shi et al., 2001). We reasoned that expressing GluR1ct-GFP should block the initiation of synaptic exchange of GluR1 in expressing neurons. Therefore, we could use GluR1ct-GFP-expressing neurons as "control" cells to monitor synaptic exchange of endogenous GluR1 in nearby nonexpressing neurons. Indeed, nonexpressing cortical layer 2/3 pyramidal

neurons had relatively larger AMPA responses compared to nearby expressing pyramidal neurons after expression of GluR1ct-GFP for 18 hr (by ~45%) (Figures 7B and 7C). In the following ~2 days, we blocked experience-dependent synaptic inputs by trimming all large whiskers of the rats expressing GluR1ct-GFP. We found that nonexpressing neurons had temporarily depressed AMPA responses before they regained the relatively larger AMPA responses seen initially (Figures 7B and 7C). NMDA responses did not differ between nonexpressing and expressing neurons in all cases, consistent with the previous finding that synaptic NMDA-Rs and AMPA-Rs are differentially regulated (Rao and Craig, 1997) and suggesting that the difference in AMPA responses between nonexpressing and expressing neurons was not due to nonspecific mechanisms. The same results were obtained if synaptic activity was blocked by cortical infusion of TTX (not shown). These results indicate that synaptic removal of endogenous GluR1 and addition of endogenous GluR2-containing AMPA-Rs mediate synaptic AMPA-R exchange in vivo.

To extend our findings, we examined the synaptic exchange of endogenous GluR4 in the intact rat barrel cortex. We expressed the GFP-tagged cytoplasmic terminus of GluR4, GluR4ct-GFP, which selectively blocks synaptic delivery of endogenous GluR4 (Kolleker et al., 2003; Zhu et al., 2000), to block the initiation of synaptic exchange of endogenous GluR4-containing AMPA-Rs in expressing neurons. We then used these GluR4ct-GFP-expressing neurons as "control" cells to monitor synaptic exchange of endogenous GluR4 in nearby nonexpressing neurons. Compared to layer 2/3 pyramidal neurons that expressed GluR4ct-GFP for 18 hr, nearby nonexpressing pyramidal neurons had relatively larger AMPA responses (by ~45%) (Figures 7D and 7E). With cortical infusion of TTX, nonexpressing neurons had temporarily depressed AMPA responses before they recuperated the relatively larger AMPA responses seen initially (Figures 7D and 7E). NMDA responses were the same between nonexpressing and expressing neurons at all time points measured. Collectively, these results indicate that synaptic removal of endogenous GluR4 and addition of endogenous GluR2-containing AMPA-Rs mediate synaptic AMPA-R exchange in vivo.

Discussion

In this study, we have demonstrated that a slow, activity-independent synaptic AMPA exchange process is essential for maintaining bidirectional plasticity. The process consists of two trafficking events: synaptic removal of AMPA-Rs with long cytoplasmic termini and synaptic refill of the newly available "slots" with AMPA-Rs with only short cytoplasmic termini. During exchange, GluR1-, GluR2L-, or GluR4-containing AMPA-Rs are removed from synapses with a rate time constant of ~17 hr, and the same amount of GluR2-containing AMPA-Rs are added into synapses with a rate time constant of ~16 hr. In the end, these two trafficking events maintain transmission strength unaltered but result in a partial depression of AMPA responses in the middle of the exchange. The data indicate that molecule(s) other than AMPA-Rs, such as the hypothesized "slot" proteins, code and maintain transmission efficacy.

Synaptic AMPA-R Exchange and Bidirectional Plasticity

During LTP and LTD, two of the major processes that constitute bidirectional plasticity (Bear and Linden, 2000; Malinow, 2003), different AMPA-Rs are delivered and removed from synapses. How do synapses maintain continuous capacity for further bidirectional plasticity? One solution is that newly delivered AMPA-Rs with long cytoplasmic termini, such as GluR1-, GluR2L-, and GluR4-containing AMPA-Rs, will be exchanged with AMPA-Rs with only short cytoplasmic termini, such as GluR2-containing AMPA-Rs (Kolleker et al., 2003; Takahashi et al., 2003; Zhu et al., 2000). Such an exchange process not only supplies new synaptic GluR2-containing AMPA-Rs, important for new synaptic depression (Chung et al., 2003; Zhu et al., 2002), but also removes GluR1-, GluR2L-, and GluR4-containing AMPA-Rs, which appear to be used for new synaptic potentiation (Kolleker et al., 2003; Park et al., 2004; Qin et al., 2005). Our results support this notion because synaptic AMPA-R exchange restores the ability of synapses to generate new LTP and LTD after potentiation or depression.

