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Neuromodulators Control the Polarity of Spike-Timing-Dependent Synaptic Plasticity

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SUMMARY

Near coincidental pre- and postsynaptic action potentials induce associative long-term potentiation (LTP) or long-term depression (LTD), depending on the order of their timing. Here, we show that in visual cortex the rules of this spike-timing-dependent plasticity are not rigid, but shaped by neuromodulator receptors coupled to adenylyl cyclase (AC) and phospholipase C (PLC) signaling cascades. Activation of the AC and PLC cascades results in phosphorylation of postsynaptic glutamate receptors at sites that serve as specific "tags" for LTP and LTD. As a consequence, the outcome (i.e., whether LTP or LTD) of a given pattern of pre- and postsynaptic firing depends not only on the order of the timing, but also on the relative activation of neuromodulator receptors coupled to AC and PLC. These findings indicate that cholinergic and adrenergic neuromodulation associated with the behavioral state of the animal can control the gating and the polarity of cortical plasticity.

INTRODUCTION

Bidirectional modifications of cortical synapses through mechanisms like long-term potentiation (LTP) and long-term depression (LTD) are believed to be essential for the refinement of connectivity during development and memory storage in adults. Selective induction of LTP and LTD has been traditionally achieved by varying the presynaptic firing rate or the postsynaptic membrane potential during conditioning (Malenka and Bear, 2004). Recent studies indicate that near coincident pre- and postsynaptic firing also induces plasticity: LTP is induced when the presynaptic spike precedes postsynaptic firing, and LTD is induced when postsynaptic firing precedes the presynaptic spike (Bi and Poo, 1998; Feldman, 2000; Froemke and Dan, 2002; Fu et al., 2002; Markram et al., 1997; Sjostrom and Nelson, 2002). Because the timing between pre- and postsynaptic firing specifies synaptic change and polarity, spike-timing-dependent plasticity (STDP) has become an attractive mechanism to model naturally occurring plasticity, in particular experience-induced changes in the receptive field properties of cortical cells (Celikel et al., 2004; Froemke and Dan, 2002; Fu et al., 2002; Song and Abbott, 2001).

It is well established that STDP varies across synapses (Markram et al., 1997; Feldman, 2000), and even opposite STDP rules might apply for the same axons when contacting different cells (Tzounopoulos et al., 2007). Remarkably, the question of how the timing rules are dynamically regulated remains relatively unexplored. One study in CA1 synapses reported that β-adrenergic agonists increase the temporal window for spike-timing-dependent LTP (Lin et al., 2003). Indeed, neuromodulators are attractive candidates to regulate STDP, as they can control the biophysical properties of dendrites, including the dynamics of spike backpropagation (Sandler and Ross, 1999; Tsubokawa and Ross, 1997), and can influence the state of kinases and phosphatases implicated in synaptic plasticity. In addition, it is well established that experience-induced plasticity in cortex depends critically on neuromodulatory input (Bear and Singer, 1986; Kilgard and Merzenich, 1998). Some of these neuromodulators, like acetylcholine and norepinephrine, regulate and even gate the induction of cortical LTP and LTD with traditional conditioning protocols (Choi et al., 2005; Thomas et al., 1996). Therefore, we examined the role of neuromodulators in the induction of STDP in layer II/III pyramidal cells of the rodent visual cortex. Our findings indicate that multiple receptors coupled to adenylyl cyclase (AC) and phospholipase C (PLC) intracellular cascades control the polarity of STDP.

RESULTS

We studied postsynaptic responses in visual cortical layers II/III evoked by layer IV stimulation in brain slices

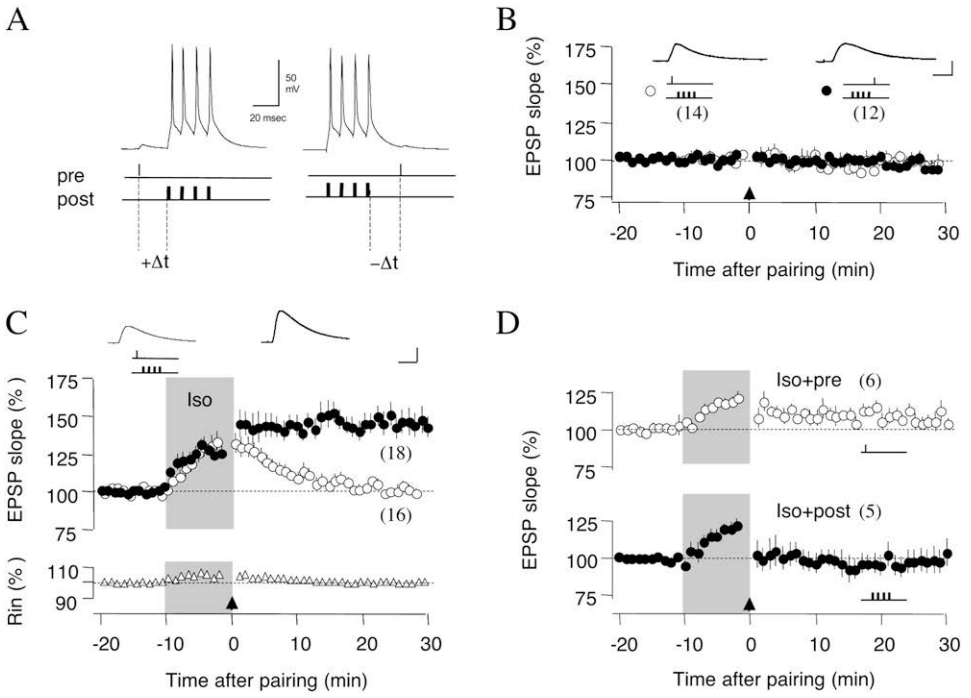

Figure 1. Selective Gating of Associative LTP by β-Adrenergic Receptor Agonists

(A) Associative plasticity induction paradigms: pre-post (left) and post-pre (right) stimulation pairings. (Top) Traces of EPSPs and action potentials; (bottom) stimulation schematics.

(B) In normal ACSF, pairing at +20 ms (open circles) or −20 ms (filled circles) does not induce lasting changes in the EPSPs.

(C) Bath application of the β-adrenergic agonist isoproterenol (Iso: 10 μM, gray box) reversibly increases EPSP slope (open circles) and allows induction of LTP with pairing at +20 ms (filled circles). Traces (top) are averages of ten responses recorded before (left) and 30 min. after the induction of LTP (right). Scale bars: 5 mV, 10 ms. (Bottom graph) Changes in input resistance (Rin) for the +20 ms pairing experiments.

(D) No LTP is induced when isoproterenol is applied with presynaptic activation (open circles) or postsynaptic firing alone (filled circles). The number of experiments in (A)–(D) is indicated in parentheses, the time of pairing is indicated by a black triangle.

All results are shown as averages ± SEM.

from 3-week-old rats. Spike-timing-dependent plasticity was induced in layer II/III cells by pairing presynaptic stimulation (in layer IV) with postsynaptic burst firing evoked by four brief (2 ms duration, 10 ms apart) suprathreshold current pulses (Figure 1A). These pairing epochs were delivered for 2 min at 1 Hz. As shown in Figure 1B, under our standard experimental conditions, these pairings induced no long-lasting changes in synaptic efficacy in the layer IV to layer II/III inputs in visual cortex. We observed no lasting changes when the presynaptic activation preceded the postsynaptic burst (+20 ms: 100.6% ± 2.8% of baseline at 30 min, n = 14, p = 0.864; +10 ms: 98.7%, n = 8, p = 0.170; +5 ms: 98.0% ± 2.5%, n = 6, p = 0.714) or when the postsynaptic burst preceded presynaptic activation (−20 ms: 95.6% ± 3.5%, n = 12, p = 0.175; −10 ms: 95.6% ± 5.7%, n = 7, p = 0.094). This absence of long-term changes in synaptic efficacy was surprising in light of reports of robust associative plasticity in other cortical synapses (Sjostrom et al., 2003). The differences in STDP could be due to differences in experimental conditions or in stimulation protocols. In any case, we exploited the fact that under our standard conditions associative pairings produce little change in synaptic transmission, which allowed us to unambiguously interpret results of synaptic changes induced with neuromodulators.

Receptors Coupled to the Adenylyl Cyclase Cascade Specifically Gate Associative LTP

We first studied the effects of activation of β-adrenergic receptors, which are coupled to the AC cascade and promote LTP (Lin et al., 2003; Thomas et al., 1996). We found that a brief bath application of the agonist isoproterenol (10 μM, 10 min) induced a transient potentiation of postsynaptic responses that was reversed after the drug was washed out of the bath (Figure 1C; 101.8% ± 2.4%, n = 16, p = 0.560). This transient potentiation was converted to persistent potentiation when an associative pairing stimulus (pre then post: +20 ms) was delivered at the end of the drug application (LTP: 144.7% ± 8.2%, n = 18, p < 0.001). No lasting changes were induced when isoproterenol was applied in conjunction with either postsynaptic firing or presynaptic activation alone (Figure 1 D), indicating that isoproterenol permits the induction of an associative form of LTP.

