nature neuroscience

A single fear-inducing stimulus induces a transcription-dependent switch in synaptic AMPAR phenotype

Yu Liu[1], Luigi Formisano[2,5], Iaroslav Savtchouk[1,3], Yukihiro Takayasu[2,5], Gábor Szabó[4], R Suzanne Zukin[2] & Siqiong June Liu[1,3]

Changes in emotional state are known to alter neuronal excitability and can modify learning and memory formation. Such experience-dependent neuronal plasticity can be long-lasting and is thought to involve the regulation of gene transcription. We found that a single fear-inducing stimulus increased *GluR2* (also known as *Gria2*) mRNA abundance and promoted synaptic incorporation of GluR2-containing AMPA receptors (AMPARs) in mouse cerebellar stellate cells. The switch in synaptic AMPAR phenotype was mediated by noradrenaline and action potential prolongation. The subsequent rise in intracellular Ca^{2+} and activation of Ca^{2+}-sensitive ERK/MAPK signaling triggered new *GluR2* gene transcription and a switch in the synaptic AMPAR phenotype from GluR2-lacking, Ca^{2+}-permeable receptors to GluR2-containing, Ca^{2+}-impermeable receptors on the order of hours. The change in glutamate receptor phenotype altered synaptic efficacy in cerebellar stellate cells. Thus, a single fear-inducing stimulus can induce a long-term change in synaptic receptor phenotype and may alter the activity of an inhibitory neural network.

The ability of the nervous system to respond to a transient stimulus with a persistent change in the efficacy of synaptic transmission depends on the regulation of gene transcription[1,2]. The best understood postsynaptic modification involves a change in the phosphorylation state and number of AMPARs[3–6], but may also involve long-lasting changes in AMPAR subunit composition and Ca^{2+} permeability[4,7,8]. These changes arise not only as a consequence of regulated receptor trafficking, but also of local dendritic protein synthesis[3–12]. Given the importance of experience-dependent gene transcription in learning and memory, an alteration in AMPAR transcription represents a powerful means to produce a long-lasting change in synaptic AMPARs and activity of entire neuronal circuits. However, whether experience can regulate AMPAR transcription remains unclear.

The stress hormone noradrenaline mediates memory consolidation by emotion[13]. During emotional arousal, noradrenaline is released from neurons arising in the locus coeruleus and lateral brain stem tegmentum and produces its effects at synapses throughout the CNS[14,15]. In the cerebellum, these fibers terminate primarily in the molecular and Purkinje/granule cell layers, where noradrenaline acts via β-adrenergic receptors to increase the action potential firing rate of inhibitory stellate cells[16,17] and alter the spontaneous firing of Purkinje neurons[14,15]. Noradrenaline has a central role in motor learning and fear-related memories, which affect synaptic transmission in the cerebellum[18–21]. Noradrenaline also produces powerful effects on synaptic plasticity, which is widely believed to be a cellular substrate for learning and memory. In the hippocampus and visual cortex, noradrenaline lowers the threshold for induction of long-term potentiation by facilitating phosphorylation and synaptic delivery of GluR1-containing AMPARs[22,23]. However, the ability of noradrenaline to alter synaptic AMPAR phenotype is unclear.

Cerebellar stellate cells spontaneously fire action potentials of brief duration and express GluR2-lacking AMPARs, which is characteristic of inhibitory interneurons[24,25]. Synaptic AMPARs lacking the edited GluR2 subunit have high Ca^{2+} permeability, rapid decay kinetics and are blocked by intracellular polyamines[26]. These properties allowed us to directly follow the synaptic incorporation of GluR2 subunits in cerebellar stellate cells. Targeted expression of GluR2 in inhibitory interneurons disrupts long-range synchrony of gamma oscillations in the hippocampus[27]. Thus, an alteration in *GluR2* gene expression could have profound consequences on neuronal function and the activity of neuronal circuits. We examined the effect of emotional arousal in the intact mouse on AMPAR GluR2 transcription and synaptic AMPAR phenotype. We found that a single fear-inducing stimulus acted via β-adrenergic receptors to increase *GluR2* mRNA abundance and alter synaptic AMPAR phenotype in cerebellar stellate cells. We further elucidated the mechanism by which the switch in AMPAR phenotype occurred. Noradrenaline prolonged the action potential duration. The subsequent rise in intracellular Ca^{2+} activated the Ca^{2+}-sensitive ERK/MAPK pathway, which drives transcription of GluR2 and synaptic incorporation of GluR2-containing AMPARs at parallel fiber to stellate cell synapses. This transcription-dependent form of synaptic plasticity may underlie brain processing of fear-inducing stimuli.

[1]Department of Biology, Pennsylvania State University, University Park, Pennsylvania, USA. [2]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York, USA. [3]Department of Cell Biology and Anatomy, Neuroscience Center, Louisiana State University Health Science Center, New Orleans, Louisiana, USA. [4]Laboratory of Molecular Biology and Genetics, Institute of Experimental Medicine, Budapest, Hungary. [5]Present address: Universita Degli Studi Di Napoli Federico II, Via Pansini 5 Torre Biologica, Naples, Italy (L.F.), and Department of Otolaryngology, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan (Y.T.). Correspondence should be addressed to S.J.L. (sliu@lsuhsc.edu).

Received 27 April; accepted 20 November; published online 27 December 2009; corrected 17 January 2010 (details online); doi:10.1038/nn.2474

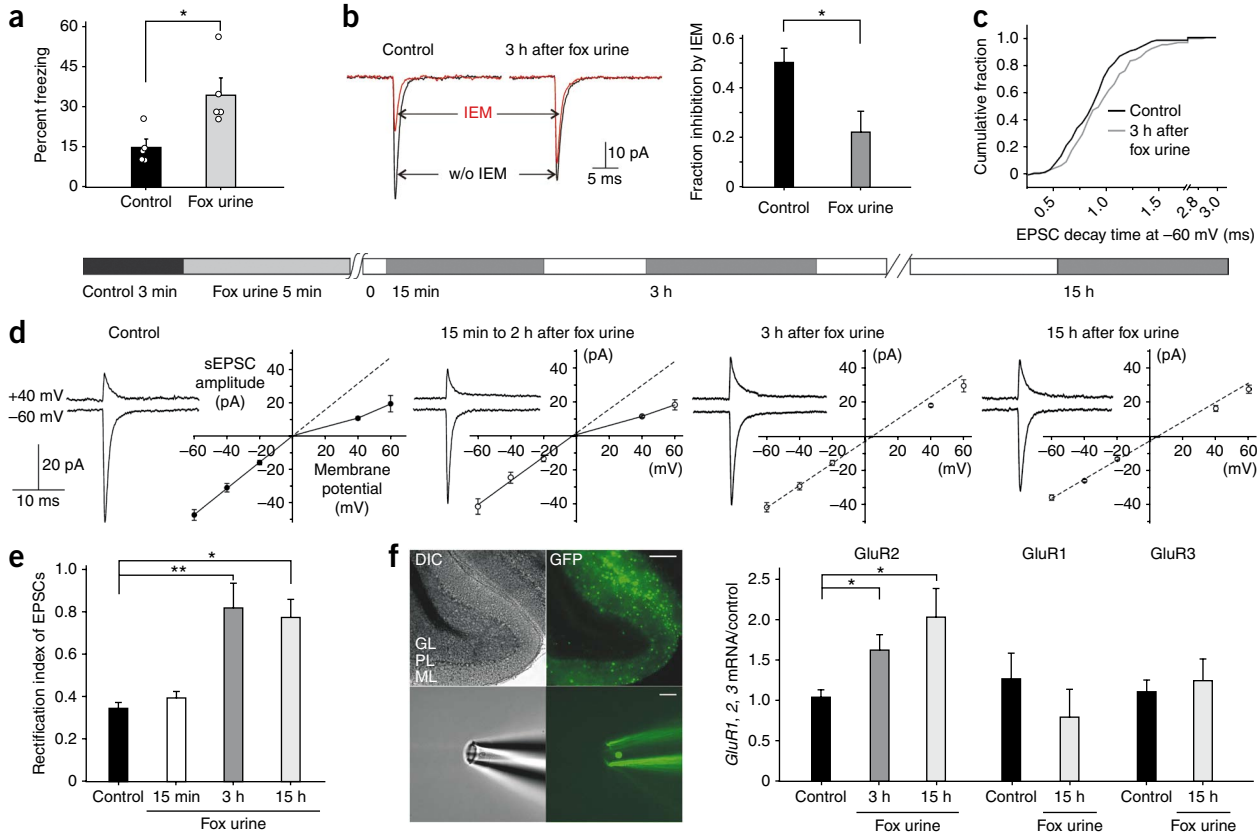

Figure 1 An olfactory stimulus altered synaptic AMPA receptor subtype and expression of *GluR2* mRNA in stellate cells. (**a**) A natural olfactory stimulus, fox urine, caused fear (measured as a freezing response). Percentage freezing was calculated during the 3-min control and 5-min fox urine exposure periods ($n = 5$, $^*P < 0.01$). (**b**) Inhibition of EPSCs at –60 mV by IEM-1460 (100 μM, $n = 3$, $P < 0.05$). (**c**) Cumulative distribution of decay time constants of EPSCs at –60 mV of individual synaptic events from five cells under each condition (Kolmogorov-Smirnov test, $P < 0.0001$, control versus 3 h). (**d**) Synaptic currents and *I-V* relationship when spermine was included in the pipette in stellate cells. Control cells had an *I-V* relationship with pronounced inward rectification, suggesting the presence of GluR2-lacking receptors. Mice were exposed to fox urine for 5 min. Cerebellar slices were prepared 15 min after olfactory stimulus. Synaptic currents were recorded within 2 h of fox urine exposure and had an inwardly rectifying *I-V* relation ($n = 4$). The synaptic current in stellate cells showed a near linear *I-V* relationship 3 h after fox urine exposure ($n = 5$, $P < 0.01$), indicating that it was mediated mainly by GluR2-containing AMPARs. When slices were prepared 15 h after fox urine exposure, the synaptic current still showed a near linear *I-V* relationship ($n = 4$). (**e**) Rectification index ($^*P < 0.01$, $^{**}P < 0.005$). (**f**) The *GluR1*, *GluR2* and *GluR3* mRNA levels in individual stellate cells were determined using real-time single-cell RT-PCR. GFP-positive neurons (~8 μm in diameter) were isolated from the cerebellar cortex of GAD65-GFP mice (control, 23 cells from 4 animals; 3 h following fox urine exposure, 18 cells; 15 h following fox urine exposure, 15 cells; $^*P < 0.05$). Scale bars represent 200 μm (top) and 20 μm (bottom). Error bars represent ± s.e.m.

