Abstract: Synapses throughout the brain are modified through associative mechanisms in which one input provides an instructive signal for changes in the strength of a second co-activated input. In cerebellar Purkinje cells, climbing fiber synapses provide an instructive signal for plasticity at parallel fiber synapses. Here we show that noradrenaline activates α2-adrenergic receptors to control short-term and long-term associative plasticity of parallel fiber synapses. This regulation of plasticity does not reflect a conventional direct modulation of the postsynaptic Purkinje cell or presynaptic parallel fibers. Instead, noradrenaline reduces associative plasticity by selectively decreasing the probability of release at the climbing fiber synapse, which in turn decreases climbing fiber-evoked dendritic calcium signals. These findings raise the possibility that targeted presynaptic modulation of instructive synapses could provide a general mechanism for dynamic context-dependent modulation of associative plasticity.
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Opposed Reviewers:
March 14, 2008

Dear Katja,

Megan and I have submitted a manuscript entitled “Noradrenergic control of associative synaptic plasticity by selective modulation of instructive signals” to be considered for publication as a report in Neuron. A great deal is known about how the induction of associative plasticity is controlled by postsynaptic spiking as well as the presynaptic and postsynaptic properties of the synapses being modified. Much less is known about whether associative plasticity can be controlled by modulating instructive synaptic inputs. We addressed this question in Purkinje cells, which are particularly well suited to studying the role of instructive synapses because climbing fiber synapses provide an instructive signal for plasticity at parallel fiber synapses. We found that noradrenaline reduces associative plasticity by selectively decreasing the probability of release at the climbing fiber synapse, which in turn decreases climbing fiber-evoked dendritic calcium signals. We are excited about these findings, which suggest that targeted presynaptic modulation of instructive synapses could be a general mechanism for dynamic context-dependent modulation of associative plasticity and learning.

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We ask that the manuscript not be sent to our friendly competitor Christian Hansel.

Sincerely Yours,

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Noradrenergic control of associative synaptic plasticity by selective modulation of instructive signals

Running title: Selective modulation of instructive signals

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Keywords: climbing fiber, Purkinje cell, cerebellum, α2-adrenergic receptors, LTD, endocannabinoids.
Synapses throughout the brain are modified through associative mechanisms in which one input provides an instructive signal for changes in the strength of a second co-activated input. In cerebellar Purkinje cells, climbing fiber synapses provide an instructive signal for plasticity at parallel fiber synapses. Here we show that noradrenaline activates α2-adrenergic receptors to control short-term and long-term associative plasticity of parallel fiber synapses. This regulation of plasticity does not reflect a conventional direct modulation of the postsynaptic Purkinje cell or presynaptic parallel fibers. Instead, noradrenaline reduces associative plasticity by selectively decreasing the probability of release at the climbing fiber synapse, which in turn decreases climbing fiber-evoked dendritic calcium signals. These findings raise the possibility that targeted presynaptic modulation of instructive synapses could provide a general mechanism for dynamic context-dependent modulation of associative plasticity.
Introduction

Associative synaptic plasticity is a candidate substrate for the formation of real-world associations (Hebb, 1949). Synaptic plasticity typically requires nearly coincident activation of a presynaptic input with postsynaptic events including depolarization and elevation of dendritic calcium (Bi and Poo, 2001; Bliss and Collingridge, 1993). Induction of associative plasticity is often triggered by instructive synaptic inputs that influence the state of the postsynaptic cell (Blair et al., 2001; Dudman et al., 2007; Ito, 2001). A great deal is known about how postsynaptic spiking, and the presynaptic and postsynaptic properties of the synapses being modified, control the induction of associative plasticity (Duguid and Sjostrom, 2006; Malenka and Bear, 2004; Nicoll, 2003; Seol et al., 2007). Much less is known about whether associative plasticity can be controlled by modulating instructive synaptic inputs.