We next explored how the order of pre- and postsynaptic firing affected associative plasticity induced with

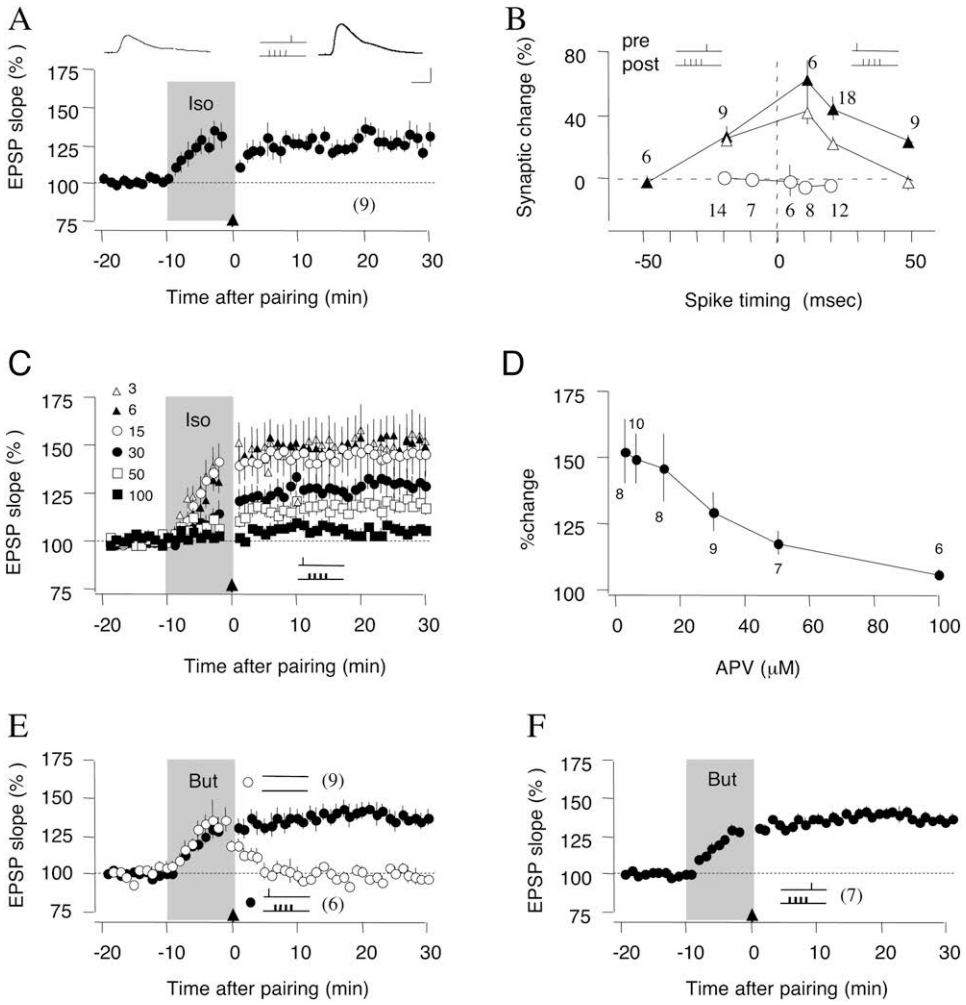

Figure 2. Receptors Coupled to the Adenylyl Cyclase Specifically Gate LTP

(A) Pairing in the reverse order (post then pre: −20 ms) after bath application of isoproterenol (gray box) results in the induction of LTP, not LTD.

(B) Summary of changes (30 min after conditioning) obtained by different time delays between pre- and postsynaptic spiking. No changes in control ACSF (open circle), only LTP in the presence 10 μM isoproterenol (filled triangles, bursts; open triangles, single action potentials). The number of experiments included in the analysis is indicated (C and D). Partial blockade of NMDAR reduces isoproterenol-promoted LTP, but does not produce LTD.

(C) Time course of the changes in the EPSP slope induced by +20 ms pairing at the end of 10 min application of isoproterenol in the presence of the indicated concentrations of APV (in μM).

(D) Summary graph showing that APV reduces associative LTP in a dose-dependent manner. The number of experiments included in the analysis is indicated.

(E and F) Bath application of the prostaglandin-E2 receptor agonist butaprost (But: 1 μM for 10 min, gray box) transiently enhances the EPSPs (open circles in [E]) and promotes induction of LTP with either pairing at +20 ms (filled circles in [E]) or −20 ms (F).

All results are shown as averages ± SEM.

isoproterenol. Surprisingly, reversing the order of pre- and postsynaptic stimulation during the pairing did not induce LTD as expected, but resulted in robust LTP (Figure 2A; post then pre at −20 ms: 126.4% ± 6.4%, n = 9, p = 0.002). Moreover, varying the timing between pre- and postsynaptic stimulation from −50 to + 50 ms in the presence of isoproterenol always produced LTP (Figure 2B; F [4,43] = 5.48, p = 0.0006). The absence of LTD was not a consequence of postsynaptic bursting because pairing presynaptic stimulation with a single postsynaptic action potential still resulted in LTP, regardless of the pre-post timing order (Figure 2B). We also considered the possibility that, in the presence of isoproterenol, the conditioning stimulation overactivated NMDA receptors beyond the range for LTD induction. However, blockade of NMDAR with varying concentrations of APV resulted in a dose-dependent reduction in LTP and still failed to induce LTD (Figures 2C and 2D), suggesting that NMDAR overactivation is unlikely to have prevented LTD. Finally, we explored the possibility that our whole-cell recording methods

prevented the induction of associative plasticity in normal ACSF and associative LTD in the presence of isoproterenol. To that end, we explored associative plasticity extracellularly in a two-input slice preparation (see Figure S1 in the Supplemental Data available with this article online) as described (Kirkwood and Bear, 1994). Associative plasticity of the layer II/III field potentials was attempted by pairing stimulation of one input (test input) with supramaximal activation of the other input (conditioning input) at different delays (see Experimental Procedures). Using this approach, we confirmed the principal findings obtained with intracellular methods: the associative pairing protocol induces LTP of the layer II/III field potentials only in the presence of isoproterenol, and the induction of associative LTP is independent of the timing relationship between the test and the conditioning pathway (Figure S1). Together, these results indicate that β-adrenergic activation selectively promotes associative LTP.

In vivo studies indicate that neuromodulatory systems can substitute for each other in supporting experience-dependent plasticity (Bear and Singer, 1986). Similarly, we have reported previously that different receptors coupled to the phospholipase C cascade can support LTD (Choi et al., 2005). Therefore, we asked whether other receptors that stimulate cAMP production also promote the induction of associative LTP. We tested prostaglandin E2 receptors, which have been recently implicated in the regulation of visual cortical LTP (Akaneya and Tsumoto, 2006). The results are shown in Figures 2E and 2F. Brief application of the PGE2 agonist butaprost (1 μM) causes a transient and reversible increase in the EPSPs (Figure 2E), but in conjunction with associative pairing, it permits the induction of LTP in a spike-timing-independent manner (+20 ms: 144.1% ± 5.4%, n = 6, $p < 0.001$; −20 ms: 135.3% ± 10.3%, n = 7, p = 0.011; Figures 2E and 2F). These results support the idea that receptors coupled to the adenylyl cyclase pathway specifically enable associative LTP.

Receptors Coupled to the Phospholipase C Cascade Specifically Gate Associative LTD

Activation of the adenylyl cyclase pathway specifically enables associative LTP, but not associative LTD. We have previously reported that activation of PLC is permissive for the induction of LTD with low-frequency stimulation and pairing protocols and that multiple PLC-coupled receptors can substitute each other in this function (Choi et al., 2005). Therefore, we tested whether stimulation of receptors coupled to the PLC cascade also enables associative LTD. We first examined the muscarinic cholinergic receptor M1. As shown in Figure 3A, applying M1 agonist McN (3 μM, 10 min) to the bath enabled the induction of LTD regardless of the order of pre- and postsynaptic activation (McN alone: 99.4% ± 2.9%, n = 17, p = 0.451; −20 ms: 70.5% ± 3.1%, n = 10, $p < 0.001$; +20 ms: 70.5% ± 3.7%, n = 6, $p < 0.001$). This form of LTD was blocked by 100 μM APV (−20 ms: 100.7% ± 1.3%, n = 8, p = 0.758, data not shown), and it did not occur when McN application was followed by either presynaptic stimulation (92.1% ± 5.9%, n = 5, p = 0.311) or postsynaptic activation alone (102.9% ± 2.1%, n = 5, p = 0.739) (Figure 3B). Thus, activation of M1 receptors enables the induction of associative LTD. Like the induction of associative LTP with isoproterenol, varying the timing between pre- and postsynaptic activation in the presence of McN always resulted in LTD (Figure 3C). Methoxamine (10 μM), an agonist of α1-adrenergic receptors coupled to PLC, also enabled the induction of LTD with either pre-post or post-pre associative paradigms (−20 ms: 86.4% ± 1.9%, n = 5, p = 0.002; +20 ms: 84.4% ± 4.4%, n = 5, p = 0.68, p = 0.021; Figure 3D). Altogether, these results show that activation of the PLC pathway allows the induction of associative LTD in a manner independent of the timing sequence of the pre- and postsynaptic spikes.

Coactivation of β-Adrenergic and M1 Receptors Enable STDP

Modification rules based on the exact timing between pre- and postsynaptic spikes are the cardinal feature of associative plasticity (Song and Abbott, 2001). Our results indicate that distinct signaling pathways can specifically promote associative LTP (by AC) or LTD (by PLC), but in a manner independent of spike-timing order (Figures 2B and 3C). Therefore, we examined whether coactivating these two pathways enables the spike-timing dependence of associative plasticity. As shown in Figure 4A, when 3 μM McN and 10 μM isoproterenol were coapplied, the timing between pre- and postsynaptic activation produced the expected polarity of plasticity. LTP occurred only when postsynaptic firing followed presynaptic activation, which was larger at shorter intervals. On the other hand, pairing in the reverse order induced only LTD (Figure 4B). We explored how the relative content of neuromodulator agonists in the mixture affects associative plasticity. Reducing isoproterenol to 1 μM significantly reduced the magnitude of LTP at positive delays (two-way ANOVA: $F[1,77] = 7.6$, $p = 0.0072$; Figure 4B). Moreover, pairing with a fixed delay (Δt = +20 ms) induced LTD or LTP, depending on the isoproterenol concentration in the mixture ($F[4,41] = 19.8$, $p < 0.001$; Figure 4C). Thus, the outcome (i.e., polarity and magnitude) of associative conditioning depends on both the timing of pre- and postsynaptic activation and the relative balance of neuromodulators.