LTP and LTD have been extensively examined in various preparations. However, even for the same rodent hippocampal CA3→CA1 synapses, different investigators have reported LTP and LTD with very different magnitudes. Here, we show that activity-independent synaptic AMPA-R exchange "recharges" synapses and increases their capacity for generating new LTP and LTD. These results suggest that the amounts of inducible LTP and LTD at particular synapses depend also on the amounts of synaptic activity and/or plasticity that occurred previously at these synapses, which may vary depending on a number of physiological factors, including hormones and behavioral states (e.g., see Qin et al. [2005]). Thus, the large variances found in magnitudes of maximal LTP and LTD may reflect differences in experimental preparations (e.g., whether the acute slices are prepared in early morning or late afternoon). In support of this idea, the maximal amounts of LTP and LTD found at CA3→CA1 synapses in hippocampal culture slices by different investigators using different experimental approaches are quite comparable (e.g., Bagal et al. [2005], Brown et al. [2005], Esteban et al. [2003], Kamenetz et al. [2003], Kolleker et al. [2003], Seidenman et al. [2003], Tomita et al. [2005], and Zhu et al. [2002]), consistent with the fact that synaptic activity is relatively constant in culture slices (McKinney et al., 1999; Zhu et al., 2000).

Synaptic AMPA-R Exchange and Other Trafficking Processes

How synaptic AMPA exchange initiates and completes are unknown. Here, we report that synaptic exchange of GluR1 with GluR2-containing AMPA-Rs requires synaptic delivery of GluR1 receptors. Moreover, LTP-inducing stimuli, Ras activity, and experience-dependent and -independent spontaneous synaptic activities all drive synaptic delivery of AMPA-Rs with long cytoplasmic termini and all initiate synaptic AMPA-R exchange. Likely, as previously proposed (Malinow, 2003), synaptic insertion of AMPA-Rs with long cytoplasmic termini brings

with them and/or triggers subsequent synaptic delivery of "slot" proteins. Refilling synapses during AMPA-R exchange requires GluR2-containing AMPA-Rs because the process fails in *GluR2* knockout mice (Figure 5). Because synaptic AMPA-R exchange occurs in absence of GluR1 (Figure 4), synaptic refilling seems to require GluR2/3, but not GluR1/2 AMPA-Rs.

Synaptic removal of AMPA-Rs during synaptic AMPA-R exchange differs from synaptic removal of AMPA-Rs during depotentiation. Although synaptic GluR1 and GluR2L are removed during depotentiation, the process is synaptic activity-dependent (Zhu et al., 2005). In contrast, synaptic removal of AMPA-Rs during exchange requires no synaptic activity (this study; see also Kolleker et al. [2003], Takahashi et al. [2003], and Zhu et al. [2000]). Moreover, depotentiation, which occurs only within a short window (~0.5–2 hr) after long-term potentiation (Bear and Linden, 2000; Zhou and Poo, 2004), removes synaptic GluR1 and GluR2L at the rapid time course of ~15–20 min (Zhu et al., 2005). On the other hand, we show here that synaptic AMPA-Rs are removed with a slower rate time constant of ~17 hr during exchange. The unanswered question is whether the removed pools of AMPA-Rs from the exchange and/or depotentiation processes will be recycled for reinsertion during LTP (Park et al., 2004).

Synaptic AMPA-R exchange displays properties different from synaptic AMPA-R cycling. Although both processes add GluR2-containing AMPA-Rs back into synapses and require no synaptic activity, they differ significantly in rate time constants. Whereas synaptic addition of GluR2-containing AMPA-Rs during exchange has a rate time constant of ~16 hr, GluR2-containing AMPA-Rs rapidly cycles between synaptic and nonsynaptic sites with a rate time constant of ~20–25 min (Lee et al., 2002; Luscher et al., 1999; Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998; Zhu et al., 2000). Thus, addition of GluR2-containing AMPA-Rs during exchange and cycling may employ different endosomal pools/secretory pathways (Horton and Ehlers, 2004). Alternatively, synaptic AMPA-R exchange may share the fast synaptic insertion pathway with synaptic AMPA-R cycling when adding AMPA-Rs back to synapses. If this is the case, the proportion of nonsynaptic AMPA-Rs in the cycling pool is likely very low, and additional GluR2-containing AMPA-Rs must first be added into this pool at a slow rate constant (i.e., ~15–18 hr) during the exchange. Consistent with this idea, a recent study reports that synaptic responses recover at a slow rate time constant of ~16 hr after pharmacological inactivation of all surface AMPA-Rs (Adesnik et al., 2005). In any case, our results suggest the existence of at least two activity-independent trafficking pools of GluR2-containing AMPA-Rs, with receptors in one pool cycling rapidly between synaptic and nonsynaptic sites and receptors in the other pool moving slowly toward synapses.