Cerebellar Purkinje cells (PCs) are well suited to studying the role of instructive synapses in the regulation of associative plasticity. PCs receive two very different classes of excitatory inputs: weak synaptic inputs from roughly 100,000 granule cell parallel fibers (PFs) (Eccles et al., 1966b), and a strong synaptic input from a single climbing fiber (CF) (Eccles et al., 1966a). The CF provides an important instructive signal that controls the induction of associative plasticity at the PF synapse and which is thought to be important for motor learning (Gilbert and Thach, 1977; Kitazawa et al., 1998; Raymond and Lisberger, 1998). Activation of the CF synapse elicits a characteristic postsynaptic complex spike that elevates calcium throughout PC dendrites (Schmolesky et al., 2002). Activation of PFs followed by complex spikes within several hundred milliseconds leads to rapid synaptic suppression resulting from endocannabinoid release from PCs and retrograde activation of type 1 cannabinoid (CB1) receptors (Brenowitz and Regehr, 2005). Repetition of this stimulus for minutes induces cerebellar long-term depression (LTD) of PF synapses onto PCs (Ito, 2001; Safo and Regehr, 2005). Previous studies have suggested that altering the strength of CF inputs to PCs can provide a way to control the induction of associative plasticity (Coesmans et al., 2004), but the circumstances under which such regulation might occur remain unclear.

The cerebellum receives monoaminergic inputs from neuromodulatory centers throughout the brain. They, together with mossy fibers and CFs, comprise the three classes of cerebellar afferent input, and are a relatively poorly understood element of the cerebellar circuitry (Schweighofer et al., 2004). Anatomical studies indicate that noradrenergic fibers
originate in the locus coeruleus and course through all layers of the cerebellar cortex (Bloom et al., 1971; Hokfelt and Fuxe, 1969; Kimoto et al., 1978; Olson and Fuxe, 1971; Schroeter et al., 2000), forming varicosities closely apposed to PC dendrites (Landis and Bloom, 1975). Noradrenergic inhibition of PCs can be elicited through electrical stimulation of the locus coeruleus in vivo (Siggins et al., 1971b).

Here we ask whether neuromodulation can control the induction of associative plasticity through selective regulation of instructive signals conveyed to PCs by CFs. We find that noradrenaline acts through α2-adrenergic receptors to decrease the probability of release at the CF synapse. This in turn decreases CF-evoked dendritic calcium transients and interferes with the induction of short-term and long-term associative plasticity of PF synapses. We conclude that noradrenaline controls synaptic plasticity of PF synapses through selective regulation of instructive signals, thereby providing a mechanism that could allow for dynamic, context-dependent regulation of learning.

Results

Noradrenaline alters complex spike waveform and associated calcium elevation

We tested the effects of noradrenaline (NA) on CF-evoked responses in PCs. Whole-cell current-clamp recordings were made from PCs with a potassium-based internal solution containing the fluorescent calcium indicator fura-2 (200 µM). An electrode placed in the granule cell layer stimulated CFs and elicited characteristic PC complex spikes (Fig. 1A, black trace), which involve widespread depolarization and the activation of calcium, sodium, and potassium conductances (Schmolesky et al., 2002). As shown for a representative experiment, we found that noradrenaline (5 μM) had clear effects on the complex spike waveform, decreasing the number of evoked spikelets (Fig. 1A, gray trace). Simultaneous calcium imaging revealed that CF activation transiently elevated calcium throughout PC dendrites (Fig. 1B, black trace), and that NA reduced the CF-evoked dendritic calcium transient (Fig. 1B, gray trace). Across cells, NA significantly reduced the number of spikelets (Fig. 1C, left, n=6, p < 0.01, paired T-test) and reduced PC dendritic calcium transients by 36±8% (Fig. 1C, middle, n=6 paired T-test p < 0.05) (n=6,).
NA decreases release probability at CF synapses by activating α2-adrenergic receptors

The alteration in CF-evoked responses by noradrenaline could reflect either changes in the properties of the CF synapse or changes in any of the active conductances in PCs that generate the complex spike. We examined synaptic responses by making whole-cell voltage-clamp recordings with a Cs-based internal solution to minimize the contributions of active postsynaptic conductances. CFs were stimulated by pairs of stimuli separated by 30 ms.

