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Neocortical LTD via Coincident Activation of Presynaptic NMDA and Cannabinoid Receptors

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Summary

There is a consensus that NMDA receptors (NMDARs) detect coincident pre- and postsynaptic activity during induction of long-term potentiation (LTP), but their role in timing-dependent long-term depression (tLTD) is unclear. We examine tLTD in neocortical layer 5 (L5) pyramidal pairs and find that tLTD is expressed presynaptically, implying retrograde signaling. CB1 agonists produce depression that mimics and occludes tLTD. This agonist-induced LTD requires presynaptic activity and NMDAR activation, but not postsynaptic Ca^{2+} influx. Further experiments demonstrate the existence of presynaptic NMDARs that underlie the presynaptic activity dependence. Finally, manipulating cannabinoid breakdown alters the temporal window for tLTD. In conclusion, tLTD requires simultaneous activation of presynaptic NMDA and CB1 receptors. This novel form of coincidence detection may explain the temporal window of tLTD and may also impart synapse specificity to cannabinoid retrograde signaling.

Introduction

Long-term depression (LTD) is a persistent weakening of synaptic strength thought to be involved in learning and development (Cline, 1998; Linden and Connor, 1995). At cortical synapses, LTD is reliably induced by low-frequency stimulation of presynaptic afferents (Dudek and Bear, 1992; Mulkey and Malenka, 1992) or by pairing presynaptic stimulation with modest depolarization (Artola et al., 1990). More recent experiments have revealed that LTD also occurs when postsynaptic firing repeatedly precedes presynaptic firing within a temporal window that varies from 20 to 200 ms or more, depending on the experimental conditions (Bi and Poo, 1998; Debanne et al., 1994, 1998; Feldman, 2000; Levy and Steward, 1983; Markram et al., 1997b; Nishiyama et al., 2000; Sjöström et al., 2001; Zhang et al., 1998).

Although induction of tLTD is known to depend on activation of NMDARs (Bi and Poo, 1998; Feldman, 2000; Markram et al., 1997b; Sjöström et al., 2001), the factors determining the temporal window for tLTD remain elusive. Some have argued that postsynaptic before presynaptic activity produces tLTD because this temporal order yields sublinear summation of postsynaptic action potential (AP) and EPSP-evoked Ca^{2+} influx (Koester and Sakmann, 1998), perhaps in part by Ca^{2+}-mediated inactivation of postsynaptic NMDA currents (Legendre et al., 1993; Medina et al., 1996). Others have suggested that the slow, prolonged Ca^{2+} influx produced by the combination of the back-propagating AP and the NMDAR component of the EPSP promotes LTD (Bi and Poo, 1998).

The expression mechanisms underlying tLTD are not well understood. At some synapses, LTD is expressed postsynaptically (Wang and Linden, 2000), whereas at others it is expressed presynaptically (Egger et al., 1999; Zakharenko et al., 2002). The locus of expression may also depend on induction protocol (Oliet et al., 1997) and animal age (Kemp et al., 2000). Pre- and postsynaptic expression mechanisms hold different implications for neural coding. Presynaptic changes in the probability of release can alter the reliability (Otmakhov et al., 1993) and short-term plasticity of central synapses (Markram and Tsodyks, 1996). In contrast, postsynaptic changes in transmitter sensitivity alter the gain of transmission but do not typically alter reliability or dynamics (Buonomano, 1999; Pananceau et al., 1998; Selig et al., 1999).

The site of expression also holds important implications for the mechanisms of induction. Plasticity expressed postsynaptically can depend entirely on biochemical pathways within a postsynaptic neuron. In contrast, presynaptic expression of forms of plasticity that, like tLTD, require postsynaptic activity (Markram et al., 1997b; Sjöström et al., 2001) necessitates the existence of a retrograde messenger. Prior studies of candidate retrograde messengers in plasticity have focused mainly on their roles in LTP (Tao and Poo, 2001). Recently, however, endocannabinoids have been shown to act as retrograde messengers for LTD in basal ganglia (Gerdeman et al., 2002; Robbe et al., 2002), amygdala (Marsicano et al., 2002), and hippocampus (Chevaleyre and Castillo, 2003).