RESULTS

Emotional stress alters AMPAR subtype and elevates *GluR2* mRNA

Exposing mice to fox urine, a natural olfactory stimulus, causes innate fear and promotes noradrenaline release in the brain[22,28]. To examine whether emotional stress can induce an alteration in synaptic AMPAR properties, we exposed mice to fox urine for 5 min and monitored locomotor activity. A single exposure to fox urine induced a freezing behavior, indicative of fear (**Fig. 1a**). We next monitored excitatory postsynaptic currents (EPSCs) at parallel fiber to stellate cell synapses in cerebellar slices from mice at indicated times after exposure to the fear-inducing stimulus. To examine synaptic AMPAR properties, we first used IEM-1460, a subunit-selective blocker of Ca^{2+}-permeable AMPARs. In control cells, application of IEM-1460 (100 μM) reduced the EPSC amplitude at –60 mV by ~50% ($n = 3$; **Fig. 1b**). At 3 h after exposure to fox urine, the block by IEM-1460 was greatly diminished (~20%, $P < 0.05$; **Fig. 1b**), which is consistent with a stress-induced switch in AMPAR phenotype from GluR2 lacking to GluR2 containing.

We then measured spontaneous EPSCs (sEPSCs) as a function of holding potential in the presence of the polyamine spermine. Inclusion of spermine in the pipette solution causes a voltage-dependent block of AMPARs lacking GluR2 and produces a characteristic inwardly rectifying *I-V* relationship[4,7,8]. Synaptic incorporation of GluR2-containing AMPARs is detected as decreased rectification of AMPAR-mediated EPSCs. The sEPSC provides a measure of intrinsic activity of all synapses onto stellate cells and enables comparison of the mean synaptic current over the prolonged time during which the fear-inducing stimulus alters synaptic strength.

In stellate cells from control mice, the sEPSC amplitude was reduced at positive membrane potentials. This inwardly rectifying *I-V* relation implies that the sEPSCs were mediated mainly by GluR2-lacking, Ca^{2+}-permeable AMPARs (**Fig. 1**). In contrast, in stellate cells from mice exposed to fox urine, synaptic currents recorded at 3 h after exposure showed a near linear *I-V* relation (**Fig. 1d**), indicating that they were mediated mainly by GluR2-containing AMPARs. Although fox urine exposure increased the sEPSC amplitude from 10.7 ± 0.9 pA (control) to 18.2 ± 0.8 pA at +40 mV ($n = 5$, $P < 0.001$), there was little or no change in the sEPSC amplitude at –60 mV. Fox urine exposure increased the rectification index from predominantly inwardly

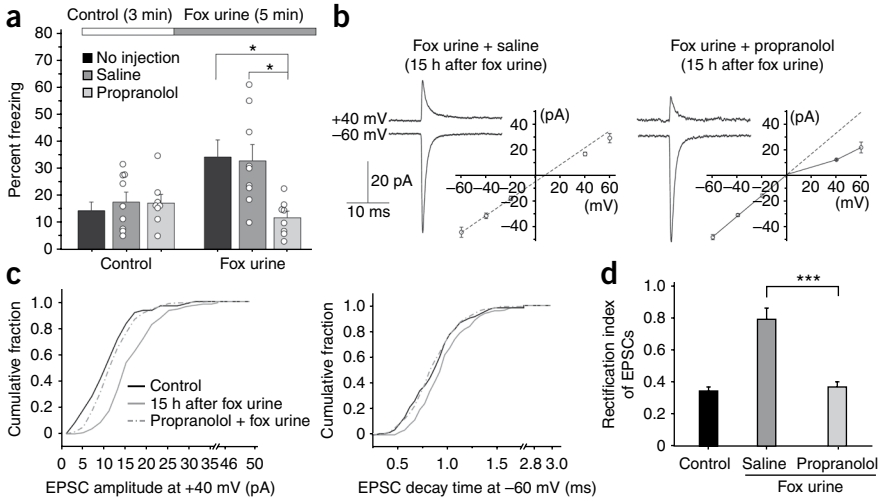

Figure 2 β-adrenergic receptors mediated the olfactory stimulus–induced change in synaptic AMPA receptor subtype. Mice were injected with propranolol or saline (as control) 15–30 min before the fox urine exposure. (**a**) Percentage freezing was calculated during the 3-min control and 5-min fox urine exposure periods ($n = 5$, $^*P < 0.01$). (**b**) Slices were prepared 15 h after fox urine exposure. Synaptic currents and *I-V* relationship of EPSCs in stellate cells from the mice pre-injected with saline ($n = 6$) and propranolol ($n = 6$) are shown. (**c**) Cumulative distribution of EPSC amplitude at +40 mV and decay time constant of EPSC at –60 mV of individual synaptic events from six cells under each condition (Kolmogorov-Smirnov test, $P < 0.0001$, 15 h versus propranolol). (**d**) Rectification index ($^{***}P < 0.001$). Error bars represent ± s.e.m.

rectifying (0.34 ± 0.03; $n = 5$) to nearly linear (0.82 ± 0.11; $n = 5$; $P < 0.01$; **Fig. 1e**). Fox urine exposure also prolonged the decay time constant of the EPSC at −60 mV (**Fig. 1c**), consistent with the slower time course of EPSCs mediated by GluR2-containing AMPARs versus GluR2-lacking AMPARs[27,29].

Although activity-dependent regulation of AMPAR trafficking occurs within 15–30 min of parallel fiber stimulation[29,30], the stress-induced switch in synaptic AMPAR phenotype was delayed. Synaptic currents had an inwardly rectifying I-V relation as late as 2 h after fox urine exposure (0.39 ± 0.03, $n = 4$; **Fig. 1d**,**e**). Thus, the appearance of GluR2-containing AMPARs at stellate cell synapses does not occur until 2–3 h after a fear-inducing stimulus. The switch in synaptic AMPAR phenotype was long-lasting in that synaptic currents had a near linear *I-V* relationship (0.78 ± 0.08, $n = 4$; $P < 0.01$ versus control, **Fig. 1d**,**e**), elevated amplitude at +40 mV (16.6 ± 1.7 pA; $P < 0.01$) and characteristically long decay time at −60 mV (**Fig. 2**) as late as 15 h after the fear-inducing stimulus.

The long delay between the fear-inducing stimulus and the increase in synaptic GluR2 is consistent with a requirement for transcription-dependent alterations. To examine whether fox urine exposure alters *GluR2* mRNA expression, we measured *GluR2* mRNA abundance in individual GFP-positive stellate cells from glutamic acid decarboxylase (GAD)-65–GFP knock-in mice by quantitative real-time single-cell reverse transcription–PCR (RT-PCR). Fox urine exposure increased *GluR2* mRNA levels in stellate cells by 63 ± 19% ($P < 0.05$) at 3 h and 103 ± 36% ($P < 0.05$) at 15 h (**Fig. 1f**). The stress-induced change in *GluR2* mRNA was subunit-specific in that *GluR1* and *GluR3* (also known as *Gria1* and *Gria3*, respectively) mRNA were unaltered (**Fig. 1f**). Thus, incorporation of functional GluR2-containing AMPARs at synapses is associated with an increase in *GluR2* mRNA content of individual stellate cells. These results do not, however, distinguish between transcription of new mRNA versus enhanced stability of existing mRNA.

We reasoned that during emotional arousal noradrenaline released onto cerebellar stellate cells might activate β-adrenergic receptors, which are expressed by stellate cells and mediate fear-induced freezing behavior (**Fig. 2a**)[22,31]. The β-adrenergic receptor blocker propranolol, injected 15–30 min before fox urine exposure, largely abolished stress-induced increase in GluR2-containing AMPAR levels at stellate cell synapses (stress and propranolol, 0.37 ± 0.03, $n = 6$, $P < 0.01$ versus stress alone; stress and saline, 0.80 ± 0.07, $n = 6$, $P < 0.0005$ versus stress and propranolol; **Fig. 2b–d**). Thus, noradrenaline and β-adrenergic receptors probably mediate the stress-induced change in synaptic AMPAR phenotype.