In control conditions, CF-EPSCs exhibited marked paired-pulse depression as previously described (Eccles et al., 1966a). We found that NA (5 μM) preferentially reduced the amplitude of the first of the pair of CF-EPSCs (Fig. 1D, gray trace), increasing the paired-pulse ratio by 35±3% (n=6, p<0.01). This decrease in paired-pulse depression is consistent with NA acting presynaptically to decrease the probability of release at the CF synapse (Foster and Regehr, 2004; Kreitzer and Regehr, 2001; Maejima et al., 2001; Takahashi and Linden, 2000; Wadiche and Jahr, 2001).

We used selective agonists and antagonists to pharmacologically characterize the involvement of various adrenergic receptors in modulating CF-EPSCs. α1, α2, and β adrenergic receptors are expressed in cerebellum (Nicholas et al., 1996). Previous studies have shown that β adrenergic receptors modulate PC output through direct postsynaptic effects on PCs (Hoffer et al., 1971; Siggins et al., 1971a) as well as through the augmentation of GABAergic inhibition of PCs (Mitoma and Konishi, 1999; Yeh and Woodward, 1983). We found that the α2-receptor antagonist yohimbine reversed the effects of noradrenaline on the amplitude and paired-pulse ratio of CF-EPSCs (Fig. 1D).

Additionally, the α2-receptor agonist UK14304 mimicked the effects of noradrenaline (Fig. 1D). UK14304 reduced EPSC amplitude and paired-pulse depression to a similar extent as NA (Fig. 1E, Supplementary Fig. 1, 36±4% increase in PPR, n=5, p=.87). In contrast, application of the α1 and β-adrenergic receptor agonists phenylephrine and isoproterenol (10 μM each) did not affect EPSC amplitude or paired-pulse ratio (Supplementary Fig. 1). Thus, the decrease in probability of release at CF synapses by noradrenaline is mediated by α2-adrenergic receptors.

We next asked whether the effects of noradrenaline on CF-evoked complex spikes and dendritic calcium transients could be accounted for by presynaptic modulation of the CF synapse. We found that the effects of noradrenaline on complex spikes (Fig. 1C, left) and
postsynaptic calcium transients were also reversed by yohimbine (Fig. 1C, middle), indicating that they, like the effects on CF-EPSCs, were mediated by α2-receptors. To assess possible postsynaptic effects of α2-receptors (Nicholas et al., 1996; Scheinin et al., 1994), which are coupled to $G_{i/o}$ type G proteins (Hein, 2006), we substituted the non-hydrolyzable GTPγS for the GTP in our internal solution. GTPγS did not affect the ability of noradrenaline to modulate CF-evoked calcium transients (42±17% reduction with GTPγS, $n=4$, $p=.73$; Fig. 1C, middle). However, it blocked the endocannabinoid-mediated suppression of CF synapses triggered by the mGluR1 agonist DHPG that is known to require postsynaptic G-proteins (Fig. 1C right, $n=3$, Maejima et al., 2001). Thus, the effects of noradrenaline on CF-evoked complex spikes and dendritic calcium transients appear to result from a presynaptic decrease in transmitter release probability mediated by α2-receptors.

Neuromodulators can either act directly or indirectly to modulate transmission (Maejima et al., 2001; Varma et al., 2001; Vogt and Regehr, 2001). We therefore determined whether activation of α2-adrenergic receptors indirectly modulated release at CF synapses through other signaling systems and receptors (Kreitzer and Regehr, 2001; Kulik et al., 1999; Takahashi et al., 1995). Coapplication of antagonists of type II mGluRs, GABA$_B$Rs, adenosine A$_1$Rs, and cannabinoid CB1Rs (MCPG, 500 μM; CGP 55845A, 2 μM; DPCPX, 5 μM; AM251, 5 μM) did not affect the ability of NA to decrease the EPSC and alter paired-pulse plasticity (39±13% increase in PPR, $n=4$, $p=.72$; Supplementary Fig. 1). This indicates that noradrenaline does not act indirectly in a manner that requires the activation of any of these signaling systems and is consistent with noradrenaline decreasing the probability of release by acting directly on CF terminals. Such a decrease in transmitter release is a well-known function of α2-adrenergic receptors (Bertolino et al., 1997; Delaney et al., 2007; Hein, 2006; Langer, 1977; Leao and Von Gersdorff, 2002).