Neuromodulators Phosphorylate AMPA Receptors at Specific Sites Required for LTP and LTD

Pre- and postsynaptic forms of LTP and LTD induced with STDP protocols have been described in several cortical synapses (Bender et al., 2006; Froemke et al., 2005; Hardingham and Fox, 2006; Sjostrom et al., 2004; Markram et al., 1997). Therefore, we used paired-pulse stimulation to examine the locus of expression of associative LTP and LTD promoted by neuromodulators. We found that the induction of associative LTP and LTD was not accompanied by changes in the responses to paired-pulse stimulation (50 ms; Figures S2A and S2B), suggesting that neuromodulators do not change the probability of neurotransmitter

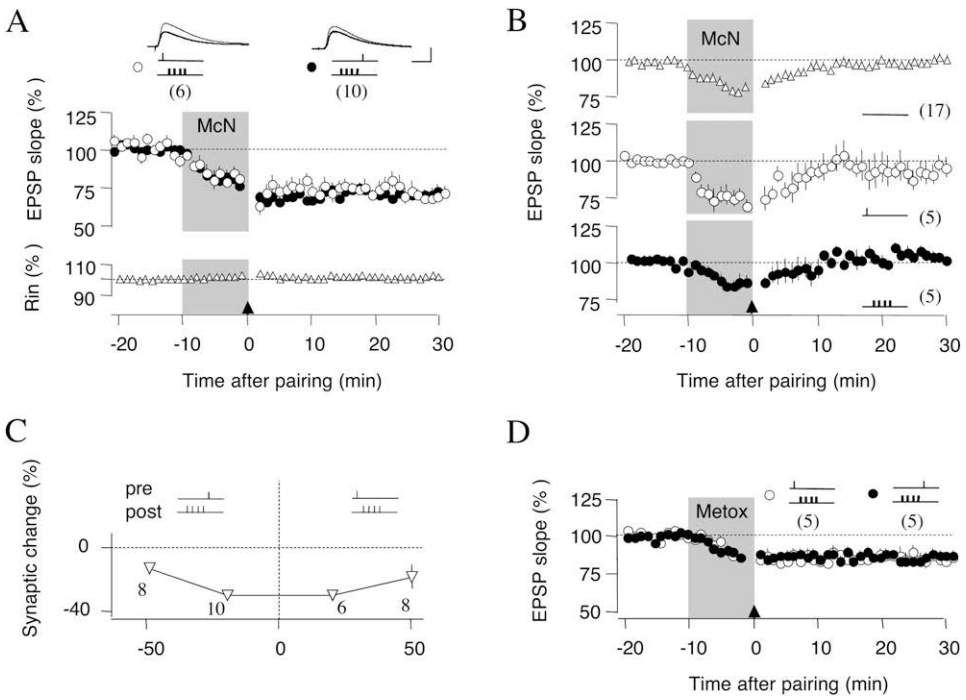

Figure 3. Receptors Coupled to Phospholipase C Specifically Gate LTD

(A) Bath application of the M1 muscarinic agonist McN (3 μM, 10 min) allows induction of LTD with +20 (open cicles) or −20 ms (solid circles) pairing. Traces are average of ten consecutive responses recorded before (thin traces) and after pairing (thick traces). Scale bars: 5 mV, 10 ms.

(B) Application of McN alone (open triangles) or in conjuction with presynaptic activation (open circles) or postsynaptic firing alone (filled circles) causes no LTD.

(C) Changes (30 min after conditioning) obtained by pairing with different time delays between pre- and postsynaptic spiking.

(D) Bath application of the α1 adrenergic agonist methoxamine (Metox: 10 μM for 10 min, gray box) allows induction of LTP with either pairing at +20 ms (open circles) or −20 ms (filled circles).

All results are shown as averages ± SEM.

release from the presynaptic terminal. This was somewhat surprising in view of recent evidence for presynaptic forms of LTD in layer II/III triggered by endocannabinoids (Bender et al., 2006; Nevian and Sakmann, 2006; Sjostrom et al., 2003). However, under our experimental conditions, the cannabinoid receptor antagonist (10 mM AM251; Figure S2C) did not block the induction of associative LTD, suggesting that neuromodulators do not promote presynaptic LTD.

Regulation of AMPA receptor phosphorylation is essential for postsynaptic forms of LTP and LTD (Esteban et al., 2003; Lee et al., 2000, 2003). Therefore, we investigated whether the neuromodulators enable associative plasticity by acting at this step. We focused on two phosphorylation sites on the GluR1 subunit of the AMPA receptors: S845, a PKA consensus site; and S831, a putative PKC consensus site (Roche et al., 1996). Immunoblot analysis of slices exposed to isoproterenol (10 μM, 10 min) revealed a substantial phosphorylation increase at the S845 site (0 min post-Iso: 231% ± 19.7%, n = 11; control: 100% ± 6.6%, n = 9; t test: $p < 0.001$) that persisted for more than 1 hr (60 min post-Iso: 246% ± 14.9%, n = 11; control: 100% ± 8.5%, n = 11; t test: $p < 0.001$), but no change at the S831 site (Figure 5). Exposure to McN, (5 μM, 10 min) resulted in an increase in S845 for at least 30 min (0 min post-McN: 193% ± 37.3%, n = 9; control: 100% ± 5.0%, n = 8; t test: $p < 0.04$; 30 min post-McN: 150% ± 21.7%, n = 12; control: 100% ± 4.8%, n = 12; t test: $p < 0.05$) and a transient increase in S831 (control: 100% ± 5.8%, n = 15; 0 min post-McN: 127% ± 10.6%, n = 15; t test: $p < 0.05$; control: 100% ± 7.2%, n = 8; 30 min post-McN: 109% ± 8.6%, n = 9; t test: $p > 0.4$). These results suggest that phosphorylation at the S845 site may be important for both LTP and LTD, while phosphorylation at the S831 site may be involved in LTD. Consistent with these results, we found that inclusion of adenylyl cyclase and PKA blockers in the recording pipette prevented the induction of associative LTP and LTD (Figures S3A and S3B).

Loss of Associative LTP and LTD in Mice Lines Lacking S845 or S831 on the GluR1 Subunit

The possible requirement of phosphorylation of S845 and S831 on the GluR1 subunit in associative LTD was somewhat surprising because these sites have been implicated in LTP in the hippocampus (Barria et al., 1997; Lee et al., 2000). To directly test the role of phosphorylation at S845 and S831 sites in associative plasticity, we used

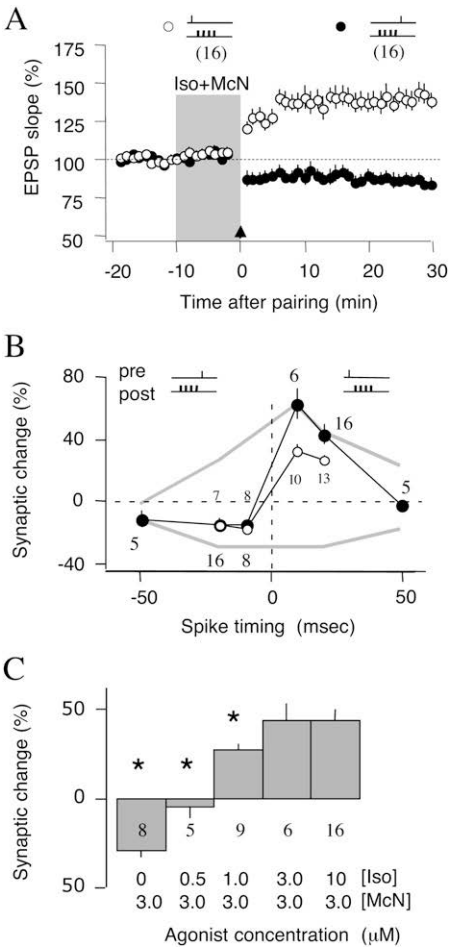

Figure 4. Coapplication of β-Adrenergic and M1 Muscarinic Agonists Enables STDP

(A) In the presence of agonist mixture (10 μM isoproterenol, 3 μM McN), pairing with a +20 ms delay induces LTP (open circles), while pairing with a −20 ms delay induces LTD (filled circles). The delay at each experiment was decided by a coin flip.

(B) Changes in the EPSPs (30 min after conditioning) elicited by pairing with different time delays after 10 min perfusion with the agonist mixture of McN (3 μM) and isoproterenol (10 μM, filled circles; 1 μM, open circles).

(C) Increasing the content of isoproterenol in the mixture results in a graded transition from LTD to LTP. A timing delay of +20 ms was used for the paired stimulation.

All results are shown as averages ± SEM.

two knockin mice lines (KO) in which serine (S) at each of these sites was substituted by alanine (A) to prevent phosphorylation. As expected, brief applications of isoproterenol (10 μM, 10 min) and McN (3 μM, 10 min) increase phosphorylation of S845 and S831 in mouse visual cortex (Figure S4). We also confirmed that application of the neuromodulators alone only transiently change postsynaptic responses in mouse layer II/III pyramidal cells (Figure S4).

The effects of the two agonists on the induction of associative plasticity in the two mouse lines are summarized in Figure 6. In the *S845A* line, both forms of associative plasticity, LTP (KO: 97% ± 1.8%, n = 4, 8; wt: 137.5% ± 7.6%, n = 3, 6; p = 0.003) and LTD (KO: 101.7% ± 7.4%, n = 5, 10; wt: 76.2% ± 3.1%, n = 3, 8; p < 0.001), were absent in the KO animals, yet robust in their wild-type littermates (Figures 6A and 6B). Interestingly, the transient effects of the neuromodulators, which can be suppressed by intracellular blockade of PKA (Figure S3), were also absent in the *S845A* line. This suggests that S845 is involved in both the short- and long-term effects of the neuromodulators. On the other hand, alanine substitution at the S831 site affected LTD (KO: 102.6% ± 6.2%, n = 5, 8; wt: 74.8% ± 6.9%, n = 3, 5; p = 0.012) but not LTP (KO: 142.6% ± 6.2%, n = 4, 7; wt: 150.5% ± 6.9%, n = 5, 8; p = 0.41). These results indicate that the expression of associative LTP requires phosphorylation at S845, whereas associative LTD requires phosphorylation at both S845 and S831.