Noradrenaline alters AMPARs and membrane excitability

We next examined whether direct application of noradrenaline to brain slices can mimic the fear-induced switch in AMPAR phenotype at stellate cell synapses. Application of noradrenaline (10 μM) via the bath perfusate (at 36 °C) produced a modest increase in sEPSC frequency (control, 0.37 ± 0.16; noradrenaline, 0.58 ± 0.34 Hz; $n = 6$, $P = 0.24$). To avoid a contribution of altered glutamate or GABA release, we incubated slices in the presence of kynurenic acid (1 mM) to block AMPARs and NMDARs, and picrotoxin (100 μM) to block $GABA_A$Rs (**Supplementary Fig. 1**). Kynurenic acid and picrotoxin (3 h) did not detectably alter the sEPSC rectification index (0.25 ± 0.07, $n = 4$, $P = 0.23$ versus no treatment). Application of noradrenaline (3 h) in the presence of kynurenic acid and picrotoxin caused a switch in the *I-V* relation of the sEPSC (**Fig. 3**) and evoked EPSC (**Fig. 3c**) from inwardly rectifying to near linear. Although noradrenaline increased the sEPSC amplitude from 7.8 ± 1.7 (control) to 16.3 ± 1.1 pA at +40 mV ($n = 8$, $P < 0.001$), it produced little or no change in EPSC amplitude at −60 mV (control, −46.9 ± 6.7, $n = 4$; noradrenaline, −39.0 ± 1.6 pA, $n = 8$). Noradrenaline increased the rectification index from inwardly rectifying (control, 0.25 ± 0.07) to nearly linear (noradrenaline, 0.74 ± 0.10; $P < 0.01$; **Fig. 3d**). Noradrenaline also prolonged the decay time constant of sEPSCs at −60 mV (control, 0.95 ± 0.04; noradrenaline, 1.29 ± 0.09 ms; $P < 0.02$; **Fig. 3b**). Together, these results strongly suggest that noradrenaline promotes synaptic incorporation of GluR2-containing receptors. Noradrenaline treatment for 0.5 h, followed by a 2.5-h incubation, increased the rectification index of sEPSCs (0.57 ± 0.12; $n = 5$; $P < 0.05$; **Fig. 3d**). These findings indicate that noradrenaline treatment for 0.5 h is sufficient to induce the switch in synaptic AMPAR phenotype in stellate cells, although the switch is delayed relative to its induction.

Association of transmembrane AMPAR regulatory proteins such as stargazin with GluR2-lacking AMPARs can relieve the block of synaptic AMPA currents caused by intracellular spermine and increase the EPSC rectification index[32]. Two factors indicate that the noradrenaline-induced increase in rectification index is unlikely to reflect increased association of synaptic AMPARs with stargazin. First, we included 100 μM spermine in the patch pipette, which is sufficient to block Ca^{2+}-permeable AMPARs at +40 mV, even in the presence of stargazin[32]. Second, in the presence of stargazin, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) acts as a partial agonist at AMPARs and elicits an inward current that is diagnostic for association of AMPARs with transmembrane AMPAR regulatory proteins[33]. In control cells, CNQX (10 μM with 100 μM cyclothiazide, to reduce AMPAR desensitization)

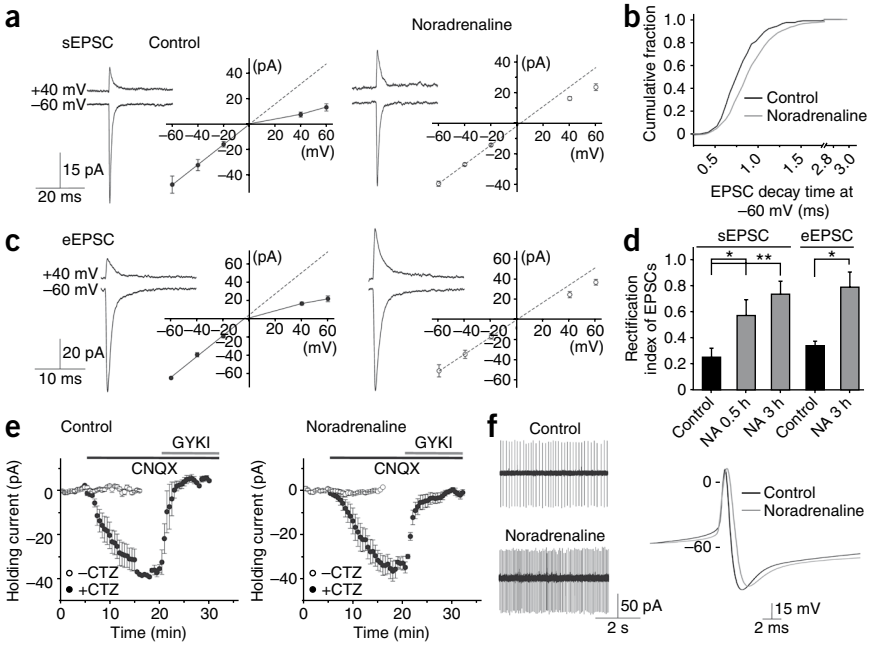

Figure 3 Noradrenaline induced a change in synaptic AMPA receptor phenotype. (**a**) Average sEPSCs had an inwardly rectifying *I-V* relationship in controls and became more linear following noradrenaline treatment (control, $n = 4$; noradrenaline treatment, $n = 8$). Cerebellar slices were incubated with kynurenic acid (1 mM) and picrotoxin (100 μM) in the absence (control) or presence of noradrenaline (10 μM, 3 h). Following each treatment, noradrenaline and kynurenic acid were washed out before we recorded sEPSCs. (**b**) The decay time of sEPSCs at –60 mV increased following noradrenaline treatment (Kolmogorov-Smirnov test, $P < 0.0001$). The cumulative distribution of decay time constant of EPSC at –60 mV of individual synaptic events from four control cells and eight noradrenaline-treated cells is shown. (**c**) Noradrenaline also induced a change in the *I-V* relationship of evoked EPSCs at the parallel fiber to stellate cell synapse (control, $n = 4$; noradrenaline treatment, $n = 5$). (**d**) Summary of rectification index of EPSCs. Cerebellar slices were incubated with 10 μM noradrenaline (NA) for 3 h and 0.5 h (2.5 h in picrotoxin and kynurenic acid control, $n = 5$; picrotoxin and kynurenic acid control, $n = 4$). (**e**) CNQX (10 μM) and cyclothiazide (100 μM) evoked inward currents of comparable amplitude in control ($n = 4$) and noradrenaline-treated cells ($n = 5$, two-way ANOVA test, $P = 0.39$). (**f**) Noradrenaline (10 μM) increased the frequency (left) and duration (right) of spontaneous action potentials in stellate cells at 36 °C (frequency, $n = 5$, $P < 0.05$; duration, $n = 5$, $P < 0.005$). Error bars represent ± s.e.m. $^*P < 0.05$, $^{**}P < 0.005$.

produced a modest inward current (−32.1 ± 5.3 pA, $n = 4$), which was not altered by noradrenaline (−33.9 ± 2.9 pA, $n = 5$; **Fig. 3e**).

To examine the mechanisms by which noradrenaline alters AMPA EPSCs in stellate cells, we examined the effect of noradrenaline on the frequency and duration of spontaneous action potentials. Application of noradrenaline increased the frequency of spontaneous action potentials in cerebellar stellate cells by 146 ± 33% (from 12.1 ± 3.7 to 29.6 ± 9.8 Hz; $n = 5$; $P < 0.05$; **Fig. 3f** and **Supplementary Fig. 2**), consistent with previous findings[16,17,31]. Noradrenaline also prolonged the duration of action potentials, defined as the action potential half-width (control, 0.97 ± 0.06; noradrenaline, 1.27 ± 0.06 ms; $n = 5$, $P < 0.005$), and reduced the amplitude of the after-hyperpolarization (from −26.2 ± 5.4 to −22.7 ± 5.5 mV, $P < 0.05$; **Fig. 3f** and **Supplementary Fig. 2**). Noradrenaline acts via β-adrenergic receptors to promote formation of cAMP, which activates the hyperpolarization-activated inward current I_h and increases spike frequency in cerebellar stellate cells[31]. We reasoned that the noradrenaline-induced changes in action potential waveform might arise as a result of an increase in I_h. Consistent with this, the I_h inhibitor ZD7288 (10 μM) did not alter action potential duration, but abolished the noradrenaline-induced spike broadening (**Supplementary Table 1** and **Supplementary Fig. 2**). Thus, the increase in spike frequency and spike broadening arise, at least in part, as a result of an increase in I_h current.

Spike broadening increases synaptic GluR2 receptors

These results indicate that noradrenaline increases action potential firing frequency and prolongs action potential duration in stellate cells. To examine whether either change alone is sufficient to trigger the switch in AMPAR phenotype, we selectively altered action potential frequency or duration. First, we increased spike frequency while maintaining constant spike duration. Block of inhibitory transmission by application of picrotoxin (100 μM) increased action potential firing frequency by 203 ± 72% ($n = 5$, $P < 0.05$; **Supplementary Fig. 3**), with no change in duration or after-hyperpolarization (**Supplementary Table 2**). Application of picrotoxin (3 h) did not alter the rectification index or sEPSC amplitude at stellate cell synapses (**Fig. 4** and **Supplementary Fig. 3**). Thus, an increase in action potential frequency, but not duration, does not alter synaptic AMPAR phenotype.

Next, we increased spike duration while maintaining constant spike frequency. The potassium channel blocker tetraethylammonium (TEA, 1 mM) increased action potential duration (control, 1.3 ± 0.07; TEA, 1.8 ± 0.14 ms; $n = 9$, $P < 0.0005$; **Fig. 4a**) and reduced the amplitude of the after-hyperpolarization (from −30.2 ± 3.3 to −14.4 ± 3.5 mV; $P < 0.0001$), but not the frequency of spontaneous action potentials (**Supplementary Table 3**) in postsynaptic stellate cells. We examined the effect of TEA on the synaptic AMPAR phenotype in stellate cells. Application of TEA (1 mM with kynurenic acid and picrotoxin, 3 h) altered the *I-V* relation of the sEPSC from inwardly rectifying to nearly linear and increased the rectification index (control, 0.29 ± 0.03, $n = 5$; TEA, 0.88 ± 0.09, $n = 6$; $P < 0.005$; **Fig. 4b**,**c**), indicative of a switch from GluR2-lacking to GluR2-containing AMPARs. Although TEA increased the sEPSC amplitude at +40 mV (control, 7.9 ± 0.5; TEA, 18.5 ± 1.6 pA; $n = 6$, $P < 0.005$), there was little or no change at −60 mV. However, TEA increased the decay time of sEPSCs at −60 mV (**Fig. 4d**). These results suggest that TEA promotes synaptic incorporation of GluR2-containing receptors. Although TEA treatment for 1 h did not alter the sEPSC rectification index (0.31 ± 0.03, $n = 3$; **Fig. 4c**), TEA treatment for 1 h, followed by a 2-h incubation in control solution, significantly increased the rectification index of synaptic currents (0.60 ± 0.09, $n = 3$, $P < 0.05$ versus control). Thus, although brief (1 h) application of TEA is sufficient to trigger the switch in AMPAR phenotype, the expression of GluR2-containing AMPARs at stellate cell synapses does not occur until >1 h.