**Neuromodulation of the CF synapse disrupts short-term associative plasticity**

CFs play a central role in inducing associative synaptic plasticity in PCs (Ito, 2001). PF activity followed within a few hundred milliseconds by CF activation has been shown to induce short-term associative plasticity at PF synapses (Brenowitz and Regehr, 2005). This plasticity involves endocannabinoid release from PCs and activation of presynaptic CB1Rs, leading to the modulation of presynaptic calcium channels and the suppression of
presynaptic transmitter release at PF synapses (Brown et al., 2004; Kreitzer and Regehr, 2001).

We investigated whether α2-receptor-mediated modulation of the CF synapse could regulate short-term associative plasticity. We made whole cell current-clamp recordings from PCs at 34°C with a potassium-based internal solution. PF-EPSPs were measured with test pulses presented at 0.5 Hz before and after a conditioning train that consisted of a burst of PF-stimuli, either alone or followed by 3 CF-stimuli (Fig. 2A). The amplitudes of PF-EPSPs before and after the conditioning trains were compared. PF-only trains generally result in post-tetanic potentiation, a transient enhancement of PF-EPSPs (Beierlein et al., 2007; Zucker and Regehr, 2002). When the number of PF stimuli is increased, the enhancement can be overcome by endocannabinoid-mediated synaptic suppression (Brown et al., 2003). For these experiments, we adjusted the number of PF stimuli in the conditioning trains (3 to 7 stimuli) to produce minimal enhancement or suppression of the EPSP when presented alone (Fig. 2A inset, black vs. gray). In control conditions, PF+CF conditioning trains resulted in a transient 42±7% suppression of PF-EPSP amplitude (Fig. 2A and 2B, red), while PF-only conditioning trains did not (Fig. 2A and 2B, black).

We examined the effect of α2-adrenergic receptor activation on short-term associative plasticity (Fig. 2C and 2D). α2-adrenergic receptor activation did not affect the balance between short-term enhancement and suppression observed following presentation of PF-only conditioning trains (black), but significantly reduced the synaptic suppression observed following PF+CF trains (red), from 42±7% to 15±11% (n=7, p<0.05, paired T-test). The decrease in associativity caused by α2-receptor activation was reversed upon drug washout (Fig. 2E and 2F). Thus, the activation of α2-adrenergic receptors interfered specifically with the induction of associative short-term plasticity at PF-PC synapses.

The selective disruption of associative plasticity observed during activation of α2-receptors is consistent with its suppression of CF synapses (Fig. 1). However, there are two other possibilities. First, direct effects of noradrenaline on PF synapses could affect plasticity. Second, α2-receptors in PCs (Nicholas et al., 1996; Scheinin et al., 1994) might interfere with plasticity through postsynaptic actions such as regulation of dendritic excitability (Rancz and Hausser, 2006) that might only be revealed during stimulus conditions that cause plasticity.

We assessed the effects of α2-receptor activation on PF synapses in voltage-clamp with a Cs-based internal solution. We found that neither noradrenaline nor yohimbine
altered the amplitude or paired-pulse ratio of PF-EPSCs (Fig. 3A). This suggests that the
decrease in associative plasticity shown in Fig. 2 does not involve presynaptic regulation of
PF synapses.