Neuromodulators Prime the Induction of Associative Plasticity

The persistence of GluR1 phosphorylation (Figure 5) prompted us to ask whether synapses remain susceptible to associative plasticity after removal of the neuromodulators. In a first set of experiments, paired stimulation was applied 15 min after wash out of the agonists (McN or isoproterenol), which is after the disappearance of the acute effects of the drugs on the EPSP amplitude. As shown in Figures 7A and 7B, +20 ms pairing after washing out isoproterenol induced robust LTP (167.2% ± 9.2%, n = 10, p < 0.001; Figure 7A), and −20 ms pairing after McN induced robust LTD (64.1% ± 1.8%, n = 9, p < 0.001; Figure 7B). These results indicate that neuromodulators "prime" the synapses into a plastic state.

Prolonged whole-cell recordings can negatively affect the induction of plasticity. Therefore, to evaluate the duration of the priming effect, we applied and washed the agonists before making the seal. This allowed us to initiate the recordings at longer times after wash out of the drugs (Figure 7C). The results, shown in Figures 7C and 7D, indicate that priming of associative plasticity lasted up to 30 min. Interestingly, in agreement with the longer persistence of GluR1 phosphorylation induced by isoproterenol than McN (Figure 5), the priming of LTP lasted longer than the priming of LTD. Together, these results indicate that a brief application of neuromodulators can "prime" synapses to remain in a plastic state.

DISCUSSION

Our results indicate that receptors coupled to the AC and PLC signaling cascades "prime" the induction of associative LTP and LTD, but in a manner independent of spike timing. Induction of bidirectional STDP requires the coactivation of both cascades. In line with these findings, neuromodulators phosphorylate at least two sites on the AMPA receptor GluR1 subunit (S845 and S831) that are specifically required for the expression of LTP and LTD. We propose that neuromodulators can control STDP by

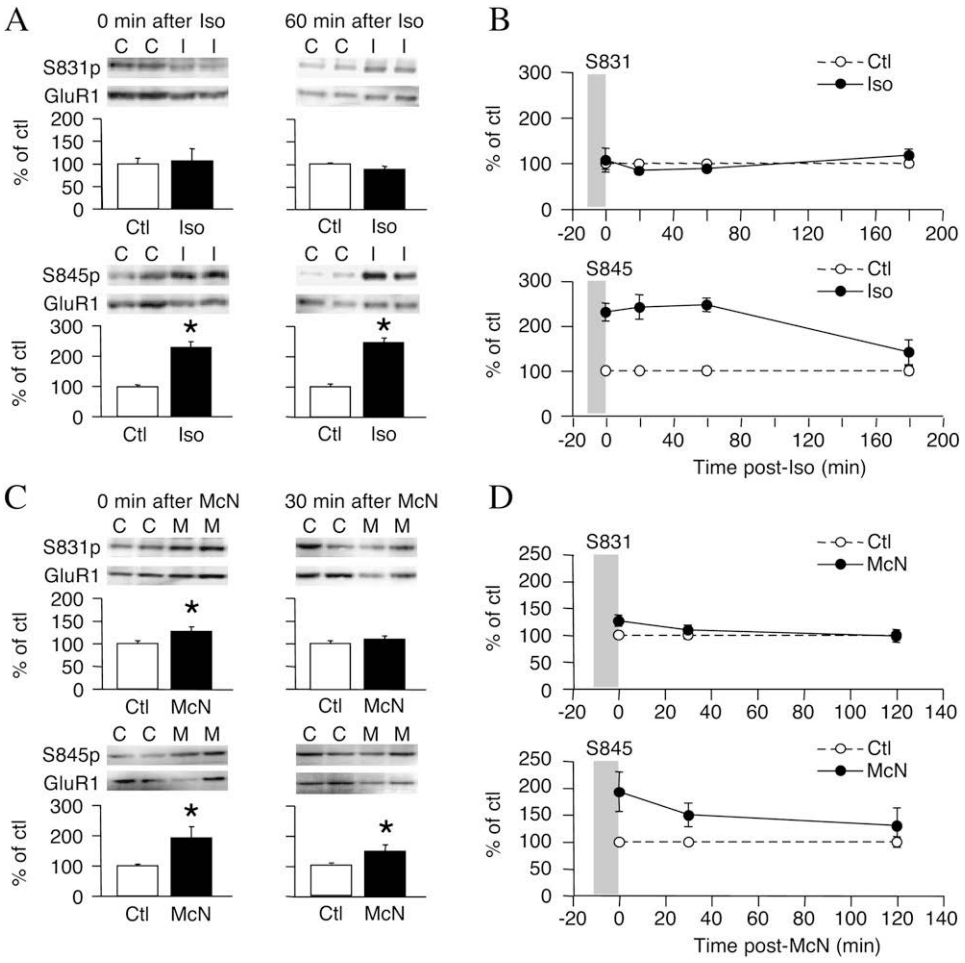

Figure 5. Neuromodulators Increase Phosphorylation of AMPA Receptor GluR1 Subunit

(A and B) Isoproterenol treatment (10 μM, 10 min) produces an increase in phosphorylation of GluR1-S845 for at least 1 hr without much change in GluR1-S831.

(C and D) McN application (3 μM, 10 min) transiently increases phosphorylation of GluR1 S831 and S845. Note that GluR1-S845 phosphorylation increase was still observed at 30 min post-McN application, while GluR1-S831 phosphorylation was not.

All results are shown as averages ± SEM.

phosphorylating AMPA receptors at sites that serve as specific tags for the induction of LTP and LTD. In this scenario, if these phosphorylation tags limit the magnitude of LTP and LTD, then the relative activation of AC- and PLC-coupled receptors becomes a determining factor for the polarity of STDP.

The notion that phosphorylation at S845 is required for associative LTP is consistent with the idea that phosphorylation at this site primes the induction of LTP (Esteban et al., 2003; Oh et al., 2006). In contrast, the exact role of S845 and S831 phosphorylation in associative LTD is less clear. Phosphorylation at these sites is reduced after the induction of LTD with electrical stimulation in hippocampus (Lee et al., 2000) or visual deprivation in visual cortex (Heynen et al., 2003), which supports the prevailing view that expression of LTD requires dephosphorylation of these sites. On the other hand, three lines of evidence suggest that phosphorylation of S845 and S831 is required for the induction of associative LTD: (1) these sites are phosphorylated by neuromodulators that promote LTD (Figure 5; see also Delgado and O'Dell, 2005), (2) associative LTD is lost in mice lacking S845 and S831 sites (Figure 6; see also Lee et al., 2003), and (3) inhibitors of PKA and adenylyl cyclase block the induction of LTD (Figure 5; also Crozier et al., 2007; Fischer et al., 2004). The possibility that phosphorylation and dephosphorylation of these sites might play distinct roles during induction and expression of LTD remains to be determined.

The specific gating of associative LTP and LTD reported here is consistent with previous reports showing separate induction pathways for LTP and LTD in cortical STDP (Bender et al., 2006; Nevian and Sakmann, 2006; Sjostrom et al., 2003). However, these studies described a form of LTD that involves a reduced probability of release

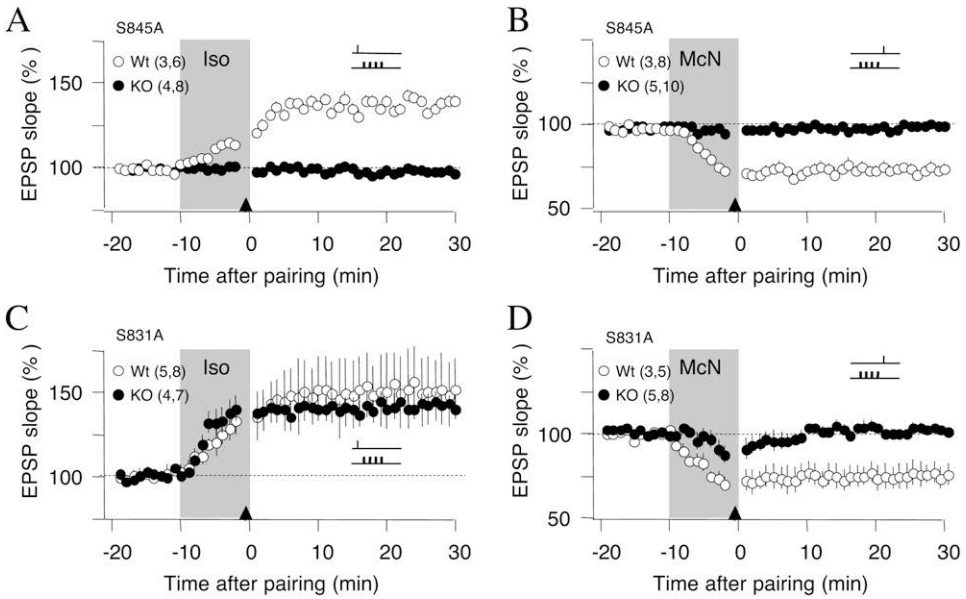

Figure 6. Associative Plasticity in Transgenic Mouse Lacking S845 or S831 Phosphorylation Sites

In S845A mice (KO, filled circles; wt littermates, open circles), both associative LTP induced with isoproterenol and +20 ms pairing (A) and associative LTD induced with McN and −20 ms pairing (B) are lost. In S831A mice, associative LTP is normal (C), while associative LTD is lost (D). The experimenter was blind to the genotype of the animals. The number of mice and slices is indicated in parenthesis. All results are shown as averages ± SEM.

triggered by the retrograde action of endocannabinoids. In contrast, LTD reported here is postsynaptic and independent of cannabinoids and hence most likely represents a different form of plasticity. It is possible that the induction of presynaptic LTD requires additional processes that are not activated under our experimental conditions. It remains unclear whether these two mechanisms coexist at the same synapses.

A noticeable difference between previous studies and ours is that associative plasticity requires exogenous neuromodulators under our experimental conditions. This might relate to differences in basal levels of endogenous agonist, AC and PLC activity, and/or AMPAR phosphorylation. Variations in the preparation and maintenance of the slices can affect the levels of basal AMPAR phosphorylation (Ho et al., 2004). On the other hand, a rise in intracellular Ca^{2+} alone can stimulate AC and PLC activity (Horne and Dell'Acqua, 2007; Thore et al., 2004). Thus, with stronger synaptic stimulation or under increased dendritic excitability, it is plausible that the Ca^{2+}-stimulated PLC and AC might be activated sufficiently to support associative plasticity.