Most forms of LTP depend critically on postsynaptic NMDARs (Bi and Poo, 2001; Magee and Johnston, 1997). Although presynaptic NMDARs receptors have previously been found in neocortex by immuno-electron microscopy (Aoki et al., 1994; Charton et al., 1999; DeBiasi et al., 1996), their role in long-term plasticity—if any—is unknown. Presynaptic NMDARs modulate neurotransmitter release in the cerebellum (Glitsch and Marty, 1999), spinal cord (Liu et al., 1997), and entorhinal cortex (Berretta and Jones, 1996; Woodhall et al., 2001; see MacDermott et al., 1999, for review), and there is also evidence for a role of presynaptic NMDARs in cerebellar LTD (Casado et al., 2002).

Here we report that induction of tLTD in L5 pairs changes short-term depression (STD) and the coefficient of variation (CV) consistent with a presynaptic site of expression. We also find that tLTD requires activity-dependent postsynaptic release of an endocannabinoid, which diffuses retrogradely and binds to presynaptic CB1 cannabinoid receptors. tLTD is produced only if the presynaptic neuron is active when endocannabinoid is bound to CB1 receptors. This presynaptic activity dependence relies on presynaptic NMDA autoreceptors that detect the release of glutamate. Thus, the coinci-

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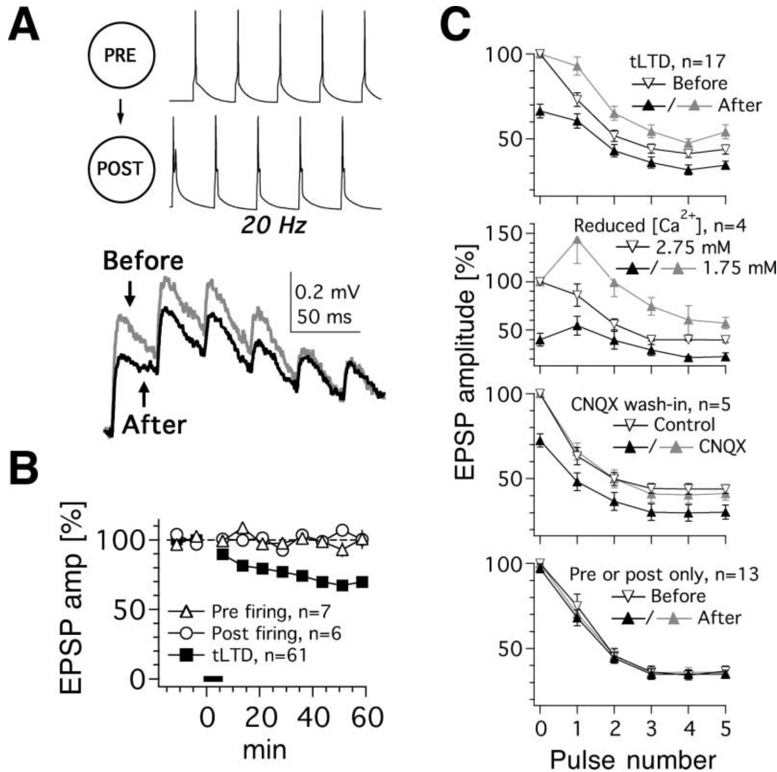

Figure 1. tLTD Reduces STD in a Manner Consistent with Presynaptic Expression

(A) Sample paired recording for which post-before-pre spiking (25 ms interval) at 20 Hz (top) reduced the first response but not the last (bottom).

(B) This induction protocol reliably produced tLTD (squares), relative to presynaptic (triangles) or postsynaptic (circles) controls. In control pairs, both cells fired 30 Hz trains during baseline, and only one cell fired during induction. Horizontal line at 0 min denotes induction period. Induction was carried out at 0.1–20 Hz.

(C) STD was reduced after tLTD induction (−10 to −25 ms interval, 4–20 Hz) and Ca^{2+} reduction, but not after CNQX wash-in. Unpaired presynaptic or postsynaptic spiking yielded no depression and did not alter STD (both conditions pooled). Gray triangles are identical to black triangles but are renormalized to emphasize changes in STD independently of changes in amplitude.

dent activation of presynaptic NMDA and CB1 receptors signal tLTD. This constitutes a novel form of presynaptic NMDAR-mediated coincidence detection.