This study, together with a number of previous studies, indicates that at least five distinct synaptic AMPA-R trafficking processes exist at synapses. Three of the processes, including activity-dependent synaptic delivery of AMPA-Rs with long cytoplasmic termini, activity-dependent synaptic removal of AMPA-Rs with only short cytoplasmic termini, and activity-dependent synaptic removal of AMPA-Rs with long cytoplasmic termini, mediate LTP, LTD, and depotentiation. The other two processes, including activity-independent synaptic AMPA-R exchange and activity-independent synaptic AMPA-R cycling, function to maintain the capacity for bidirectional plasticity and transmission efficacy in the face of continuous synaptic AMPA-R trafficking and protein turnover.

Synaptic AMPA-R Exchange and "Slot" Proteins

How synaptic AMPA-R exchange maintains transmission efficacy is unclear. It is possible that during exchange, GluR2-containing AMPA-Rs get into synapses and make a one-to-one replacement of synaptic GluR1-, GluR2L-, or GluR4-containing AMPA-Rs. It is also possible that synaptic delivery of GluR1-, GluR2L-, and GluR4-containing AMPA-Rs brings with them "slot" proteins (Malinow, 2003), which allow GluR2-containing AMPA-Rs to refill the empty "slots" after GluR1-, GluR2L-, and GluR4-containing AMPA-Rs leave synapses. The temporarily depressed AMPA responses during synaptic AMPA-R exchange indicate that GluR1-, GluR2L-, and GluR4-contaning AMPA-Rs leave synapses before GluR2-containing AMPA-Rs fill in. This view is further supported by our findings that synaptic refilling of GluR2-containing AMPA-Rs after removal of AMPA-Rs with long cytoplasmic termini are required for completing exchange and maintaining transmission unaltered after exchange. Because the ultimate transmission strength does not change after the exchange, synaptic efficacy must be "memorized" by molecule(s) other than AMPA-Rs, in particular when AMPA-R-mediated transmission is temporarily depressed. Our results thus provide experimental evidence supporting the "slot" theory: "slot" proteins, instead of AMPA-Rs, code and maintain transmission efficacy. The remaining puzzle is what are the "slot" proteins. Proteomic analysis and functional characterization of proteins binding to cytoplasmic termini of all AMPA-R subunits (e.g., 4.1N [Hayashi et al., 2005] or similar proteins) promise to reveal their identity.

Experimental Procedures

Constructs of Recombinant Proteins and Expression

Constructs, made as previously described (Kolleker et al., 2003; Zhu et al., 2000, 2002), were expressed in CA1 neurons in hippocampal culture slices or layer 2/3 pyramidal neurons in the barrel cortex by using Sindbis virus or biolistics transfection. Hippocampal culture slices were prepared from postnatal 5–7 day old rats or mice, infected with virus immediately (for GluR4-GFP) or after 6–8 days in vitro, and incubated in culture media and 5% CO_2 for 18–114 hr before experiments. Expression level of GluR4-GFP was measured following a previous study (Zhu et al., 2000). For in vivo expression, postnatal 14–16 (P14–P16) old mice and rats were initially anesthetized by an intraperitoneal injection of ketamine and xylaxine (10 and 2 mg/kg, respectively) (cf. Qin et al. [2005]). Animals were then placed in a stereotaxic frame, and a hole ~1×1 mm was opened above the center of the right barrel cortex. A glass pipette was used to penetrate into layer 2/3 according to stereotaxic coordinates, and ~50 nl of viral solution was delivered by pressure injection. After injection, animals were allowed to recover from the anesthesia and then returned to their dams. To manipulate synaptic activity during expression of recombinant proteins, either all large whiskers on the contralateral face were trimmed (cf. Takahashi et al. [2003]), or ~100 nl TTX (100 μM) was infused into several places close to the virus injection site with a glass pipette every

8 hr. About 24–72 hr after expression, 350 μm thick thalamocortical slices were prepared from the infected brains as previously described (Agmon and Connors, 1991). Slices that contained the thalamus and barrel cortex were incubated at 37.0ºC ± 0.5ºC in oxygenated physiological solution for ~1 hr before recordings.