Another interesting observation is that in the presence of neuromodulators the time window for coincidence detection is much wider than previously reported. For example, in the presence of isoproterenol, the temporal window for LTP is at least 50 ms, whereas in most other studies done in the absence of any neuromodulators it is typically less than 10–15 ms. In the Schaffer collateral to CA1 synapses in the hippocampus, isopreteronol has also been reported to extend the time window for associative LTP (Lin et al., 2003). The coincidence time window of 10–15 ms for LTP induction is considerably shorter than the duration of the NMDAR-synaptic response, which is typically 50–100 ms. We propose that in the absence of neuromodulators, LTP requires activation of PLC and AC triggered by intracellular Ca^{2+} signals. This would imply that the appropriate Ca^{2+} elevation would only be met when the postsynaptic spike coincides with the peak of NMDAR activation, which is between 10 and 15 ms. In contrast, neuromodulators that directly activate PLC and AC through G proteins would by-pass this Ca^{2+} constraint and extend the coincidence-detection window to a duration closer to the time course of the actual NMDAR current.

Neuromodulators like acetylcholine and norepinephrine are essential for experience-induced cortical plasticity. Besides regulating excitability, neuromodulators might also enhance the backpropagation of action potentials (Hoffman and Johnston, 1999; Tsubokawa and Ross, 1997) and/or other dendritic events implicated in associative plasticity (Lisman and Spruston, 2005). Indeed, there is evidence that the timing rules of STDP can be affected by factors limiting dendritic excitability and backpropagation, like synaptic location (Letzkus et al., 2006) and stimulation regime (Wittenberg and Wang, 2006). In contrast, our findings indicate that neuromodulators prime the induction of plasticity by affecting steps that are downstream from the activation of NMDA receptors. A unique feature of such a mechanism is that neuromodulators

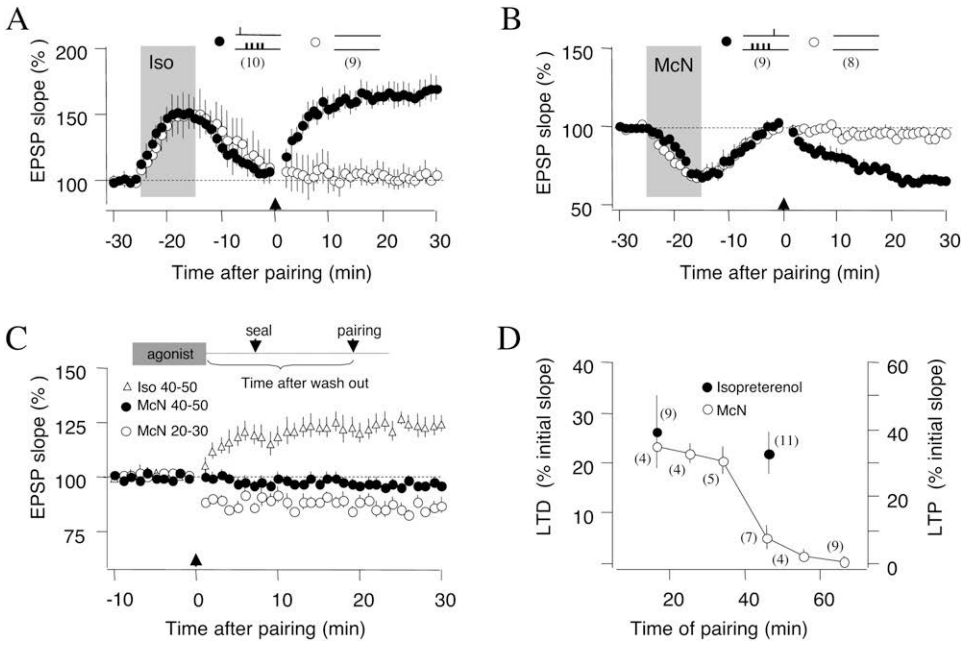

Figure 7. Neuromodulators "Prime" the Induction of Associative Plasticity

(A and B) Associative pairing (+20 ms in [A], −20 ms in [B]) applied 15 min after wash out of the neuromodulators (filled circles: 10 μM isoproterenol in [A], 3 μM McN in [B]). Neuromodulators without paired stimulation induce only transient changes in the responses (open circles).

(C and D) Priming of associative plasticity persists at least 30 min in experiments where whole-cell recordings were initiated after washing out the neuromodulators.

(C) The experimental sequence is depicted on the top. Pairing with −20 ms interval induce LTD when applied 20–30 min (open circles) but not 40–50 min (filled circles) after McN wash out, whereas LTP can be induced with pairing (+20 ms) delivered 40–50 min (open triangles) after isoproterenol washout.

(D) Average synaptic changes induced by pairing protocol delivered at different times after wash out of neuromodulators.

All results are shown as averages ± SEM.

might increase the propensity for modification without affecting synaptic transmission. This differs from previously described mechanisms of metaplasticity that target NMDA receptors because these receptors can potentially play a role in neural transmission in vivo (Krukowski and Miller, 2001; Sato et al., 1999). The mechanism supported by our results would allow neuromodulators to regulate neural plasticity without altering neural processing.

The independent control of associative LTP and LTD has fundamental consequences: the polarity and magnitude of associative plasticity becomes codetermined by the timing of pre- and postsynaptic firing and the relative activation of the AC and PLC cascades. Thus, pre- and postsynaptic firing pairing might induce LTP or LTD, depending on which of these cascades is active. It is interesting to speculate that the differences in the basal level of neuromodulation in the cortical slices might contribute to the variation in the robustness of associative plasticity across laboratories and also to the differences in the shape of the timing-dependent modification rules (Feldman, 2000; Markram et al., 1997). Furthermore, we surmise that in vivo spike-timing-dependent rules may not be rigid but are shaped by complex interactions of the neuromodulatory inputs.

EXPERIMENTAL PROCEDURES

Visual cortical slices (300 μm) from 3- to 4-week-old Long-Evans rats and C57BL/6 mice were prepared as described previously (Kirkwood and Bear, 1994). Briefly, slices were cut in ice-cold dissection buffer containing (in mM) 212.7 sucrose, 5 KCl, 1.25 NaH_2PO_4, 10 $MgCl_2$, 0.5 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose, bubbled with 95% O_2/5% CO_2 (pH 7.4). Individual slices were transferred to normal artificial cerebrospinal fluid (ACSF) for at least an hour prior to recording. Normal ACSF is similar to the dissection buffer except that sucrose is replaced by 124 mM NaCl, $MgCl_2$ is lowered to 1 mM, and $CaCl_2$ is raised to 2 mM. One cell per slice was used.

Electrophysiology

Visualized whole-cell current-clamp recordings were made from layer II/III regular-spiking pyramidal cells using a MultiClamp 700A amplifier (Molecular Devices). Borosillicate glass recording pipettes (4–6 MΩ) were filled with intracellular solution containing (in mM) 130 (K)Gluconate, 10 KCl, 0.2 EGTA, 10 HEPES, 4 (Mg)ATP, 0.5 (Na)GTP, and 10 (Na)Phosphocreatine (pH adjusted to 7.25 with KOH, 280–290 mOsm). Only cells with membrane potentials more negative than −65 mV, series resistance < 20 MΩ (8–18 MΩ, compensated at 80%), and input resistance larger than 100 MΩ were studied. Cells were excluded if input resistance changed >15% over the entire experiment, with the exception of changes during bath application of the agonists. Data were filtered at 2 kHz and digitized at 5 kHz using Igor Pro (WaveMetrics Inc., Lake Oswego, OR).

Synaptic responses were evoked every 20 s by stimulating layer IV with 0.2 ms pulses delivered through concentric bipolar stimulating electrodes (FHC). Intensity was adjusted to evoke 4–6 mV responses. Synaptic strength was quantified as the initial slope (the first 2 ms) of the EPSP. Mean baseline slope was calculated from 20 consecutive sweeps before the start of drug application. LTP and LTD were attempted by pairing presynaptic activation with four action potentials (100 Hz) evoked by passing suprathreshold depolarizing current steps through the recording electrode (~1 nA, 2 ms). Extracellular recordings were done in 400 micron slices in which a vertical cut in the lower half of the cortical depth served to separate two independent inputs (Kirkwood and Bear, 1994). Field potential recordings were done in layer II/III with a patch pipette filled with ACSF. Stimulation electrodes were placed at each side of the cut at the middle of the cortical depth. One side of the cut was weakly stimulated (stimulus intensity adjusted to evoke a half-maximal response) and served as the test pathway. The other side served as the conditioning pathway, and it was strongly stimulated during associative pairing (bursts of four pulses, 100 Hz, at twice the intensity to evoke the maximal response). Associative pairing consisted of 200 pairing epochs (one burst paired with stimulation of the test pathway at different delays) delivered at 1 Hz.

Most drugs, including 4-[*N*-(3-Chlorophenyl) carbamoyloxy]-2-butynyltrimethylammonium chloride (McN), methoxamine, isoproterenol, butaprost, and 2-amino-5-phosphonopentanoic acid (APV), were purchased from Sigma (St. Louis, MO). SQ 22536 and PKIamide were purchased from Tocris. Isoproterenol was applied with 10 μM sodium ascorbate to prevent oxidation of the drug. Only data from slices with stable recordings (<5% change over the baseline period) were included in the final analysis. All data are presented as average ± standard error of the mean normalized to the preconditioning baseline. For comparisons, the LTD or LTP magnitude was taken as the average of the last 5 min recorded.