To examine a possible contribution of mGluRs and $GABA_B$Rs to the switch in AMPAR phenotype, we applied TEA in the presence

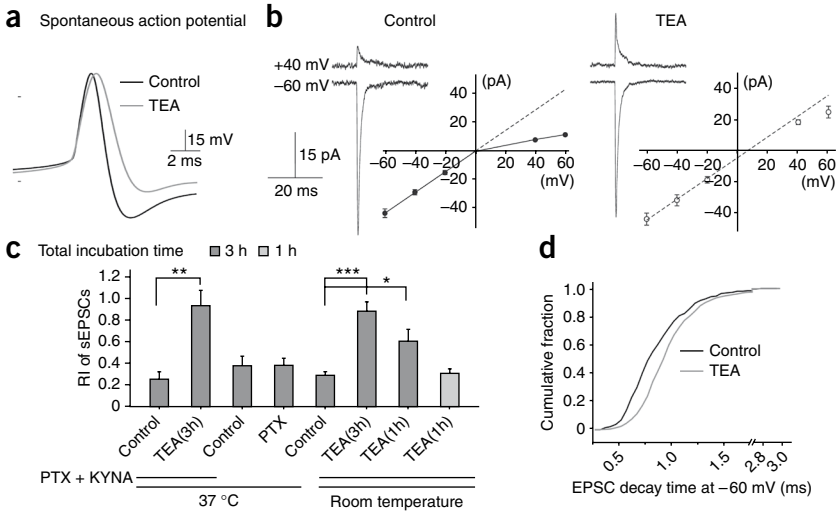

Figure 4 Increasing the action potential duration in stellate cells induces a change in rectification of the *I-V* relationship. (**a**) The duration of spontaneous action potentials in cerebellar stellate cells increased during bath application of TEA at 22–25 °C. (**b**) sEPSCs had a nearly linear *I-V* relationship following TEA treatment (control, $n = 5$; TEA treatment, $n = 6$). Cerebellar slices were incubated with kynurenic acid (1 mM) and picrotoxin (100 μM) in the absence (control) or presence of TEA (1 mM, 3 h). (**c**) Summary of rectification index (RI) of EPSCs. Cerebellar slices were incubated with 100 μM picrotoxin ($n = 5$; control, $n = 4$) or with 1 mM TEA for 3 h (at 37 °C, $n = 3$; at 22–25 °C, $n = 6$). (**d**) The decay time of sEPSCs increased following TEA treatment (Kolmogorov-Smirnov test, $P < 0.0001$). $^*P < 0.05$, $^{**}P < 0.005$ and $^{***}P < 0.0005$. Error bars represent ± s.e.m.

of the group I/II mGluR inhibitor MCPG (1 mM) and the $GABA_BR$ inhibitor SCH50911 (10 μM) for 3 h. Neither mGluR nor $GABA_BR$ blockade altered the TEA-induced switch in AMPAR phenotype (TEA, MCPG and SCH50911, 0.77 ± 0.04, $n = 4$, $P < 0.0001$ versus control). Moreover, TEA did not alter the frequency of sEPSCs (**Supplementary Table 3**), indicating that TEA does not affect transmitter release from presynaptic granule cells. During TEA treatment (3 h), the action potential duration remained prolonged and the spontaneous action potential frequency was unaltered relative to that in the absence of TEA (**Supplementary Table 3**). These findings suggest that action potential broadening contributes to the noradrenaline-induced increase in synaptic GluR2 expression.

Enhanced Ca^{2+} entry during action potentials is required

Spike broadening could increase Ca^{2+} entry via voltage-gated Ca^{2+} channels during action potentials. To examine whether the noradrenaline-induced increase in action potential duration is associated with enhanced Ca^{2+} entry, we measured Ca^{2+} currents in stellate cells under voltage-clamp using action potential waveforms that mimicked either action potential in control or in the presence of noradrenaline. Ca^{2+} entry during a noradrenaline action potential (defined as current integrated over action potential duration) was ~40% greater than that of a control action potential waveform ($n = 5$; control action potential, 176 ± 32; noradrenaline action potential, 229 ± 34 pA ms; $P < 0.05$; **Fig. 5a**). The L-type Ca_v1 blocker nifedipine (20 μM) blocked 62.0 ± 4.6% ($n = 5$) of the Ca^{2+} current during a control action potential waveform (**Fig. 5a**), indicating that a substantial fraction of the Ca^{2+} current associated with an action potential is mediated by L-type channels.

Application of nifedipine alone increased the rectification index of the EPSC (nifedipine, 0.49 ± 0.06, $n = 5$, $P < 0.05$ versus control; **Fig. 5**) to a lesser extent than noradrenaline (noradrenaline, 0.89 ± 0.05, $n = 8$, $P < 0.05$ nifedipine versus noradrenaline). However, nifedipine blocked the noradrenaline-induced switch in AMPAR phenotype, as assessed by the sEPSC amplitude at +40 mV (noradrenaline and nifedipine, 11.3 ± 0.6 pA, $n = 6$, $P < 0.005$ versus noradrenaline) and rectification index (noradrenaline and nifedipine, 0.35 ± 0.03, $P < 0.005$ versus noradrenaline; $P < 0.05$ versus nifedipine alone; **Fig. 5b,c**). These findings are consistent with nifedipine-induced block rather than occlusion of the noradrenaline effect and indicate that Ca^{2+} entry via L-type Ca^{2+} channels is required for the noradrenaline-induced switch in AMPAR phenotype at stellate cell synapses.

Activation of ERK and gene transcription is required

Ca^{2+} entry via L-type Ca^{2+} channels can activate Ca^{2+}-sensitive ERK/MAPK signaling, leading to activation of gene transcription[34,35]. To determine whether ERK/MAPK signaling is required for the increase

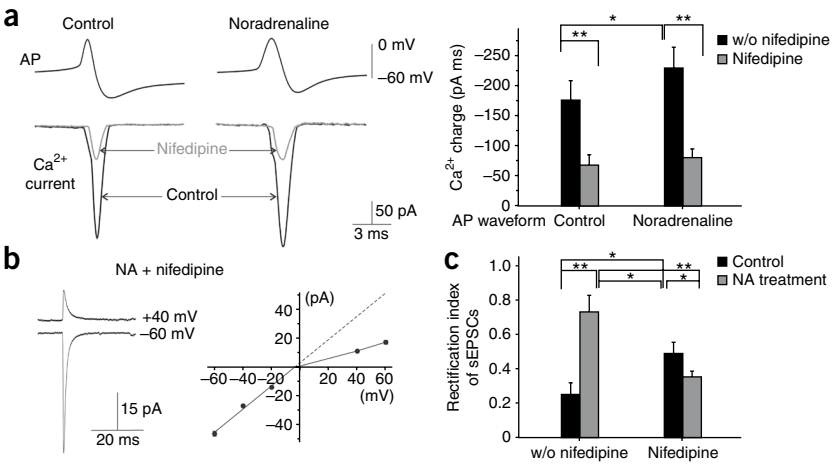

Figure 5 Noradrenaline increases Ca influx during the action potential and Ca entry via L-type Ca channels is required for noradrenaline-induced change in AMPAR phenotype. (**a**) The duration of Ca^{2+} currents was enhanced using noradrenaline action potential as the voltage command, compared with control ($n = 5$). Nifedipine (20 μM) blocked most of the Ca^{2+} current using control action potential and noradrenaline action potential as the voltage commands ($n = 5$). AP, action potential. (**b**) Following a 3-h incubation with noradrenaline and nifedipine, sEPSCs had an inwardly rectifying *I-V* relationship ($n = 6$). (**c**) Summary of rectification index of EPSCs (nifedipine alone, $n = 5$). $^*P < 0.05$, $^{**}P < 0.005$. Error bars represent ± s.e.m.