Electrophysiology

Simultaneous whole-cell in vitro recordings were obtained from pairs of nearby infected and noninfected CA1 pyramidal neurons from hippocampal culture slices or layer 2/3 pyramidal neurons from thalamocortical slices (Larkum and Zhu, 2002; Zhu et al., 2000), under visual guidance by fluorescence and transmitted light illumination, with two Axopatch-200B amplifiers (Axon Instruments, Foster City, CA). Whole-cell in vivo recordings from layer 2/3 pyramidal neurons in the barrel cortex of anesthetized P14–P17 mice and rats were obtained with an Axoclamp 2B amplifier as previously described (Larkum and Zhu, 2002; Zhu et al., 2004). Synaptic responses, LTP, and LTD were induced and measured as previously described (Qin et al., 2005; Zhu et al., 2002). Synaptic delivery of GluR1 in vitro was made by applying a classic CA1 LTP-inducing paradigm, which includes ten repetitions of 25 ms train of pulses at 400 Hz (Worley et al., 1993). The stimuli were simultaneously delivered to the four stimulating electrodes (Figure 4A). All results are reported as mean ± SEM, and statistical significances of the means ($p < 0.05$) were determined by Wilcoxon and Mann-Whitney Rank Sum nonparametric tests for paired and unpaired samples, respectively.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/50/1/75/DC1/.

Acknowledgments

We thank Drs. José Esteban and Robert Malinow and members of the Zhu laboratory for helpful comments and discussions; Dr. Yinghua Zhu for help with some experiments. This study is supported in part by the National Institutes of Health and Whitehall Foundation. S.G.M. receives a National Institutes of Health Predoctoral Training Fellowship, and J.J.Z. is an Alfred P. Sloan Fellow.

Received: June 25, 2005
Revised: December 31, 2005
Accepted: February 22, 2006
Published: April 5, 2006

References

Adesnik, H., Nicoll, R.A., and England, P.M. (2005). Photoinactivation of native AMPA receptors reveals their real-time trafficking. Neuron *48*, 977–985.

Agmon, A., and Connors, B.W. (1991). Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. Neuroscience *41*, 365–379.

Bagal, A.A., Kao, J.P., Tang, C.M., and Thompson, S.M. (2005). Long-term potentiation of exogenous glutamate responses at single dendritic spines. Proc. Natl. Acad. Sci. USA *102*, 14434–14439.

Bear, M.F., and Linden, D.J. (2000). The mechanisms and meaning of long-term synaptic depression in the mammalian brain. In The Synapse, M.M. Cowan, T.C. Südhof, and C.F. Stevens, eds. (Baltimore, MD: The Johns Hopkins University Press), pp. 455–517.

Bolshakov, V.Y., Carboni, L., Cobb, M.H., Siegelbaum, S.A., and Belardetti, F. (2000). Dual MAP kinase pathways mediate opposing forms of long-term plasticity at CA3–CA1 synapses. Nat. Neurosci. *3*, 1107–1112.

Bredt, D.S., and Nicoll, R.A. (2003). AMPA receptor trafficking at excitatory synapses. Neuron *40*, 361–379.

Brown, T.C., Tran, I.C., Backos, D.S., and Esteban, J.A. (2005). NMDA receptor-dependent activation of the small GTPase Rab5 drives the removal of synaptic AMPA receptors during hippocampal LTD. Neuron *45*, 81–94.

Chung, H.J., Steinberg, J.P., Huganir, R.L., and Linden, D.J. (2003). Requirement of AMPA receptor GluR2 phosphorylation for cerebellar long-term depression. Science *300*, 1751–1755.

Collingridge, G.L., Isaac, J.T., and Wang, Y.T. (2004). Receptor trafficking and synaptic plasticity. Nat. Rev. Neurosci. *5*, 952–962.

English, J.D., and Sweatt, J.D. (1997). A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. J. Biol. Chem. *272*, 19103–19106.

Esteban, J.A., Shi, S.H., Wilson, C., Nuriya, M., Huganir, R.L., and Malinow, R. (2003). PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nat. Neurosci. *6*, 136–143.