Immunoblot Analysis

Visual cortical slices were homogenized in ice-cold lysis buffer (20 mM $NaPO_4$, 300 mM NaCl, 10 mM EDTA, 10 mM EGTA, 10 mM sodium pyrophosphate, 50 mM NaF, 1 mM Na_3VO_4, 1 μM okadaic acid, and protease inhibitor cocktail [Pierce]), and crude membranes were prepared as previously described (Lee et al., 2000). SDS-PAGE gels were transferred to polyvinyl difluoride (PVDF) membranes (Immobilon, Millipore) and blocked for ~1 hr in blocking buffer (1% bovine serum albumin and 0.1% Tween-20 in phosphate-buffered saline [PBS], pH 7.4) and subsequently incubated for 1–2 hr in primary antibodies diluted in blocking buffer to yield the effective concentration as tested prior to the experiments. After five times 5 min washes in blocking buffer, the blots were incubated for 1 hr in secondary antibody conjugated to alkaline phosphatase diluted 1:10,000 in blocking buffer. The blots were washed five times 5 min and developed using enhanced chemifluorescence substrate (ECF substrate, Amersham). The ECF blots were scanned and quantified using the Versa Doc 3000 gel imaging system (Bio Rad). Signal obtained using phosphorylation site-specific antibody (P) was normalized to total GluR1 (C) measured by reprobing the blot with GluR1-C-terminal antibody. The P/C ratio of each sample on a blot was normalized to the average of control samples to obtain the percent of control values, which were compared between control and experimental samples using the unpaired Student's t test.

Generation of GluR1 Serine 831 and Serine 845 Mouse Lines

Mutant mice carrying a single mutation at GluR1 serine 831 or serine 845 were generated as descried previously (Lee et al., 2003). Amino acid substitutions to alanine at each site were introduced by PCR mutagenesis in each targeting vector. Linearized targeting vectors were electroporated into R1 ES cells (Dr. A. Nagy, Mount Sinai Hospital, Toronto, Canada). Recombinant clones of correct homologous recombination, confirmed by Southern blot analysis, were injected into C57 BL/6 blastocyst followed by chimera mice production at the Transgenic Facility of Johns Hopkins University School of Medicine. After germ-line transmission, heterozygote mice were bred to CMV (cytomegalovirus promoter)-Cre mice to delete the neo^r cassette, utilizing the *Cre-loxP* system (provided by Dr. A. Nagy, Mount Sinai Hospital, Toronto, Canada), and the *Cre* gene was bred out in the next generation. Homozygous and wild-type mice were obtained by intercrossing of heterozygous mice.

Statistical Analysis

The significance of LTP and LTD was assessed using the paired Student's t test. Other comparisons used unpaired t test or the ANOVA test.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/55/6/919/DC1/.

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REFERENCES

Akaneya, Y., and Tsumoto, T. (2006). Bidirectional trafficking of prostaglandin E2 receptors involved in long-term potentiation in visual cortex. J. Neurosci. *26*, 10209–10221.

Barria, A., Muller, D., Derkach, V., Griffith, L.C., and Soderling, T.R. (1997). Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. Science *276*, 2042–2045.

Bear, M.F., and Singer, W. (1986). Modulation of visual cortical plasticity by acetylcholine and noradrenaline. Nature *320*, 172–176.

Bender, V.A., Bender, K.J., Brasier, D.J., and Feldman, D.E. (2006). Two coincidence detectors for spike timing-dependent plasticity in somatosensory cortex. J. Neurosci. *26*, 4166–4177.

Bi, G.Q., and Poo, M.M. (1998). Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. J. Neurosci. *18*, 10464–10472.

Celikel, T., Szostak, V.A., and Feldman, D.E. (2004). Modulation of spike timing by sensory deprivation during induction of cortical map plasticity. Nat. Neurosci. *7*, 534–541.

Choi, S., Chang, J., Jiang, B., Seol, G.H., Min, S.S., Han, J.S., Shin, H.S., Gallagher, M., and Kirkwood, A. (2005). Multiple receptors coupled to PLC gate LTD in visual cortex. J. Neurosci. *25*, 11433–11443.

Crozier, R.A., Wang, Y., Liu, C.H., and Bear, M.F. (2007). Deprivation-induced synaptic depression by distinct mechanisms in different layers of mouse visual cortex. Proc. Natl. Acad. Sci. USA *104*, 1383–1388.

Delgado, J.Y., and O'Dell, T.J. (2005). Long-term potentiation persists in an occult state following mGluR-dependent depotentiation. Neuropharmacology *48*, 936–948.

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

Feldman, D.E. (2000). Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. Neuron *27*, 45–56.

Fischer, Q.S., Beaver, C.J., Yang, Y., Rao, Y., Jakobsdottir, K.B., Storm, D.R., McKnight, G.S., and Daw, N.W. (2004). Requirement for the RIIbeta isoform of PKA, but not calcium-stimulated adenylyl cyclase, in visual cortical plasticity. J. Neurosci. *24*, 9049–9058.

Froemke, R.C., and Dan, Y. (2002). Spike-timing-dependent synaptic modification induced by natural spike trains. Nature *416*, 433–438.

Froemke, R.C., Poo, M.M., and Dan, Y. (2005). Spike-timing-dependent synaptic plasticity depends on dendritic location. Nature *434*, 221–225.

Fu, Y.X., Djupsund, K., Gao, H., Hayden, B., Shen, K., and Dan, Y. (2002). Temporal specificity in the cortical plasticity of visual space representation. Science *296*, 1999–2003.

Hardingham, N., and Fox, K. (2006). The role of nitric oxide and GluR1 in presynaptic and postsynaptic components of neocortical potentiation. J. Neurosci. *26*, 7395–7404.

Heynen, A.J., Yoon, B.J., Liu, C.H., Chung, H.J., Huganir, R.L., and Bear, M.F. (2003). Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation. Nat. Neurosci. *6*, 854–862.

Ho, O.H., Delgado, J.Y., and O'Dell, T.J. (2004). Phosphorylation of proteins involved in activity-dependent forms of synaptic plasticity is altered in hippocampal slices maintained in vitro. J. Neurochem. *91*, 1344–1357.

Hoffman, D.A., and Johnston, D. (1999). Neuromodulation of dendritic action potentials. J. Neurophysiol. *81*, 408–411.

Horne, E.A., and Dell'Acqua, M.L. (2007). Phospholipase C is required for changes in postsynaptic structure and function associated with NMDA receptor-dependent long-term depression. J. Neurosci. *27*, 3523–3534.

Kilgard, M.P., and Merzenich, M.M. (1998). Cortical map reorganization enabled by nucleus basalis activity. Science *279*, 1714–1718.

Kirkwood, A., and Bear, M.F. (1994). Hebbian synapses in visual cortex. J. Neurosci. *14*, 1634–1645.

Krukowski, A.E., and Miller, K.D. (2001). Thalamocortical NMDA conductances and intracortical inhibition can explain cortical temporal tuning. Nat. Neurosci. *4*, 424–430.

Lee, H.K., Barbarosie, M., Kameyama, K., Bear, M.F., and Huganir, R.L. (2000). Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature *405*, 955–959.

Lee, H.K., Takamiya, K., Han, J.S., Man, H., Kim, C.H., Rumbaugh, G., Yu, S., Ding, L., He, C., Petralia, R.S., et al. (2003). Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell *112*, 631–643.

Letzkus, J.J., Kampa, B.M., and Stuart, G.J. (2006). Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. J. Neurosci. *26*, 10420–10429.

Lin, Y.W., Min, M.Y., Chiu, T.H., and Yang, H.W. (2003). Enhancement of associative long-term potentiation by activation of beta-adrenergic receptors at CA1 synapses in rat hippocampal slices. J. Neurosci. *23*, 4173–4181.

Lisman, J., and Spruston, N. (2005). Postsynaptic depolarization requirements for LTP and LTD: a critique of spike timing-dependent plasticity. Nat. Neurosci. *8*, 839–841.

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

Markram, H., Lubke, J., Frotscher, M., and Sakmann, B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science *275*, 213–215.

Nevian, T., and Sakmann, B. (2006). Spine Ca2+ signaling in spike-timing-dependent plasticity. J. Neurosci. *26*, 11001–11013.

Oh, M.C., Derkach, V.A., Guire, E.S., and Soderling, T.R. (2006). Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. J. Biol. Chem. *281*, 752–758.

Roche, K.W., O'Brien, R.J., Mammen, A.L., Bernhardt, J., and Huganir, R.L. (1996). Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron *16*, 1179–1188.

Sandler, V.M., and Ross, W.N. (1999). Serotonin modulates spike backpropagation and associated [Ca2+]i changes in the apical dendrites of hippocampal CA1 pyramidal neurons. J. Neurophysiol. *81*, 216–224.

Sato, H., Hata, Y., and Tsumoto, T. (1999). Effects of blocking non-N-methyl-D-aspartate receptors on visual responses of neurons in the cat visual cortex. Neuroscience *94*, 697–703.

Sjostrom, P.J., and Nelson, S.B. (2002). Spike timing, calcium signals and synaptic plasticity. Curr. Opin. Neurobiol. *12*, 305–314.

Sjostrom, P.J., Turrigiano, G.G., and Nelson, S.B. (2003). Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. Neuron *39*, 641–654.

Sjostrom, P.J., Turrigiano, G.G., and Nelson, S.B. (2004). Endocannabinoid-dependent neocortical layer-5 LTD in the absence of postsynaptic spiking. J. Neurophysiol. *92*, 3338–3343.

Song, S., and Abbott, L. (2001). Cortical development and remapping through spike timing-dependent plasticity. Neuron *32*, 339–350.

Thomas, M.J., Moody, T.D., Makhinson, M., and O'Dell, T.J. (1996). Activity-dependent β-adrenergic modulation of low frequency stimulation induced LTP in the hippocampal CA1 region. Neuron *17*, 475–482.

Thore, S., Dyachok, O., and Tengholm, A. (2004). Oscillations of phospholipase C activity triggered by depolarization and Ca2+ influx in insulin-secreting cells. J. Biol. Chem. *279*, 19396–19400.

Tsubokawa, H., and Ross, W.N. (1997). Muscarinic modulation of spike backpropagation in the apical dendrites of hippocampal CA1 pyramidal neurons. J. Neurosci. *17*, 5782–5791.