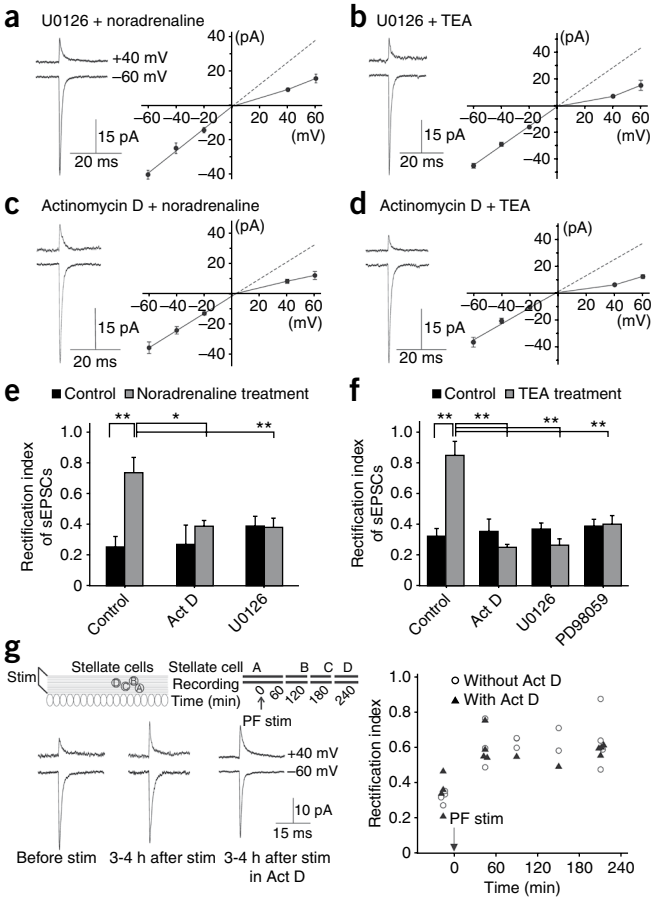

Figure 6 Activation of ERK-dependent pathways and gene transcription are required for the noradrenaline and action potential broadening–induced change in sEPSC rectification. (**a**) The *I-V* relationship of sEPSCs remained inwardly rectifying in the presence of an MEK1/2 inhibitor, U0126 (2 μM, $n = 5$), which was included during the noradrenaline treatment. (**b**) The *I-V* relationship was unaltered if U0126 (2 μM, $n = 3$; 20 μM, $n = 5$) was present during TEA treatment. (**c**) The presence of actinomycin D (Act D, 25 μM) during noradrenaline treatment prevented noradrenaline-induced change in sEPSC rectification ($n = 5$). (**d**) Inclusion of actinomycin D (25 μM) during TEA treatment blocked TEA-induced change in sEPSC rectification (actinomycin D and TEA, $n = 5$). (**e**) Summary of rectification index (actinomycin D alone, $n = 3$; U0126 control, $n = 4$). (**f**) Summary of rectification index (actinomycin D alone, $n = 5$; DMSO control, $n = 4$; DMSO and TEA, $n = 5$; U0126 control, $n = 5$; PD98059 control, $n = 3$; PD98059 and TEA, 10 μM, $n = 2$; 25 μM, $n = 5$). No differences in sEPSC amplitude were observed between the two different concentrations of each inhibitor and therefore the data were pooled. (**g**) Burst stimulation of parallel fibers (PFs) induced a change in sEPSC rectification that lasted for 4 h (before stimulation, RI = 0.32 ± 0.02; 30–60 min after stimulation, RI = 0.62 ± 0.1; 3–4 h after stimulation, RI = 0.64 ± 0.08; $n = 4$, $P < 0.03$). Actinomycin D did not prevent the change in rectification (before stimulation, RI = 0.34 ± 0.03; 30–60 min after stimulation, RI = 0.61 ± 0.05; 3–4 h after stimulation; RI = 0.59 ± 0.01; $n = 4$, $P < 0.02$). Recordings were made sequentially from a group of stellate cells located in the same region of the molecular layer. $^*P < 0.05$, $^{**}P < 0.005$. Error bars represent ± s.e.m.

in synaptic GluR2, we applied noradrenaline in the presence of the selective MEK1/2 inhibitor U0126. U0126 did not itself alter the rectification index of sEPSCs, but abolished the noradrenaline-induced switch in sEPSC *I-V* relation from inwardly rectifying to near linearity (**Fig. 6**) and the increase in EPSC amplitude observed at +40 mV ($P < 0.0005$, $n = 8$). U0126 also blocked the TEA-induced change in the EPSC rectification (**Fig. 6b**,**f**). The ERK inhibitor PD98059 also prevented the TEA-induced change in the sEPSC properties (**Fig. 6f**). These findings implicate ERK/MAPK signaling in the switch from GluR2-lacking to GluR2-containing AMPARs.

To determine whether new gene transcription is required for the noradrenaline-induced increase in GluR2-containing AMPARs at synapses, we applied noradrenaline in the presence of the transcriptional inhibitor actinomycin D (25 μM). Actinomycin D alone (3 h) did not alter the rectification index of synaptic currents (**Fig. 6e**), but abolished the noradrenaline-induced switch in *I-V* relation from inwardly rectifying to nearly linear (noradrenaline, 0.74 ± 0.10; noradrenaline and actinomycin D, 0.39 ± 0.03; $n = 5$, $P < 0.05$; **Fig. 6c**,**e**). Actinomycin D also blocked the noradrenaline-dependent increase in sEPSC amplitude at +40 mV (8.0 ± 1.4 pA, $n = 5$, $P < 0.001$ versus noradrenaline). Thus, the noradrenaline-induced increase in GluR2-containing AMPARs at stellate cell synapses, assessed electrophysiologically, is transcription dependent. If noradrenaline enhances GluR2 expression via increasing action potential duration, we would expect that actinomycin D would also abolish the TEA-induced switch in AMPAR phenotype. Indeed, actinomycin D blocked the TEA-induced increase in synaptic GluR2-containing receptors (TEA, 0.85 ± 0.09, $n = 5$; TEA and actinomycin D, 0.25 ± 0.02, $n = 5$; $P < 0.05$; **Fig. 6d**,**f**). Thus, action potential broadening alone is sufficient to increase GluR2 transcription.

High-frequency stimulation of presynaptic parallel fibers can induce a rapid change in synaptic AMPARs from Ca^{2+}-permeable to Ca^{2+}-impermeable receptors in cerebellar stellate cells. This effect is triggered by activation of NMDARs and AMPARs and requires interaction between GRIP and GluR2 subunits[29,30,36,37]. To examine whether the switch in AMPAR phenotype induced by synaptic stimulation requires new gene transcription, we examined the effect of actinomycin D on this phenomenon. We stimulated parallel fibers with 100 bursts of 4 depolarizations (at 37 °C) over a period of 10 min and measured the synaptic currents at −60 and +40 mV. High-frequency stimulation induced an increase in EPSC amplitude ratio that was detectable by 30–60 min and persisted for at least 4 h ($n = 4$, $P < 0.03$; **Fig. 6g**). Parallel fiber stimulation increased the amplitude of the synaptic current at +40 mV from 8.6 ± 1.8 pA (control) to 17.8 ± 3.5 pA (3–4 h after stimulation, $n = 4$, $P < 0.05$), without a change in the current amplitude at −60 mV. Thus, the parallel fiber stimulation triggered a rapid increase in synaptic GluR2-containing AMPARs that persisted for several hours. Continuous perfusion of actinomycin D throughout the experiment, however, did not affect the stimulation-induced change in rectification of sEPSCs (**Fig. 6g**). Thus, unlike the noradrenaline- or spike broadening–induced switch in AMPAR subunit composition that occurs on the order of hours in a transcription-dependent manner, the parallel fiber stimulation–induced change in synaptic AMPAR phenotype occurs on the order of minutes[29,30] and is transcription independent.

Noradrenaline increases the level of *GluR2* mRNA expression

We next examined whether noradrenaline increases the expression of *GluR2* mRNA in stellate cells. Toward this end, we incubated acute rat cerebellar slices in noradrenaline (3 h, with kynurenic acid and picrotoxin) and then processed cerebellar sections for *in situ* hybridization. Noradrenaline markedly increased the number of cells expressing *GluR2* mRNA and the intensity of *GluR2* mRNA expression in individual cells in the molecular layer (**Fig. 7**). Actinomycin D prevented the noradrenaline-induced increase in *GluR2* mRNA (**Fig. 7c**,**i**,**l**). At this age (P18–21), the vast majority of neurons in the molecular layer of the cerebellar cortex were inhibitory