Gil, Z., Connors, B.W., and Amitai, Y. (1999). Efficacy of thalamocortical and intracortical synaptic connections: quanta, innervation, and reliability. Neuron *23*, 385–397.

Hayashi, T., Rumbaugh, G., and Huganir, R.L. (2005). Differential regulation of AMPA receptor subunit trafficking by palmitoylation of two distinct sites. Neuron *47*, 709–723.

Hayashi, Y., Shi, S.H., Esteban, J.A., Piccini, A., Poncer, J.C., and Malinow, R. (2000). Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science *287*, 2262–2267.

Horton, A.C., and Ehlers, M.D. (2004). Secretory trafficking in neuronal dendrites. Nat. Cell Biol. *6*, 585–591.

Kamenetz, F., Tomita, T., Hsieh, H., Seabrook, G., Borchelt, D., Iwatsubo, T., Sisodia, S., and Malinow, R. (2003). APP processing and synaptic function. Neuron *37*, 925–937.

Kolleker, A., Zhu, J.J., Schupp, B.J., Qin, Y., Mack, V., Borchardt, T., Kohr, G., Malinow, R., Seeburg, P.H., and Osten, P. (2003). Glutamatergic plasticity by synaptic delivery of GluR-B(long)-containing AMPA receptors. Neuron *40*, 1199–1212.

Larkum, M.E., and Zhu, J.J. (2002). Signaling of layer 1 and whisker-evoked Ca2+ and Na+ action potentials in distal and terminal dendrites of rat neocortical pyramidal neurons in vitro and in vivo. J. Neurosci. *22*, 6991–7005.

Lee, S.H., Liu, L., Wang, Y.T., and Sheng, M. (2002). Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. Neuron *36*, 661–674.

Lee, S.H., Simonetta, A., and Sheng, M. (2004). Subunit rules governing the sorting of internalized AMPA receptors in hippocampal neurons. Neuron *43*, 221–236.

Liao, D., Zhang, X., O'Brien, R., Ehlers, M.D., and Huganir, R.L. (1999). Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. Nat. Neurosci. *2*, 37–43.

Luscher, C., Xia, H., Beattie, E.C., Carroll, R.C., von Zastrow, M., Malenka, R.C., and Nicoll, R.A. (1999). Role of AMPA receptor cycling in synaptic transmission and plasticity. Neuron *24*, 649–658.

Malenka, R.C., and Bear, M.F. (2004). LTP and LTD: an embarrassment of riches. Neuron *44*, 5–21.

Malinow, R. (2003). AMPA receptor trafficking and long-term potentiation. Philos. Trans. R. Soc. Lond. B Biol. Sci. *358*, 707–714.

McKinney, R.A., Capogna, M., Durr, R., Gahwiler, B.H., and Thompson, S.M. (1999). Miniature synaptic events maintain dendritic spines via AMPA receptor activation. Nat. Neurosci. *2*, 44–49.

Meng, Y., Zhang, Y., and Jia, Z. (2003). Synaptic transmission and plasticity in the absence of AMPA glutamate receptor GluR2 and GluR3. Neuron *39*, 163–176.

Murray, H.J., and O'Connor, J.J. (2003). A role for COX-2 and p38 mitogen activated protein kinase in long-term depression in the rat dentate gyrus in vitro. Neuropharmacology *44*, 374–380.

Nishimune, A., Isaac, J.T., Molnar, E., Noel, J., Nash, S.R., Tagaya, M., Collingridge, G.L., Nakanishi, S., and Henley, J.M. (1998). NSF binding to GluR2 regulates synaptic transmission. Neuron *21*, 87–97.

Ong, W.Y., Leong, S.K., Garey, L.J., and Reynolds, R. (1996). A light- and electron-microscopic study of GluR4-positive cells in cerebral cortex, subcortical white matter and corpus callosum of neonatal, immature and adult rats. Exp. Brain Res. *110*, 367–378.

Osten, P., Srivastava, S., Inman, G.J., Vilim, F.S., Khatri, L., Lee, L.M., States, B.A., Einheber, S., Milner, T.A., Hanson, P.I., and Ziff, E.B. (1998). The AMPA receptor GluR2 C terminus can mediate a reversible, ATP- dependent interaction with NSF and alpha- and beta-SNAPs. Neuron *21*, 99–110.