Tzounopoulos, T., Rubio, M.E., Keen, J.E., and Trussell, L.O. (2007). Coactivation of pre- and postsynaptic signaling mechanisms determines cell-specific spike-timing-dependent plasticity. Neuron *54*, 291–301.

Wittenberg, G.M., and Wang, S.S. (2006). Malleability of spike-timing-dependent plasticity at the CA3-CA1 synapse. J. Neurosci. *26*, 6610–6617.

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Supplemental Data

Neuromodulators Control the Polarity of Spike-Timing-Dependent Synaptic Plasticity

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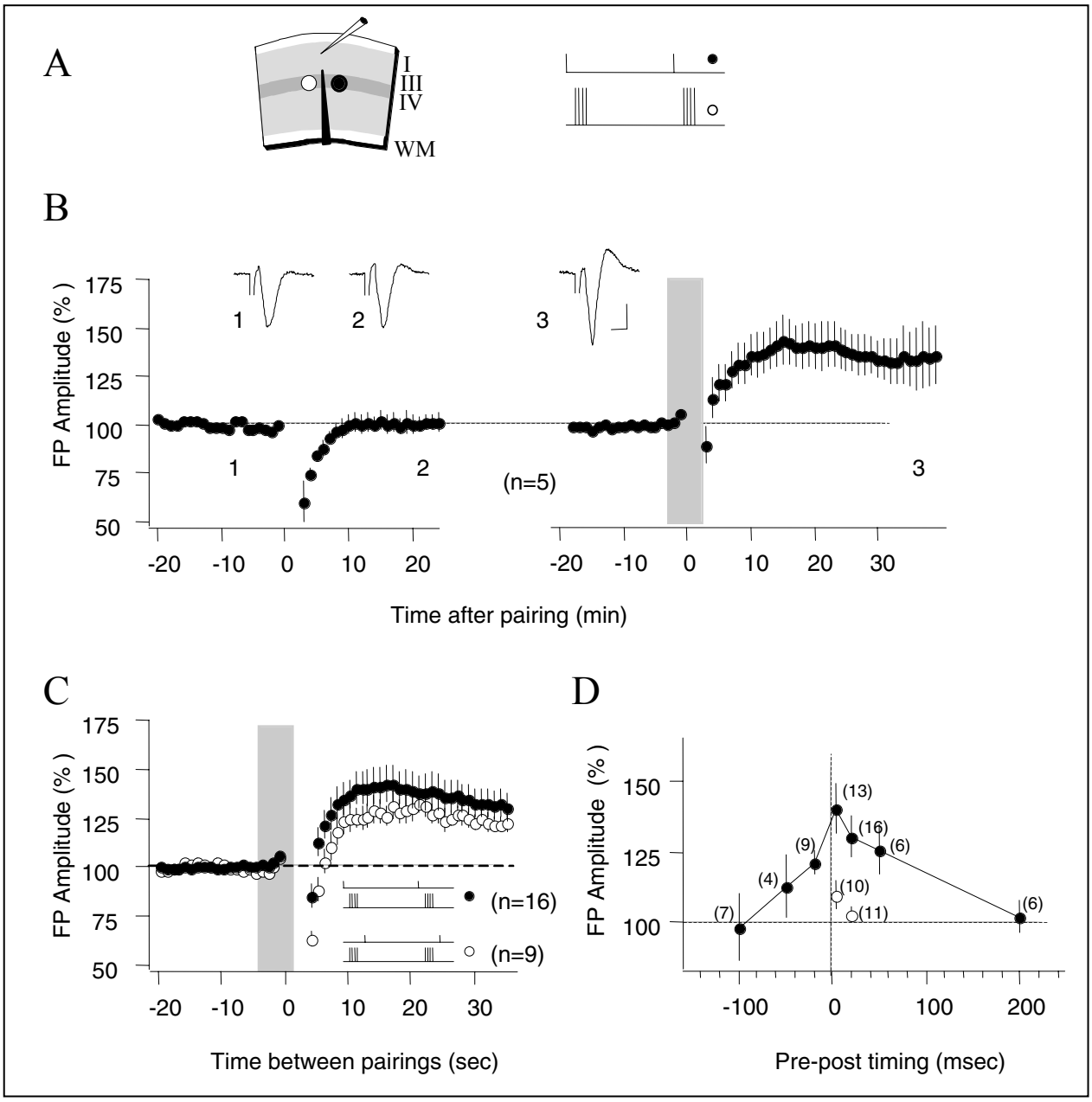
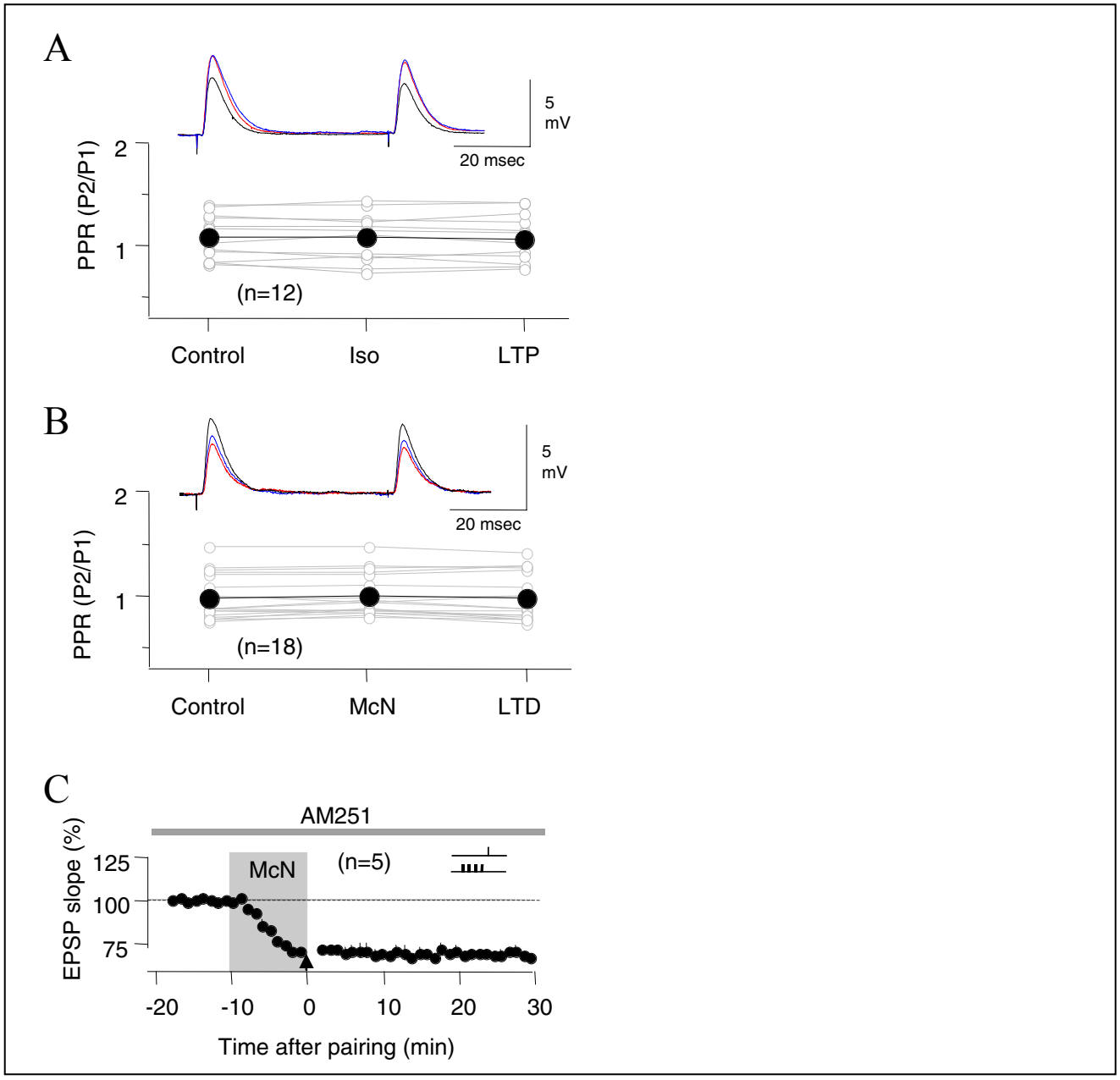
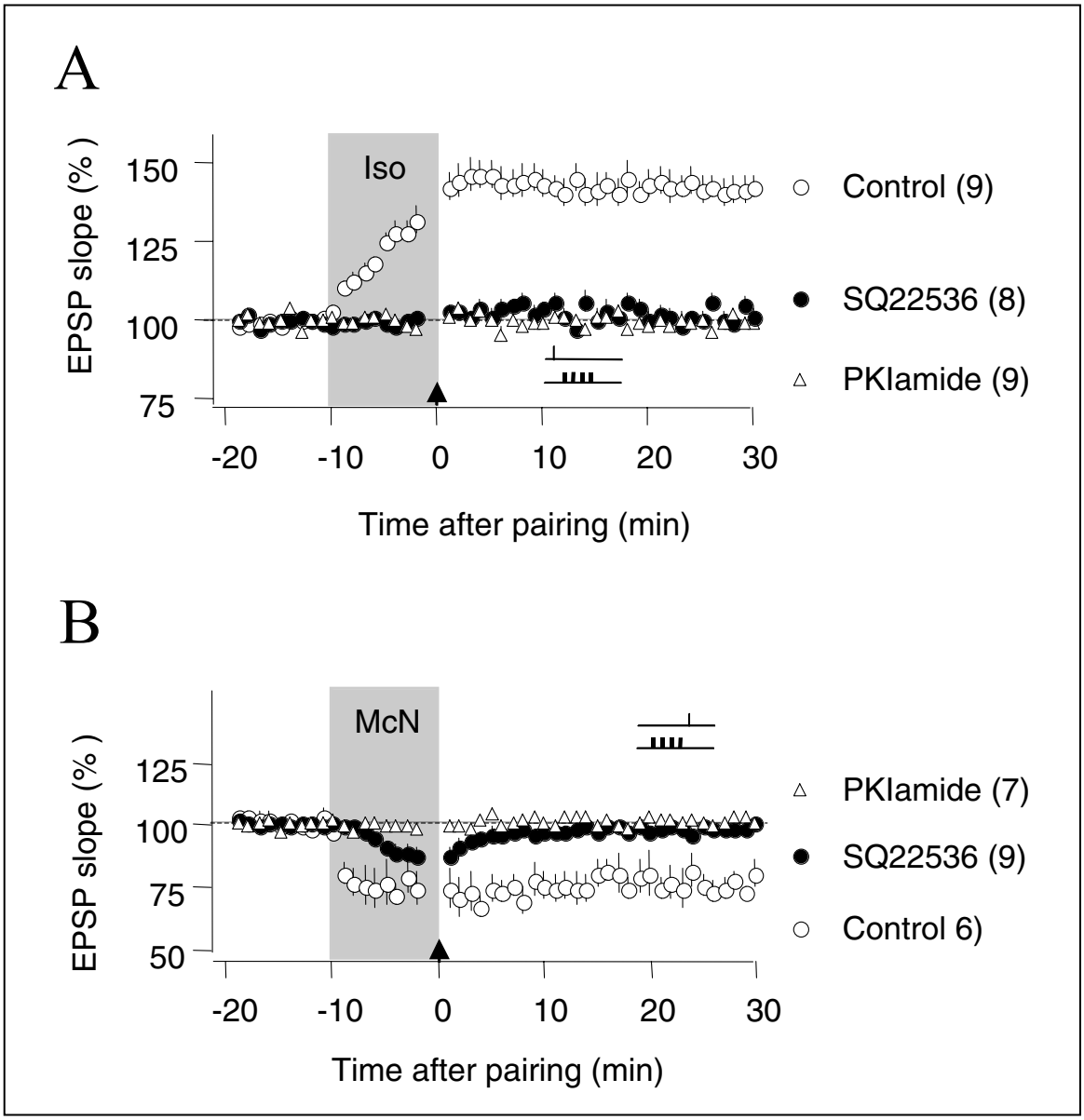