Next, we repeated experiments similar to those in Fig. 2, but with conditioning trains
consisting of 10 PF stimuli, which are sufficient to evoke short-term suppression of
excitation (SSE, Fig. 3B). SSE is extremely similar to short-term associative plasticity
involving CF and PF activation, with the exception that additional PF stimuli obviate the
need for CF activation to evoke endocannabinoid release from the postsynaptic cell (Brown
et al., 2003). If the noradrenergic effects on associative short-term plasticity were due to
postsynaptic actions, then α2-receptor activation should affect SSE as well. For these
experiments, the internal recording solution was supplemented with 500 μM Fura-FF to
measure localized dendritic calcium transients in response to PF-trains.

We compared PC responses to PF-conditioning trains in control conditions and in
the presence of an α2-receptor agonist. As shown for a representative experiment, there
was no consistent change in the response to the conditioning train (Fig. 3B, black vs. red).
The magnitude of suppression following the conditioning train, and the local calcium signal
in PC dendrites during the conditioning train, were also unaffected (Fig. 3B, insets; Fig. 3C).
Summaries (n=4, Fig. 3D and 3E) show that the lack of effect was consistent across cells.
Thus, even though α2-adrenergic receptors are likely to be expressed both in the
presynaptic cells within the inferior olive that give rise to CF afferents (Strazielle et al., 1999;
Tavares et al., 1996; Wang et al., 1996) and postsynaptically in PCs (Nicholas et al., 1996;
Scheinin et al., 1994), we conclude that they interfere with the induction of associative
plasticity primarily through a decrease in the probability of release at instructive CF
synapses.

**Activation of α2-receptors interferes with the induction of PF-LTD**

CFs also play an instructive role in the induction of long-term associative plasticity in
PCs. The repeated coactivation of PFs and CFs leads to the induction of LTD at PF-PC
synapses (Ito, 2001). We asked whether modulation of the CF synapse by α2-receptor
activation could regulate the induction of PF-LTD. The instructive signal provided by the CF
is necessary for the induction of LTD for most induction protocols, including the one used
here (Safo and Regehr, 2005): PF activation (10 at 100Hz) followed by CF activation (2 at
20 Hz) repeated every 10 s for 5 minutes (Fig. 4A and 4B).
PF-EPSP amplitudes were monitored with test pulses presented at 0.1 Hz before and after the induction protocol as shown for two representative experiments (Fig. 4A and 4B, insets). In control conditions the induction protocol resulted in long-term depression of PF-EPSP amplitude (Fig. 4C, black). In the presence of an α2-receptor agonist (Fig. 4B), the conditioning trains produced similar responses in PCs, but did not result in LTD (Fig. 4B, inset). Across cells the long-term changes in PF-EPSPs were more variable and significantly less likely to result in LTD in the presence of an α2-receptor agonist than in control conditions (p<0.05, Fig. 4C and 4D), indicating that activation of α2-receptors disrupts the induction of associative long-term depression at PF-PC synapses.

Discussion

Here we have shown that noradrenaline controls PC associative synaptic plasticity through activation of α2-adrenergic receptors. Activation of these receptors potently modulates CF-PC synapses without directly affecting PF-PC synapses. A decrease in release probability at the CF-PC synapse reduces CF-evoked postsynaptic dendritic calcium transients and interferes with the induction of short- and long-term associative, but not non-associative, plasticity at PF-PC synapses. We conclude that noradrenaline controls the induction of associative plasticity through a targeted modulation of instructive synapses.

It had not been clear whether regulation of CF synapses could provide a means of specifically and dynamically controlling the induction of associative plasticity at PF synapses. A prior study found that neuromodulators had little effect on the CF synapse (Pisani and Ross, 1999). Although some modulators of CF synapses have been identified, they also directly suppress PF synapses and are not suited to the selective regulation of associative plasticity (Takahashi and Linden, 2000). However, it had been found that long-term reductions in the strength of the CF synapse reduce CF-evoked postsynaptic calcium transients and interfere with the induction of parallel fiber LTD (Coesmans et al., 2004; Hansel and Linden, 2000; Weber et al., 2003). Those studies were the first to demonstrate that regulation of CF-PC synapses could have important consequences for the induction of associative plasticity. However, they required stimulation at 5 Hz for 30 seconds to induce LTD, which is outside the 1-2 Hz range observed in vivo (Gilbert and Thach, 1977; Raymond and Lisberger, 1998). The selective modulation of instructive signals by noradrenaline observed here raises the possibility that activity in locus coeruleus neurons
could dynamically regulate associative plasticity based on behavioral context (Aston-Jones and Cohen, 2005).