Results

tLTD Induction Reduces STD, Suggesting Presynaptic Expression

At neocortical L5 connections, postsynaptic before presynaptic spiking reliably produces LTD (Figures 1A and 1B; Markram et al., 1997b; Sjöström et al., 2001). We observed that STD during a brief 30 Hz train was invariably diminished after the induction of tLTD (Figure 1A). This effect is complementary to the enhanced STD previously observed at these synapses after LTP (Markram and Tsodyks, 1996) and suggests that tLTD is, at least in part, presynaptically expressed through a change in release probability (Zucker and Regehr, 2002).

In control experiments, we reduced the postsynaptic sensitivity to glutamate by applying concentrations of CNQX that blocked approximately one-half of the available AMPA receptors (Figure 1C). As expected, this suppressed all responses in a train to approximately the same extent, without causing a change in STD. Conversely, reducing glutamate release by lowering external $[Ca^{2+}]$ resulted in more suppression of the first response as compared to subsequent responses (Figure 1C), dramatically reducing STD. As previously demonstrated (Markram et al., 1997b; Sjöström et al., 2001), unpaired presynaptic or postsynaptic spiking did not produce tLTD (Figure 1B) and did not alter STD (Figure 1C). Similarly, switching from 0.1 to 30 Hz firing for 15 min, and then back, had no long-term effects on transmission (100% ± 6%, n = 4; $p < 0.05$), demonstrating that STD and response amplitude are stable during long periods of high-frequency firing.

A measure of the change in STD, the STD index (see Experimental Procedures) was significantly different for tLTD (−0.29 ± 0.02) compared to pre- or postsynaptic firing alone (−0.01 ± 0.01; $p < 0.01$) and to CNQX wash-in (0.05 ± 0.02; $p < 0.001$), but not compared to Ca^{2+} wash-out (−0.38 ± 0.06; $p = 0.59$), suggesting tLTD may act presynaptically to reduce glutamate release.

CV Analysis Supports Presynaptic Expression of tLTD

To test the idea that tLTD reduces release, we analyzed the coefficient of variation (CV) of the synaptic responses before and after tLTD (Faber and Korn, 1991; Larkman et al., 1992). In this analysis, pairs in which the postsynaptic sensitivity is reduced give rise to points above the diagonal, because (assuming a binomial distribution) the standard deviation and mean scale linearly as the synapse depresses. As a result, $1/CV^2$ remains unaffected as the mean decreases. Conversely, pairs in which presynaptic release is reduced give rise to points below the diagonal, because the standard deviation remains unaffected as the mean decreases (cf. Figure 2A). Other models of release may diminish the extent to which pre- and postsynaptic expression appear different as determined by CV analysis, but their relative position in the $1/CV^2$-versus-mean graph is not interchanged (Faber and Korn, 1991).

The points for all 54 pairs tested lay on or below the diagonal (Figure 2B), consistent with a presynaptic locus of expression. In the control experiments described in