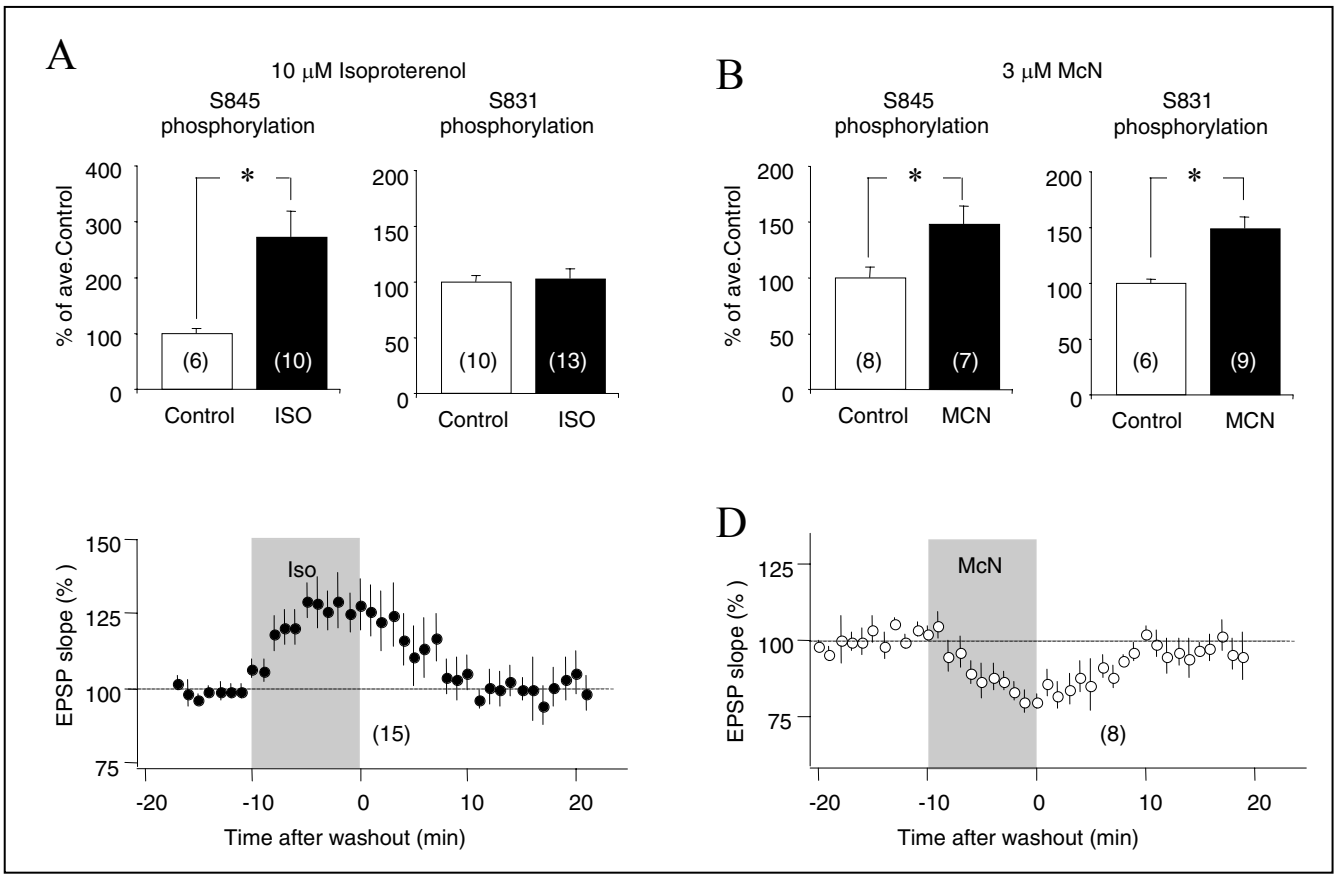
Supplementary figure 1. Bath application of 10 μM Isopreterenol gates the extracelluar induction of associative LTP. (A) Stimulus and recording arrangement. Layer II/III field potentials (FP) were recorded with a patch pipette filled with ACSF. Two independent inputs to layer II/III were recruited by stimulating at each side of vertical cut on the lower half of the slice. One pathway (black circle) was used as the test pathway and it was stimulus intensity was adjusted to evoke a half maximal FP. The pathway (white circle) pathway was stimulated only during pairing conditioning at twice the intensity to evoke the maximal FP. To induce plasticity, 200 associative pairing epochs (depicted on the right: 4 stimulation pulses at 100 Hz in the conditioning pathway, and one pulse in the test pathway) were delivered at 1 Hz. (B) Associative conditioning (at 20 msec delay) does not affects the FP amplitude in normal ACSF (99.5±4.8%, n=5. Paired t-test: p=0.88), but induces robust LTP (129.34±5.9%. Paired t-test: p=0.009) when subsequently delivered in the same slices at the end of a brief application of isoproterenol (3 min, 10 mM). (C) Associative conditioning induces robust LTP when stimulation of the test pathway either precedes the burst in the conditioning pathway (+20 msec. Filled circles, LTP: 130.0±7.3%, n=16) or when the conditioning pathway is stimulated first (-20 msec. Open circles. LTP: 120.9±4.4%, n=9. t-test: p=0.3). (D) Summary of changes (30 min after conditioning) obtained by pairing with different time delays between stimulation of the test and conditioning pathways in control ACSF (open circle) and in the presence 10 μM isoproterenol (filled circles). In the presence of isoproterenol, conditioning stimulation always produced LTP that depend on the timing ($F_{[6,48]} = 2.77$: p=0.022), but no LTD. In parentheses are the numbers of experiments included for each data point.



Supplementary figure 2. Associative LTP and LTD promoted by neuromodulators is expressed postsynaptically. (A) The paired pulse stimulation (50 msec interval) response ratio does not change during the application of isoproterenol and after the induction of associative LTP (ANOVA test: $F[2,33]=0.194$, $p=0.981$). (B) The paired pulse stimulation (50 msec interval) response ratio does not change during the application of McN and after the induction of associative LTD (ANOVA test: $F[2,51]=0.748$, $p=0.928$). Example traces in (A) and (B) are averages of 10 consecutive responses recorded in control ACSF (black trace), during application of neuromodulator (blue trace) and 30 min after the induction of plasticity (red trace). (C) LTD (induced with McN and –20msec pairing) was not blocked by the cannabinoid antagonist AM251 (5 μM, n=5, $p<0001$).



Supplementary figure 3. Associative LTP and LTD promoted by neuromodulators requires the adenylyl cyclase (AC) cascade. (A) Intracellular inclusion of either the AC blocker SQ 22536 (100μM, n=8, filled circles) or the PKA blocker PKIamide (10 μM, n=9, open triangles) prevented the induction of associative LTP (Control: n=9, open circles) by isoproterenol (+20 msec). These differences were significant ($F[2,23]=74.11$, $p<0.001$). (B) The induction of LTD with McN (Control: n=6, open circles) is prevented by including either the AC blocker SQ 22536 (100μM, n=9, filled circles) or the PKA blocker PKIamide (10 μM, n=7, open triangles) in the recording pipette. These differences were significant ($F[2,20]=37.77$, $p<0.001$).



Supplementary figure 4. Effects of neuromodulators on mouse visual cortex. (A) Isoproterenol treatment (10 μM, 10 min) produces an increase in phosphorylation of GluR1-S845 (control: 100.0±7.8, n=6; Iso: 271.8±45.9, n=10. p=0.0045) without much change in GluR1-S831 (control: 100.0±5.2, n=10; Iso: 102.8±8.8, n=13. p=0.788). (B) McN application (3 μM, 10 min) increases phosphorylation of both GluR1 S831 (control: 100.0±3.2, n=8; McN: 149.0±10.1, n=7. p=0.0022) and S845 (control: 100.0±9.3, n=6; McN: 147.9±16.0, n=9. p=0.019). (C) Bath application of isoproterenol (10 μM, gray box) reversibly increases the EPSPs (n=15). (D) Bath application of McN (3 μM, gray box) reversibly decreases the EPSPs (n=8). Asteriskcs denote statistical significance $p<0.05$.