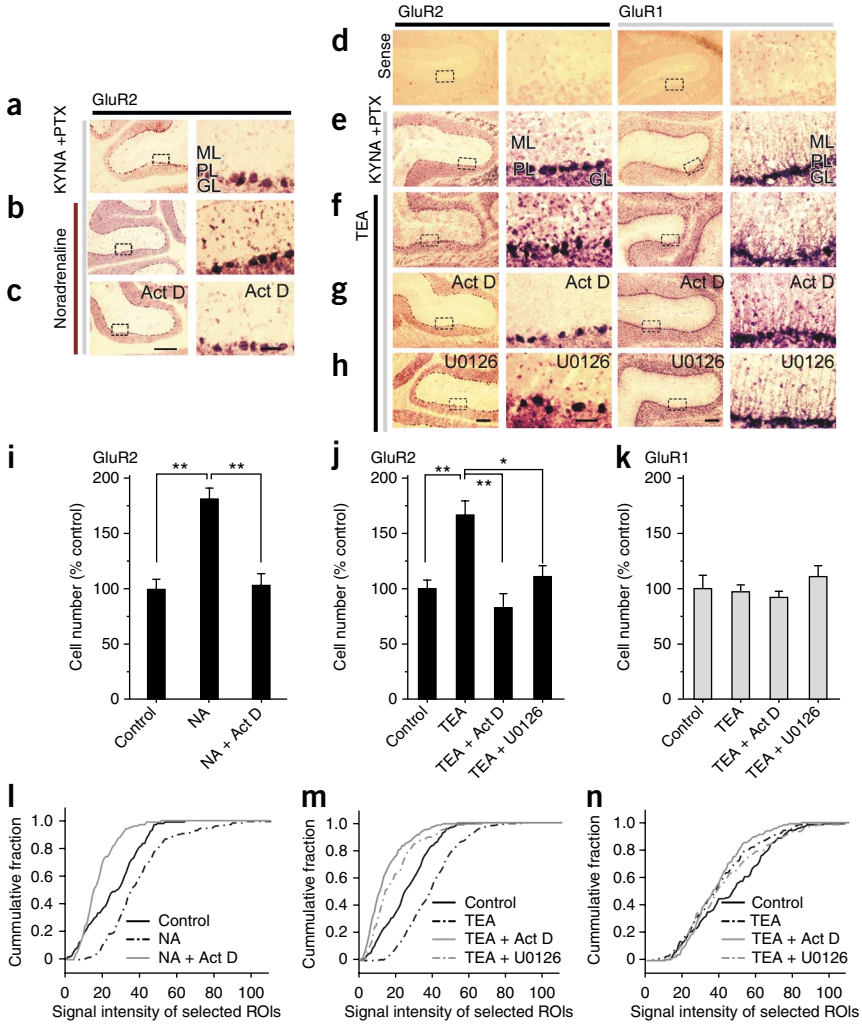

Figure 7 Noradrenaline and TEA treatment increased the level of *GluR2*, but not *GluR1*, mRNA expression ($n = 5$). (**a**–**h**) mRNA expression was assessed by *in situ* hybridization with digoxigenin-labeled RNA antisense (**a**,**c**,**e**,**h**) or sense (**d**) probes. Noradrenaline increased the level of *GluR2* mRNA expression and actinomycin D blocked the noradrenaline-induced increase in *GluR2* mRNA. TEA increased the level of *GluR2*, but not *GluR1*, mRNA expression (**f**). Actinomycin D and U0126 prevented the TEA-induced increase in *GluR2* mRNA expression in stellate cells (**g**,**h**). GL, granule cell layer; KYNA, kynurenic acid; ML, molecular layer; PL, Purkinje cell layer. Areas with labeling of higher intensity than background and containing typical stellate cells (~8 μm) were selected as regions of interest. Images are typical of $n = 5$ per group. (**i**–**k**) The number of labeled stellate cells that express high levels of *GluR2* mRNA under each condition relative to control. We used the mean of background intensity plus 2 s.d. as the threshold for positive labeling ($^{*}P < 0.05$ and $^{**}P < 0.0005$, by unpaired Student's *t* test). (**l**–**n**) Cumulative distribution of the labeling intensity of selected regions of interest after background subtraction illustrated the changes in staining intensities of positive stained cells under each treatment condition (the number of regions of interest ranged from 104 to 365 under each condition; noradrenaline versus control or noradrenaline and Act D, TEA versus control, or TEA and Act D, or TEA and U0126, Kolmogorov-Smirnov test, $P < 0.0001$). Scale bars represent 200 μM (left) and 50 μM (right). ROIs, regions of interest.

basket/stellate cells, which expressed parvalbumin (**Supplementary Fig. 4**). These data indicate that noradrenaline acts via transcription of new *GluR2* mRNA to increase the number of GluR2-containing AMPARs at parallel fiber–stellate cell synapses.

We next investigated whether lengthening the duration of postsynaptic action potential is sufficient to selectively enhance the expression of *GluR2* mRNA. TEA (3 h) induced a marked increase in *GluR2* mRNA expression in individual stellate cells and in the number of cells expressing *GluR2* mRNA (**Fig. 7e**,**f**,**j**,**m**). The change in GluR2 expression was subunit-specific in that *GluR1* mRNA expression was unaltered (**Fig. 7e**,**f**,**k**,**n**). As predicted, actinomycin D abolished the TEA-induced increase in *GluR2* mRNA expression (**Fig. 7g**,**j**,**m**). Moreover, the ERK/MAPK inhibitor U0126 also blocked the TEA-elicited increase in *GluR2* mRNA expression in stellate cells (**Fig. 7h**,**j**,**m**). These findings indicate that lengthening the action potential duration, which enhances Ca^{2+} entry, stimulates the Ca^{2+}-sensitive ERK/MAPK pathway, which in turn elevates *GluR2* mRNA expression and promotes a switch in AMPAR phenotype at stellate cell synapses.

DISCUSSION

Long-lasting, activity-dependent alterations in glutamatergic synaptic transmission require activation of transcription factors, such as CREB[1,2,38]. However, whether an experience directly regulates glutamate receptor gene expression is, as yet, unclear. We found that noradrenaline released during emotional arousal and action potential broadening, which enhance Ca^{2+} entry, selectively upregulates *GluR2* mRNA expression, an affect blocked by actinomycin D. These findings provide direct evidence that noradrenaline induces an activity-dependent, subunit-specific increase in GluR2 gene transcription. Moreover, enhanced GluR2 transcription leads to synaptic incorporation of GluR2-containing AMPARs and a switch in AMPAR phenotype at stellate cell synapses. This switch is initiated by enhanced Ca^{2+} entry via L-type voltage-gated Ca^{2+} channels and requires activation of ERK/MAPK signaling. The net result is an increase in the number of GluR2-containing receptors and a reduction in Ca^{2+} entry through AMPARs at synaptic sites. Although changing action potential frequency alone did not alter GluR2 expression, enhanced frequency in combination with action potential broadening during noradrenaline treatment may contribute to the alteration in synaptic AMPAR phenotype.

That Ca^{2+} entry through L-type Ca^{2+} channels activates ERK/MAPK signaling and promotes phosphorylation and activation of the transcription factor CREB is well established[34,35]. Both CREB and RE1-element silencing transcription factor, a transcriptional repressor that binds the proximal promoter of the *GluR2* gene and silences GluR2 expression, are implicated in the regulation of GluR2 expression in response to neuronal activity and neuronal insults[39–41]. It is therefore possible that upregulation of *GluR2* mRNA expression by noradrenaline could be mediated by one or both of these transcription factors. Our findings do not, however, rule out the possibility that noradrenaline also regulates the stability of the *GluR2* transcript, which further enhances mRNA abundance.

An important mechanism that is responsible for changes in synaptic strength involves regulation of AMPAR trafficking[3–8,12]. *Arc* mRNA, which is induced by neuronal activity and targeted to stimulated synaptic sites, is important for AMPAR trafficking[42,43]. Repetitive activation of synaptic glutamate receptors can also regulate local dendritic protein synthesis of AMPARs in a synapse-specific manner[9–11]. In contrast with these spatially localized changes, synaptic modification resulting from AMPAR gene transcription would produce a long-lasting change in synaptic AMPARs throughout an entire neuron. Thus, regulation of AMPAR gene transcription represents a powerful means of altering the activity of neuronal circuits.

A possibility suggested by our results and the work of others[26,44,45] is that neurons that display brief action potentials are likely to have low levels of *GluR2* mRNA expression[26,44,45]. Activation of Kv3 and Ca^{2+}-activated large-conductance potassium channels can shorten action-potential duration, and both channels are inhibited by 1 mM TEA[25,46]. Notably, a recent study involving gene expression profile analysis of populations of CNS neurons found an inverse relationship between the levels of Kv3 channel expression and that of GluR2 (ref. 45). This is precisely what one would predict given our findings. Generally, fast-spiking GABAergic interneurons (such as cerebellar stellate cells) express low levels of *GluR2* mRNA and high levels of potassium channels that impose a brief action-potential duration[26,44]. However, release of noradrenaline suppresses the normal pathway for regulation of AMPAR subunit composition by prolonging the action potential and promoting synaptic incorporation of GluR2-containing AMPARs and a switch in AMPAR phenotype.

Neuronal activity can regulate the synaptic incorporation of GluR2-containing receptors by facilitating their targeting to the parallel fiber–stellate cell synapse without an alteration in *GluR2* transcription. This mechanism requires activation of synaptic glutamate receptors and involves interactions with PICK1, NSF and GRIP[29,30,36,37]. Synaptic activity also selectively suppresses the synthesis of GluR1 (but not GluR2) subunits in hippocampal neurons[10] and enhances the synthesis of GluR2 in the dopamine neurons in the ventral tegmental area[9]. Our results suggest that noradrenaline increases action-potential duration and Ca^{2+} entry, which regulates *GluR2* transcription. In cerebellar stellate cells, elevated Ca^{2+} influx through L-type channels during a postsynaptic action potential elevates *GluR2* mRNA and thereby increases the number of synaptic GluR2-containing receptors. Thus, newly synthesized AMPARs are ultimately incorporated via receptor trafficking at stellate cell synapses, a mechanism that may involve interactions with PICK1 and GRIP[36,37]. This modification would be expected to reduce the Ca^{2+} permeability of synaptic AMPARs and alter short-term synaptic plasticity[26,47], producing a qualitative change in synaptic transmission. Dynamic regulation of AMPAR transcription may provide a mechanism for homeostatic control of synaptic transmission[48]. Such a transcription-dependent mechanism reveals a role for neuromodulators and postsynaptic action potentials acting over hours to determine synaptic AMPAR phenotype.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureneuroscience/.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We thank D. Grove and S. Acharjee for scientific input, and M.V.L. Bennett, B. Luscher, P.E. Castillo, L.K. Kaczmarek and M. Whim for helpful discussions and comments on the manuscript. This work was supported by National Science Foundation grant IBN-0344559 and US National Institutes of Health grant NS58867 (S.Q.J.L.) and by US National Institutes of Health grant NS46742 (R.S.Z.). R.S.Z. is the F.M. Kirby Professor in Neural Repair and Protection.

AUTHOR CONTRIBUTIONS

Y.L. designed and conducted the behavioral, electrophysiological and real-time single-cell RT-PCR experiments, prepared figures and participated in writing the manuscript. L.F. designed and carried out the *in situ* hybridization assays. I.S. performed the behavioral, real-time single-cell RT-PCR and some electrophysiology experiments, analyzed *in situ* hybridization data, and prepared figures. Y.T. helped to perform the *in situ* hybridization assays and participated in data collection and analysis. G.S. provided GAD65-GFP mice. R.S.Z. supervised the *in situ* hybridization experiments and contributed to the writing and editing of the manuscript. S.J.L. conceived and designed the study and wrote the manuscript.

Published online at http://www.nature.com/natureneuroscience/.
Reprints and permissions information is available online at http://www.nature.com/reprintsandpermissions/.