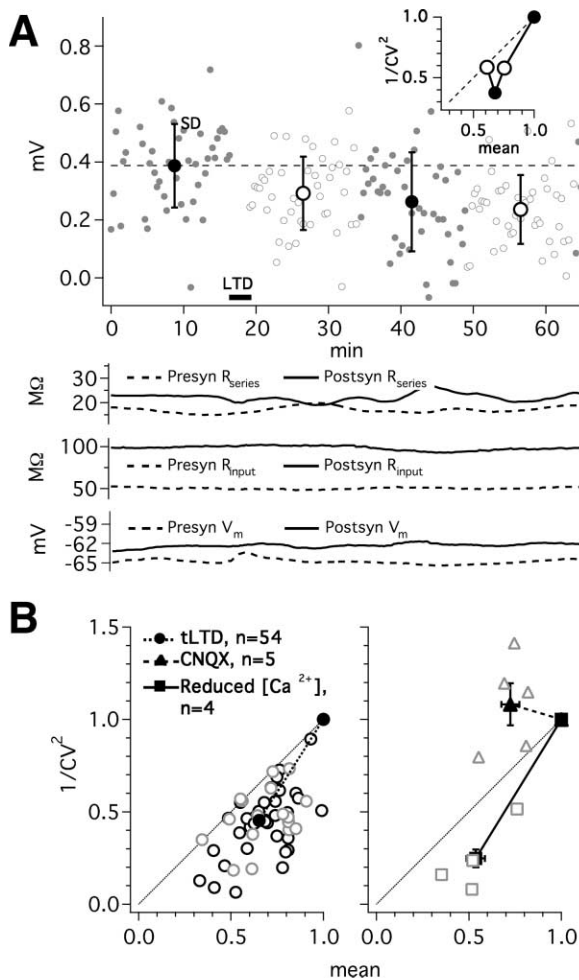

Figure 2. CV Analysis of tLTD Indicates a Presynaptic Expression Mechanism

(A) Response SD (error bars) of the sample paired recording in Figure 1A remains relatively constant as the mean amplitude decreases following tLTD induction (horizontal bar). R_{series}, R_{input}, and V_m do not change significantly. Normalized plot of $1/CV^2$ versus mean (inset top right) yield postinduction data points below the diagonal, suggesting tLTD is expressed presynaptically (see Experimental Procedures).
(B) CV analysis suggests that tLTD is presynaptically expressed (circles, left graph; dark circles [n = 37] reanalyzed from previous experiments reported in Sjöström et al., 2001). Partial block of postsynaptic AMPA receptors using CNQX (triangles, right graph) and reduced presynaptic release after Ca^{2+} reduction (squares, right graph) verified that CV analysis could correctly identify the locus of depression. Each data point corresponds to the average of postpairing data.

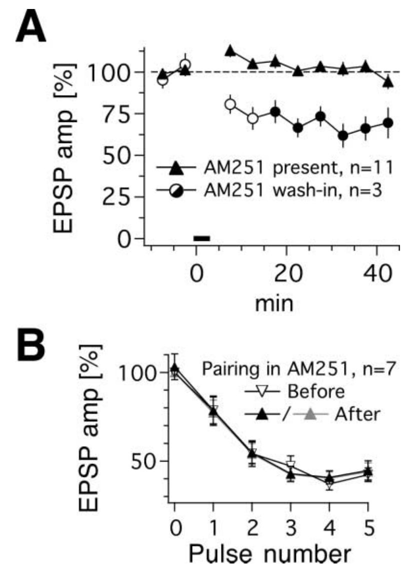

Figure 3. CB1 Receptor Antagonism Abolishes tLTD

(A) The CB1 antagonist AM251 blocks tLTD when added 15 min before induction (triangles, induction at 0.1–20 Hz with timing interval −10 or −25 ms), but does not reverse tLTD (circles, induction at 20 Hz, −25 ms) when added 15 min after induction. Filled symbols indicate the presence of AM251.
(B) STD was unaltered when tLTD induction was blocked with AM251 (symbols as in Figure 1C, induction at 10–20 Hz with timing interval −10 or −25 ms).

the previous section, reducing postsynaptic sensitivity with CNQX yielded data points above the diagonal, whereas lowering external Ca^{2+} to reduce presynaptic release yielded points below the diagonal (Figure 2B). The change in CV due to tLTD or to reduced external Ca^{2+} were significantly different compared to CNQX wash-in (ANOVA, $p < 0.001$; t tests, $p < 0.0001$ for both comparisons), but not compared to each other ($p = 0.27$).

Blocking CB1 Receptors Abolishes tLTD

The CV and STD analyses suggest that tLTD is expressed presynaptically, yet induction of tLTD requires postsynaptic activity (Markram et al., 1997b; Sjöström et al., 2001). This implies the need for a retrograde messenger, which signals postsynaptic activity back to the presynaptic terminal.

Endocannabinoids, including anandamide (AEA) and related molecules, have recently been identified as retrograde messengers that mediate short-term modulation of synapses in the hippocampus (Wilson and Nicoll, 2001), cerebellum (Kreitzer and Regehr, 2001), and neocortex (Trettel and Levine, 2003), and LTD in basal ganglia (Gerdeman et al. 2002; Robbe et al., 2002), amygdala (Marsicano et al., 2002), and hippocampus (Chevaleyre and Castillo, 2003). In these systems, activity-dependent postsynaptic release of cannabinoids modifies transmission via presynaptic CB1 receptors. CB1 receptors have been found presynaptically in L5 of visual cortex as well (Ong and Mackie, 1999). We tested the possibility that retrograde signaling during tLTD induction was mediated by presynaptic CB1 receptors in L5 pyramidal pairs by attempting to induce tLTD while blocking these receptors with the selective antagonist AM251 (Gatley et al., 1996). Under these conditions, no LTD occurred and no change in STD was observed (Figure 3; $p = 0.69$). CB1 receptor activation was required during induction but not during expression of tLTD, because washing in the antagonist after induction failed to reverse tLTD (Figure 3A; $p < 0.01$, AM251 during versus after induction). AM251 had no effect on baseline transmission (98% ± 2%, n = 4; $p = 0.82$; not shown), arguing against a direct modulatory role of CB1 receptors at these synapses (cf. Auclair et al., 2000; Misner and Sullivan, 1999; Sullivan, 1999). It was previously found in the hippocam-