Manipulation of noradrenergic inputs to the cerebellum has been shown to interfere with cerebellum-dependent motor learning (Keller and Smith, 1983; McCormick and Thompson, 1982; Pompeiano, 1998; Watson and McElligott, 1984). These findings have generally been attributed to previously-described inhibitory effects of noradrenaline on PC firing (Cartford et al., 2004; Gilbert, 1975; Schweighofer et al., 2004). Our results suggest that dysregulation of the cerebellar noradrenergic system could disrupt motor learning by interfering with CF control of plasticity at the PF-PC synapse.

Our findings establish a new way by which modulatory systems can regulate endocannabinoid-mediated mechanisms of associative plasticity. As is the case for short- and long-term plasticity of PF-PC synapses, many forms of associative plasticity throughout the brain are mediated by endocannabinoids (Chevaleyre et al., 2006). Endocannabinoid release is directly regulated by Gq-coupled receptors such as group I metabotropic glutamate receptors, oxytocin receptors and some types of muscarinic receptors (Kim et al., 2002; Maejima et al., 2001; Oliet et al., 2007). Here, we find that noradrenaline also interacts with the cannabinoid signaling system, but that the coupling is indirect and arises from modulation of the instructive signal that gates endocannabinoid release by controlling dendritic calcium levels.

The noradrenergic regulation of CF-PC synapses described here is a particularly clear example of how an instructive synapse can regulate associative plasticity. Many other types of neurons also receive anatomically distinct classes of excitatory inputs. For example, thalamic neurons receive sensory input and cortical feedback, CA3 pyramidal cells receive mossy fiber inputs and associational/commissural inputs, CA1 pyramidal cells receive perforant path and Schaffer collateral inputs and cortical cells receive thalamic inputs and recurrent excitatory collaterals (Amitai, 2001; Dudman et al., 2007; Jones, 2002; Sillito et al., 2006; Zalutsky and Nicoll, 1990). In many cases it is thought that one class of synapse can serve as an instructive signal for associative plasticity (Blair et al., 2001; Dudman et al., 2007). Moreover, different inputs are often selectively modulated (Giocomo and Hasselmo, 2007). Thus, targeted regulation of instructive signals by neuromodulators could provide a general mechanism for dynamic regulation of associative plasticity.
Experimental procedures

All animal procedures were approved by the Harvard Medical Area Standing Committee on Animals. Parasagittal cerebellar slices, 250 μm thick, were cut from the vermis of 13-19 day-old Sprague-Dawley rats as described previously (Brenowitz and Regehr, 2003; Brenowitz and Regehr, 2005). The extracellular ACSF contained: 125 mM NaCl, 26 mM NaHCO3, 25 mM glucose, 2.5 mM KCl, 1.25 mM NaH2PO4, 1 mM MgCl2, 2 mM CaCl2, and was bubbled with 95% O2/5% CO2.

Drugs were bath applied. NBQX, picrotoxin, UK14304, yohimbine, DHPG, AM251, CGP55845A, DPCPX, MCPG, phenylephrine, and isoproterenol were purchased from Tocris Bioscience (Ellisville, MO). Fura-2 and fura-FF were purchased from Invitrogen (Carlsbad, CA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Statistical significance was assessed with unpaired Student’s T-tests except where noted. Data are presented as mean±SEM.