1. Kandel, E.R. The molecular biology of memory storage: a dialog between genes and synapses. *Biosci. Rep.* **21**, 565–611 (2001).
2. West, A.E., Griffith, E.C. & Greenberg, M.E. Regulation of transcription factors by neuronal activity. *Nat. Rev. Neurosci.* **3**, 921–931 (2002).
3. Malinow, R. & Malenka, R.C. AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* **25**, 103–126 (2002).
4. Cull-Candy, S., Kelly, L. & Farrant, M. Regulation of Ca^{2+}-permeable AMPA receptors: synaptic plasticity and beyond. *Curr. Opin. Neurobiol.* **16**, 288–297 (2006).
5. Barry, M.F. & Ziff, E.B. Receptor trafficking and the plasticity of excitatory synapses. *Curr. Opin. Neurobiol.* **12**, 279–286 (2002).
6. Song, I. & Huganir, R.L. Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci.* **25**, 578–588 (2002).
7. Liu, S.J. & Zukin, R.S. Ca^{2+}-permeable AMPA receptors in synaptic plasticity and neuronal death. *Trends Neurosci.* **30**, 126–134 (2007).
8. Isaac, J.T., Ashby, M. & McBain, C.J. The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. *Neuron* **54**, 859–871 (2007).
9. Mameli, M., Balland, B., Lujan, R. & Luscher, C. Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area. *Science* **317**, 530–533 (2007).
10. Sutton, M.A. *et al.* Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* **125**, 785–799 (2006).
11. Ju, W. *et al.* Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat. Neurosci.* **7**, 244–253 (2004).
12. Bredt, D.S. & Nicoll, R.A. AMPA receptor trafficking at excitatory synapses. *Neuron* **40**, 361–379 (2003).
13. Cahill, L., Prins, B., Weber, M. & McGaugh, J.L. Beta-adrenergic activation and memory for emotional events. *Nature* **371**, 702–704 (1994).
14. Siggins, G.R., Hoffer, B.J., Oliver, A.P. & Bloom, F.E. Activation of a central noradrenergic projection to cerebellum. *Nature* **233**, 481–483 (1971).
15. Bickford-Wimer, P., Pang, K., Rose, G.M. & Gerhardt, G.A. Electrically evoked release of norepinephrine in the rat cerebellum: an in vivo electrochemical and electrophysiological study. *Brain Res.* **558**, 305–311 (1991).
16. Saitow, F., Satake, S., Yamada, J. & Konishi, S. beta-adrenergic receptor–mediated presynaptic facilitation of inhibitory GABAergic transmission at cerebellar interneuron–Purkinje cell synapses. *J. Neurophysiol.* **84**, 2016–2025 (2000).
17. Kondo, S. & Marty, A. Differential effects of noradrenaline on evoked, spontaneous and miniature IPSCs in rat cerebellar stellate cells. *J. Physiol. (Lond.)* **509**, 233–243 (1998).
18. Cartford, M.C., Samec, A., Fister, M. & Bickford, P.C. Cerebellar norepinephrine modulates learning of delay classical eyeblink conditioning: evidence for post-synaptic signaling *via* PKA. *Learn. Mem.* **11**, 732–737 (2004).
19. Sacchetti, B., Baldi, E., Lorenzini, C.A. & Bucherelli, C. Cerebellar role in fear-conditioning consolidation. *Proc. Natl. Acad. Sci. USA* **99**, 8406–8411 (2002).
20. Sacchetti, B., Scelfo, B., Tempia, F. & Strata, P. Long-term synaptic changes induced in the cerebellar cortex by fear conditioning. *Neuron* **42**, 973–982 (2004).
21. Scelfo, B., Sacchetti, B. & Strata, P. Learning-related long-term potentiation of inhibitory synapses in the cerebellar cortex. *Proc. Natl. Acad. Sci. USA* **105**, 769–774 (2008).
22. Hu, H. *et al.* Emotion enhances learning via norepinephrine regulation of AMPA receptor trafficking. *Cell* **131**, 160–173 (2007).
23. Seol, G.H. *et al.* Neuromodulators control the polarity of spike timing–dependent synaptic plasticity. *Neuron* **55**, 919–929 (2007).
24. Jonas, P., Bischofberger, J., Fricker, D. & Miles, R. Interneuron diversity series: fast in, fast out—temporal and spatial signal processing in hippocampal interneurons. *Trends Neurosci.* **27**, 30–40 (2004).
25. Rudy, B. & McBain, C.J. Kv3 channels: voltage-gated K^+ channels designed for high-frequency repetitive firing. *Trends Neurosci.* **24**, 517–526 (2001).

26. Geiger, J.R. *et al.* Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principal neurons and interneurons in rat CNS. *Neuron* **15**, 193–204 (1995).
27. Fuchs, E.C. *et al.* Genetically altered AMPA-type glutamate receptor kinetics in interneurons disrupt long-range synchrony of gamma oscillation. *Proc. Natl. Acad. Sci. USA* **98**, 3571–3576 (2001).
28. Hayley, S., Borowski, T., Merali, Z. & Anisman, H. Central monoamine activity in genetically distinct strains of mice following a psychogenic stressor: effects of predator exposure. *Brain Res.* **892**, 293–300 (2001).
29. Liu, S.Q. & Cull-Candy, S.G. Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature* **405**, 454–458 (2000).
30. Sun, L. & Liu, S.J. Activation of extrasynaptic NMDA receptors induces a PKC-dependent switch in AMPA receptor subtypes in mouse cerebellar stellate cells. *J. Physiol. (Lond.)* **583**, 537–553 (2007).
31. Saitow, F. & Konishi, S. Excitability increase induced by beta-adrenergic receptor–mediated activation of hyperpolarization-activated cation channels in rat cerebellar basket cells. *J. Neurophysiol.* **84**, 2026–2034 (2000).
32. Soto, D., Coombs, I.D., Kelly, L., Farrant, M. & Cull-Candy, S.G. Stargazin attenuates intracellular polyamine block of calcium-permeable AMPA receptors. *Nat. Neurosci.* **10**, 1260–1267 (2007).
33. Menuz, K., Stroud, R.M., Nicoll, R.A. & Hays, F.A. TARP auxiliary subunits switch AMPA receptor antagonists into partial agonists. *Science* **318**, 815–817 (2007).
34. Dolmetsch, R.E., Pajvani, U., Fife, K., Spotts, J.M. & Greenberg, M.E. Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. *Science* **294**, 333–339 (2001).
35. Deisseroth, K., Heist, E.K. & Tsien, R.W. Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. *Nature* **392**, 198–202 (1998).
36. Liu, S.J. & Cull-Candy, S.G. Subunit interaction with PICK and GRIP controls Ca^{2+} permeability of AMPARs at cerebellar synapses. *Nat. Neurosci.* **8**, 768–775 (2005).
37. Gardner, S.M. *et al.* Calcium-permeable AMPA receptor plasticity is mediated by subunit-specific interactions with PICK1 and NSF. *Neuron* **45**, 903–915 (2005).
38. Barco, A., Alarcon, J.M. & Kandel, E.R. Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell* **108**, 689–703 (2002).
39. Myers, S.J. *et al.* Transcriptional regulation of the *GluR2* gene: neural-specific expression, multiple promoters and regulatory elements. *J. Neurosci.* **18**, 6723–6739 (1998).
40. Calderone, A. *et al.* Ischemic insults derepress the gene silencer REST in neurons destined to die. *J. Neurosci.* **23**, 2112–2121 (2003).
41. Liu, S. *et al.* Expression of Ca^{2+}-permeable AMPA receptor channels primes cell death in transient forebrain ischemia. *Neuron* **43**, 43–55 (2004).
42. Rial Verde, E.M., Lee-Osbourne, J., Worley, P.F., Malinow, R. & Cline, H.T. Increased expression of the immediate-early gene arc/arg3.1 reduces AMPA receptor–mediated synaptic transmission. *Neuron* **52**, 461–474 (2006).
43. Chowdhury, S. *et al.* Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. *Neuron* **52**, 445–459 (2006).
44. Perney, T.M., Marshall, J., Martin, K.A., Hockfield, S. & Kaczmarek, L.K. Expression of the mRNAs for the Kv3.1 potassium channel gene in the adult and developing rat brain. *J. Neurophysiol.* **68**, 756–766 (1992).
45. Sugino, K. *et al.* Molecular taxonomy of major neuronal classes in the adult mouse forebrain. *Nat. Neurosci.* **9**, 99–107 (2006).
46. Salkoff, L., Butler, A., Ferreira, G., Santi, C. & Wei, A. High-conductance potassium channels of the SLO family. *Nat. Rev. Neurosci.* **7**, 921–931 (2006).
47. Rozov, A. & Burnashev, N. Polyamine-dependent facilitation of postsynaptic AMPA receptors counteracts paired-pulse depression. *Nature* **401**, 594–598 (1999).
48. Ibata, K., Sun, Q. & Turrigiano, G.G. Rapid synaptic scaling induced by changes in postsynaptic firing. *Neuron* **57**, 819–826 (2008).

ONLINE METHODS

The fox-urine exposure experiments were carried out as described previously[49]. We placed 18–23-d-old C57BL/6J mice in a cage (13 × 9 × 6 inches) for 3 min as a control. A paper towel soaked with fox urine (5 ml) was then inserted below the raised floor containing small holes at regular intervals that allow the odor to permeate into the chamber. The activities of mice were monitored with an infrared CCD camera for the entire 8-min period at a 5-Hz frame rate and stored in a computer. The movement of the experimental animal was characterized by the amount of motion that occurs between two successive frames, using a custom-written program. To determine the threshold in significant motion pixels, a lab member blind to the experimental conditions scored freezing responses. If 'movement' in a 1-s period remained below the threshold, it was considered to be a freezing episode[49]. The duration of freezing was determined and the total cumulative freezing time during each period was used to calculate the percentage freezing for both the control and stress periods. In some experiments, mice were injected with propranolol (20 mg per kg of body weight) or saline (intraperitoneal injection) 15–30 min before entering the cage. Care was taken to minimize the handling stress of the mice.

Cerebellar slices (250 μm) were obtained from 18–23-d-old C57BL/6J mice as described previously[50]. In some experiments, slices were incubated in artificial cerebrospinal fluid (ACSF; 125 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4 and 25 mM glucose, saturated with 95% O_2/5% CO_2, pH 7.3) that contained 1 mM (or 5 mM) kynurenic acid, 100 μM picrotoxin and 10 μM noradrenaline or other inhibitors (as noted) for 3 h, unless otherwise indicated. Noradrenaline and picrotoxin treatments were performed at 36 °C and TEA treatment at 36 °C (**Fig. 4c**) and at 22–25 °C (**Fig. 4**). In several experiments, 1 mM MCPG and 10 μM SCH50911 were also included during TEA treatments to block mGluRs and $GABA_B$Rs.