Park, M., Penick, E.C., Edwards, J.G., Kauer, J.A., and Ehlers, M.D. (2004). Recycling endosomes supply AMPA receptors for LTP. Science *305*, 1972–1975.

Petralia, R.S., and Wenthold, R.J. (1992). Light and electron immunocytochemical localization of AMPA-selective glutamate receptors in the rat brain. J. Comp. Neurol. *318*, 329–354.

Qin, Y., Zhu, Y., Baumgart, J.P., Stornetta, R.L., Seidenman, K., Mack, V., van Aelst, L., and Zhu, J.J. (2005). State-dependent Ras signaling and AMPA receptor trafficking. Genes Dev. *19*, 2000–2015.

Rao, A., and Craig, A.M. (1997). Activity regulates the synaptic localization of the NMDA receptor in hippocampal neurons. Neuron *19*, 801–812.

Rumpel, S., LeDoux, J., Zador, A., and Malinow, R. (2005). Postsynaptic receptor trafficking underlying a form of associative learning. Science *308*, 83–88.

Seidenman, K.J., Steinberg, J.P., Huganir, R., and Malinow, R. (2003). Glutamate receptor subunit 2 Serine 880 phosphorylation modulates synaptic transmission and mediates plasticity in CA1 pyramidal cells. J. Neurosci. *23*, 9220–9228.

Sheng, M., and Kim, M.J. (2002). Postsynaptic signaling and plasticity mechanisms. Science *298*, 776–780.

Shi, S., Hayashi, Y., Esteban, J.A., and Malinow, R. (2001). Subunit-specific rules governing ampa receptor trafficking to synapses in hippocampal pyramidal neurons. Cell *105*, 331–343.

Song, I., Kamboj, S., Xia, J., Dong, H., Liao, D., and Huganir, R.L. (1998). Interaction of the N-ethylmaleimide-sensitive factor with AMPA receptors. Neuron *21*, 393–400.

Steinberg, J.P., Huganir, R.L., and Linden, D.J. (2004). N-ethylmaleimide-sensitive factor is required for the synaptic incorporation and removal of AMPA receptors during cerebellar long-term depression. Proc. Natl. Acad. Sci. USA *101*, 18212–18216.

Takahashi, T., Svoboda, K., and Malinow, R. (2003). Experience strengthening transmission by driving AMPA receptors into synapses. Science *299*, 1585–1588.

Thomas, G.M., and Huganir, R.L. (2004). MAPK cascade signalling and synaptic plasticity. Nat. Rev. Neurosci. *5*, 173–183.

Tomita, S., Stein, V., Stocker, T.J., Nicoll, R.A., and Bredt, D.S. (2005). Bidirectional synaptic plasticity regulated by phosphorylation of stargazin-like TARPs. Neuron *45*, 269–277.

Worley, P.F., Bhat, R.V., Baraban, J.M., Erickson, C.A., McNaughton, B.L., and Barnes, C.A. (1993). Thresholds for synaptic activation of transcription factors in hippocampus: correlation with long-term enhancement. J. Neurosci. *13*, 4776–4786.

Zhou, Q., and Poo, M.M. (2004). Reversal and consolidation of activity-induced synaptic modifications. Trends Neurosci. *27*, 378–383.

Zhu, J.J., and Connors, B.W. (1999). Intrinsic firing patterns and whisker-evoked synaptic responses of neurons in the rat barrel cortex. J. Neurophysiol. *81*, 1171–1183.

Zhu, J.J., Esteban, J.A., Hayashi, Y., and Malinow, R. (2000). Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. Nat. Neurosci. *3*, 1098–1106.

Zhu, J.J., Qin, Y., Zhao, M., Van Aelst, L., and Malinow, R. (2002). Ras and Rap control AMPA receptor trafficking during synaptic plasticity. Cell *110*, 443–455.

Zhu, Y., Stornetta, R.L., and Zhu, J.J. (2004). Chandelier cells control excessive cortical excitation: characteristics of whisker-evoked synaptic responses of layer 2/3 nonpyramidal and pyramidal neurons. J. Neurosci. *24*, 5101–5108.

Zhu, Y., Pak, D., Qin, Y., McCormack, S.G., Kim, M.J., Baumgart, J.P., Velamoor, V., Auberson, Y.P., Osten, P., van Aelst, L., et al. (2005). Rap2-JNK removes synaptic AMPA receptors during depotentiation. Neuron *46*, 905–916.