Electrophysiology

Voltage clamp. Whole-cell voltage-clamp recordings were performed in PCs at room temperature using a Multiclamp 700B (Axon Instruments/ Molecular Devices, Union City, CA) and glass electrodes (1–2 MΩ) filled with an internal solution consisting of: 35 mM CsF, 100 mM CsCl, 10 mM EGTA, 10 mM HEPES. Bicuculline (20 μM) was added to the ACSF to block inhibitory currents. NBQX (250-350 nM) was included in the external solution to reduce the amplitude of the CF-EPSC and minimize voltage-clamp errors. For experiments testing the effects of GTPγS in blocking the suppression of CF synapses by DHPG, the internal solution consisted of 145 mM CsMeSO4, 15 mM HEPES, 0.2 mM EGTA, 1 mM MgCl2, 5 mM TEA-Cl, 2 mM Mg-ATP, 10 mM Phosphocreatine (tris), 2 mM QX-314, and either 0.4 mM Na-GTP or 1 mM GTPγS.

Current clamp. Recordings were performed at 34°C in ACSF containing picrotoxin (20 μM) to block inhibitory currents. CGP55845A (2 μM) was added to the ACSF for experiments in which high frequency stimulus trains were presented. Glass electrodes (2–3 MΩ) were filled with an internal solution containing: 120 mM KMeSO3, 5 mM NaCl, 2 mM MgCl2, 0.05 mM CaCl2, 0.1 mM EGTA, 10 mM HEPES, 2 mM Na2ATP, 0.4 mM NaGTP, 14 mM tris-creatine phosphate (pH 7.3). For calcium imaging experiments using Fura-2, EGTA was omitted. In some experiments GTP was replaced with GTPγS (1 mM). Small hyperpolarizing currents
were injected to prevent spontaneous spiking and maintain the resting membrane potential at a constant level throughout each experiment. Hyperpolarization was reduced during conditioning trains to permit robust spiking in response to PF stimulation. 13 of the 25 total LTD experiments were performed with the experimenter blind to the drug treatment.

**Calcium imaging**

Imaging was carried out as previously described (Brenowitz and Regehr, 2003; Brenowitz and Regehr, 2005). The ratiometric calcium indicators Fura-2 (200 μM, Fig. 1) or Fura-FF (500 μM, Fig. 3) were added to the intracellular solution to measure postsynaptic calcium transients. Images were acquired at 50 Hz with 383 nm excitation, beginning 150-250 ms prior to the onset of CF stimuli or conditioning trains. Images with excitation at the isosbestic point of Fura-2 (360 nm) or Fura-FF (357 nm) were taken immediately before and after 383 nm excitation. Fluorescence ratios were converted to calcium concentrations using a value for the $K_D$ of 131 nM for Fura-2 and 7.7 μM for Fura-FF (Brenowitz and Regehr, 2003; Grynkiewicz et al., 1985).
Figure Legends

Figure 1. Noradrenaline alters climbing fiber-evoked complex spikes, associated Purkinje cell dendritic calcium signals, and release probability at the CF-PC synapse through activation of α2-adrenergic receptors.
(A, B) A representative experiment is shown in which a CF was stimulated and the resulting complex spike waveforms (A) and dendritic calcium transients (B) were simultaneously recorded from a PC in control conditions (black) and in the presence of 5 μM noradrenaline (gray). (C, left) Summary (n = 6 PCs) of noradrenergic effects on complex spike waveforms. (C, middle) Effects of noradrenaline (NA) on dendritic calcium transients in the same PCs. Peak calcium transients in the presence of NA, following subsequent addition of the α2-adrenergic receptor antagonist yohimbine (15 μM), and in the presence of NA with GTPγS (n=4) included in the internal recording solution are each shown normalized to control conditions. NA caused a decrease in CF-evoked calcium elevation that was reversed by coapplication of yohimbine but unaffected by GTPγS. (C, right) DHPG caused a reduction in CF-EPSC that was prevented by GTPγS. (D,E) Voltage-clamp recordings of CF-EPSCs in PCs in response to pairs of CF stimuli separated by 30 ms. (D) EPSCs from a representative experiment are shown in control conditions (black) and in the presence of 5 μM NA (gray). Effects of NA and subsequent application of yohimbine (15 μM) on the first EPSC (filled circles) and paired-pulse ratio (open circles) are summarized at right (n=6). (E) Experiments similar to those in D are shown with the exception that the α2-receptor agonist UK14304 (15 μM) is used rather than NA (n=5).