Electrophysiology. Whole-cell voltage and current-clamp recordings were made with Multiclamp 700A and Axopatch 200B amplifiers (Axon Instruments). Synaptic currents, Ca^{2+} currents and action potentials were filtered at 2–5 kHz and digitized at 20 kHz.

Spontaneous excitatory postsynaptic currents were recorded from stellate cells using a cesium-based pipette solution (135 mM CsCl, 10 mM HEPES, 10 mM EGTA, 2 mM NaCl, 4 mM ATP-Mg, 5 mM TEA, 1 mM QX314 and 0.1 mM spermine, pH 7.3) in ACSF containing 100 μM picrotoxin at 22–25 °C, unless otherwise noted. Inclusion of spermine in the whole-cell pipette solution blocked AMPARs lacking GluR2 subunits (not GluR2-containing receptors) at positive potentials, producing a characteristic inwardly rectifying *I-V* relationship, and was therefore used to determine the subunit composition of synaptic receptors. To obtain an *I-V* relationship of synaptic currents, we recorded spontaneous EPSCs at several potentials. Average sEPSCs at each holding potential (typically average of 50–100 events) were measured using N version 4.0 (written by S. Traynelis, Emory University) as described previously[29]. The reversal potential was extrapolated from linear fitting of the currents at negative potentials and was about 0 pA (1.2 ± 0.4 pA, $n = 78$). We also monitored sEPSCs at 0 mV and did not detected synaptic currents. The rectification index of the *I-V* relationship was defined as the ratio of the current amplitude at +40 mV to the predicted linear value at +40 mV (extrapolated from linear fitting of the currents at negative potentials), unless otherwise noted. When the currents at positive potentials show strong inward rectification, solid lines were drawn to connect the data points at positive potentials. In a few experiments, the ratio of EPSCs at +40 mV to −60 mV ($R_{+40mV/-60mV}$) was measured and the rectification index was calculated as $1.5R_{+40mV/-60mV}$, assuming that the reversal potential is 0 mV. In some experiments, EPSCs were minimally evoked with a patch electrode containing external solution by stimulating in the molecular layer (100–140-μs pulses of 2–13 V at 0.33 Hz). CNQX-induced currents were recorded in ACSF that contained picrotoxin (100 μM), D-AP5 (50 μM), strychnine (1 μM) and TTX (0.5 μM) to block $GABA_A$, NMDA and glycine receptors, and Na^+ channels.

Spontaneous action potentials were recorded using a perforated or whole-cell patch configuration in ACSF that contained 1 mM kynurenic acid or 100 μM picrotoxin (to block ionotropic glutamatergic or inhibitory transmission, respectively). The pipette solution contained 115 mM $KMeSO_3$, 10 mM NaCl, 2 mM $MgCl_2$, 0.16 mM $CaCl_2$, 0.5 mM EGTA, 10 mM HEPES, 4 mM ATP-Na, 0.4 mM GTP-Na and 14 mM $Tris_2$-creatine phosphate (0.66 mg ml^{-1} amphotericin B for perforated patch recordings), pH 7.3. The frequency of spontaneous action potentials were recorded using a cell-attached configuration with a glass electrode filled with ACSF. The effect of noradrenaline and picrotoxin was determined at 36 °C and that of TEA at 22–25 °C.

Ca^{2+} currents evoked by single action potentials were determined using a voltage-clamp protocol that mimicked the action potential waveform at 36 °C. The waveforms of both the control action potential and the action potential in the presence of 10 μM noradrenaline were recorded in current clamp from a stellate cell. The waveforms of the average control action potential and noradrenaline action potential had action potential half widths of 0.8 ms and 1.3 ms and after-hyperpolarizations of −40 mV and −35 mV, respectively. They were therefore used as voltage commands. The pipette solution contained 119 mM CsCl, 9 mM EGTA, 10 mM HEPES, 1.8 mM $MgCl_2$, 14 mM Tris-creatine phosphate, 4 mM ATP-Mg, 0.4 mM GTP-Na, 10 mM TEA and 1 mM QX314, pH 7.3. The external solution included 10 mM TEA, 300 nM TTX, 10 μM ZD7288, 1 mM kynurenic acid and 100 μM picrotoxin to block potassium, sodium, h-currents and synaptic currents, respectively. Cd^{2+} (100 μM), a general Ca^{2+} channel blocker, was used to block Ca^{2+} channels. The Ca^{2+} current was monitored as the difference current ($I - I_{Cd}$). Ca^{2+} currents were measured in stellate cells before any treatments.

To stimulate parallel fiber inputs, we placed a parallel bipolar electrode (150-μm spacing) across the molecular layer about 200 μm from the recording electrode. The stimulus intensity ranged from 3–13 V with a stimulus duration of 120–200 μs. The stimulation protocol contained 100 sweeps of four depolarizations at 50 Hz, with a 2-s interval between two sweeps and 20 s between every ten sweeps. sEPSCs were recorded from a stellate cell before and after the simulation, followed by recordings from neighboring stellate cells. To test the effects of actinomycin D on parallel fiber stimulation–induced change, we applied ACSF that contained 25 μM actinomycin D 30 min before the recording of the first cell and parallel fiber stimulation, and continued to apply it throughout the experiment. The experiments were performed at 36 °C.

Real-time single-cell RT-PCR. Horizontal cerebellar slices (350 μm) were obtained from 18–23-d-old GAD65–GFP mice. The cerebellar cortices that were isolated from slices were incubated in extracellular solution (135 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES and 11 mM glucose, pH adjusted to 7.3 with 1 M NaOH) containing 32 U ml^{-1} papain at 37 °C for 40–50 min, washed and then triturated through a glass pipette. Individual GFP-positive cells with a diameter of 6–10 μm were picked up using a fire-polished glass electrode (that was filled with DEPC treated water containing 0.5 U RNase inhibitor) under visual guidance. The cell content was then injected into the solution (10 μl) prepared for reverse transcription (high-capacity cDNA RT kit, Applied Biosystems). The probes for GluR1, GluR2 and GluR3 (TagMan gene expression assays) were obtained from Applied Biosystems. Pre-amplification of cDNA before quantification by qPCR was performed (10 min at 95 °C, followed by 14 cycles of 15 s at 95 °C and one min at 60 °C). One-fifth of the reaction was removed and added to the qPCR mix for quantification. Two house keeping protein genes, *Gapdh* and *Ppia* (encoding cyclophilin a), were tested and showed no detectable change following fox urine exposure, and were therefore used in our experiments. Cerebellar total RNA was used as positive control. Negative control samples include bath solution without cells and omission of reverse transcription and both showed no detectable signals. Real-time PCR was performed using the ABI 7300 Sequence Detection System (Applied Biosystems) at the Genome Core Facility of the Huck Institutes for the Life Sciences, Pennsylvania State University.

***In situ* hybridization.** Transverse cerebellar slices (400 μm) from P18–21 Sprague Dawley rats were incubated with noradrenaline or TEA and inhibitors in ACSF for 3 h as described above. After incubation, slices were chilled at 4 °C, embedded, and 20-μm slices were cut by cryostat (Shandon) and fixed with paraformaldehyde (4% for 30 min, wt/vol). A digoxigenin (DIG)-labeled RNA probe was prepared by *in vitro* transcription of GluR1 and GluR2 cDNAs with T3 and T7 RNA polymerases in the presence of DIG-labeled UTP (Roche Molecular Biochemicals). *In situ* hybridization was performed as described[40]. Signals were detected by immunocytochemistry with an alkaline phosphatase–conjugated antibody to DIG, according to the DIG RNA Detection kit (Roche Molecular Biochemicals). For each treatment condition, several areas (5–10) of the molecular layer (100 × 160 μm area) of the vermis (lobules IV to VIII) from multiple slides were sampled. Areas with labeling that was more intense than background in an outline of the soma of typical stellate cells (a circle of 10 μm diameter) were selected using

doi:10.1038/nn.2474

ImageJ (version 1.38x, US National Institutes of Health) by the person blind to the experimental condition. The background was measured by selecting 14–32 (average, 24 ± 4) cell-free areas from each image. *GluR1* was used as a control. Both the labeling and subsequent quantification were carried out in a blind and unbiased manner.

Immunocytochemistry. Transverse cerebellar slices (400 μm thick) were prepared and incubated in the absence or presence of noradrenaline or TEA and inhibitors as described above. After incubation, slices were fixed with 4% paraformaldehyde and rinsed in phosphate-buffered saline (PBS, 0.03 M phosphate buffer with 0.9% NaCl, wt/vol). After fixation, slices were cut into sections (20 μm) with a cryostat (Shandon). The sections were washed thoroughly in PBS and then blocked for 2 h in PBS containing 10% normal goat serum (vol/vol), 0.5% bovine serum albumin (BSA wt/vol) and 0.2% saponin (20 mg per 10 ml of PBS). Sections were incubated overnight at 4 °C with a monoclonal antibody to parvalbumin (1:200). After washing three times with PBS containing 0.01% saponin, slices were incubated for 1 h in secondary antibody, Alexa Fluor 488 goat antibody to mouse IgG (1:200) in PBS containing 0.01% saponin. After washing in PBS, slices were mounted with Prolong-Gold and images were acquired with a FluoView 1000 confocal microscope (Olympus). All values are expressed as mean ± s.e.m. Statistical significance was assessed by a two-tailed Student's *t* test or ANOVA test.

49. Kopec, C.D. *et al.* A robust automated method to analyze rodent motion during fear conditioning. *Neuropharmacology* **52**, 228–233 (2007).
50. Liu, S.J. & Lachamp, P. The activation of excitatory glutamate receptors evokes a long-lasting increase in the release of GABA from cerebellar stellate cells. *J. Neurosci.* **26**, 9332–9339 (2006).