Figure 2. α2-receptor activation disrupts associative short-term plasticity.
PF-EPSPs were measured with test pulses presented at 0.5 Hz before and after conditioning trains consisting of either stimulation of PFs only (black) or PFs and CFs (red). (A,C,E) Responses of a PC to a PF-only train (left) and PF+CF trains (right, red) are shown for control conditions (A), in the presence of the α2 agonist UK14304 (15 μM, C), and after drug washout (E). Vertical lines beneath traces indicate timing of PF and CF stimuli. Insets display the PF-EPSPs measured in response to test pulses before (dark) and after (light) conditioning trains. (B,D,F) Summary (n=7 PCs). Average PF EPSP amplitudes in response
to test pulses are shown, normalized to the average EPSP amplitude before the conditioning train. PF+CF trains resulted in greater suppression of PF-EPSP amplitude than PF-only trains. The suppression resulting from PF+CF trains was selectively reduced by UK14304. Error bars indicate ± SEM.

**Figure 3.** Parallel fiber synapses are unaffected by α2 receptor activation. (A) PFs were activated with a pair of stimuli separated by 30 ms and the resulting EPSCs were recorded from PCs in voltage-clamp with a Cs-based internal solution in control conditions, in the presence of noradrenaline (50 μM), and in the presence of both noradrenaline and 15 μM yohimbine (n=5). Responses are normalized to the average amplitude of the first EPSC, before drug application. (B and C) A representative experiment is shown in which PFs were activated with test pulses presented at 0.5 Hz and the resulting PF-EPSPs were recorded from PCs in current-clamp with a K-based internal solution. Conditioning trains consisted of 10 PF stimuli at 100 Hz. (B) Responses to the conditioning trains and PF-EPSPs before and after the conditioning trains (insets) were measured in control conditions (top, black) and in the presence of 15 μM UK14304 (bottom, red). (C) Local dendritic calcium signals were measured in response to the conditioning train. (D and E) Summary (n=4) shows the time course of PF-EPSP amplitude (D) and the amplitude of local dendritic calcium signals (E) in control conditions (black) and in the presence of UK14304 (red). Error bars indicate ± SEM.

**Figure 4.** Activation of α2-receptors interferes with the induction of associative LTD at PF-to-PC synapses. (A) A representative experiment is shown in which LTD was induced with a protocol consisting of a train of 10 PF stimuli at 100 Hz followed by 2 CF stimuli at 20 Hz. This conditioning train was repeated 30 times every 10 seconds. Vertical lines beneath traces indicate timing of PF (black) and CF (red) stimuli. (A, inset) Average PF-EPSPs measured for the 5 min. before (black) and 15-20 min. after (gray) the induction protocol. (B) Another representative experiment conducted in the presence of 15 μM UK14304 is shown. (C) Summary of the time course and amplitude of LTD in control conditions (n=13, black) and in the presence of UK14304 (n=12, red). Error bars indicate ± SEM. (D) Cumulative histogram of the ratio of EPSP amplitudes 6-30 min. after/0-10 min. before LTD induction.
References


Figure 1
Figure 2
Figure 3
Figure 4
Supplementary Figure 1.

Summary of effects on the first CF-EPSC (A) and paired-pulse ratio (B) of: noradrenaline; UK14304; the α1- and β-adrenergic receptor agonists phenylephrine and isoproterenol (10 µM each, n=6); and noradrenaline in the presence of antagonists for CB1Rs (AM251, 5 µM), adenosine A1Rs (DPCPX, 5 µM), type I/II mGluRs (MCPG, 500 µM), and GABABRs (CGP 55845A, 2 µM) (n=4). In (A), responses are normalized to the amplitude of the first EPSC in control conditions (dashed line). Control responses are shown in black, responses in the presence of adrenergic agonists are shown in gray, and agonists + yohimbine are hatched.