pus that CB1 receptor activation increased the amount of LTP, an effect attributed to suppression of inhibition (Carlson et al., 2002). At L5-to-L5 synapses, LTP induced by strong pre- and postsynaptic depolarizations (Markram and Tsodyks, 1996) in the presence of the CB1 receptor blocker AM251 led to significantly increased potentiation (213% ± 22%, n = 9 versus 162% ± 7%, n = 31 in control pairs; $p < 0.01$; not shown), suggesting that, during LTP induction, the amount of potentiation is reduced by simultaneous induction of LTD.

Cannabinoid Receptor Agonists Produce Depression

We hypothesized that, during tLTD, postsynaptic activity is required to generate cannabinoids. In keeping with this idea, activating CB1 receptors directly by applying the specific agonist ACEA (Hillard et al., 1999) could substitute for postsynaptic activity. In the presence of ACEA, presynaptic 30 Hz firing without postsynaptic firing produced a long-lasting synaptic depression that was similar in magnitude to tLTD (Figures 4A and 4B). A similar depression (Figure 4C) was also induced when synapses were activated in the presence of the endogenous CB1 agonist AEA (Hillard, 2000). CV and STD analysis (Figures 4D and 4E) revealed that, like tLTD, cannabinoid-induced depression (cLTD) was expressed presynaptically: the STD index was significantly different when comparing ACEA/AEA wash-in (−0.24 ± 0.01; n = 25; Figure 4E) to AM251 blockade (−0.04 ± 0.02; n = 7; $p < 0.01$; Figure 3B), as was the change in CV ($p < 0.01$; Figure 4D and data not shown). cLTD and tLTD occluded; the depression arising from tLTD induction followed by 15 min AEA wash-in (77% ± 8%) was not significantly different from tLTD alone (73% ± 3%) or from LTD owing to 15 min AEA application alone (79% ± 2%) (ANOVA, $p = 0.74$; Figure 4F).

cLTD Depends on Presynaptic Activity and Presynaptic NMDA Receptors

As noted previously, tLTD requires presynaptic activity (Markram et al., 1997b; Sjöström et al., 2001), as postsynaptic activity alone produced no LTD (Figure 1B). cLTD shares with tLTD this requirement for presynaptic activity. Inactive inputs did not depress in the presence of ACEA, but once activity resumed, these same synapses depressed (Figure 4B). Similarly, 15 min AEA application in the absence of presynaptic activity produced no LTD (Figure 4C, 123% ± 13%, n = 8; $p < 0.05$ compared to AEA with activity). Because metabotropic glutamate receptors (mGluRs) have previously been implicated in some forms of LTD (Egger et al., 1999; Kemp et al., 2000; Oliet et al., 1997; Zakharenko et al., 2002), we tested the possibility that the dependence of ACEA-induced depression on presynaptic activity was mediated by glutamatergic autoreceptors. The broad-spectrum mGluR antagonist LY341495 (Sawtell et al., 1999) did not block ACEA-induced depression, however, indicating that mGluRs are not involved (Figure 4F). Surprisingly, cLTD was blocked by the selective NMDAR antagonists APV (Figure 4F; $p < 0.05$) and MK801 (104% ± 2.7%, n = 5; $p < 0.01$; data not shown). Because this depression is expressed presynaptically in the absence of postsynaptic activity, the most parsimonious interpretation is that these NMDARs are located presynaptically. NMDAR subunits have previously been identified in presynaptic terminals of rat visual cortical neurons (Aoki et al., 1994; Charton et al., 1999; DeBiasi et al., 1996). An alternative hypothesis is that a rise in Ca^{2+} owing to influx through postsynaptic NMDARs is required for cLTD. To rule this out, we perfused the postsynaptic neuron with the Ca^{2+} chelator BAPTA. As expected from its ability to block cannabinoid production (Di Marzo et al., 1994), suppression of inhibition (Kreitzer and Regehr, 2001; Trettel and Levine, 2003), and LTD (Egger et al., 1999; Mulkey and Malenka, 1992), postsynaptic BAPTA blocked induction of tLTD (Figure 4F). However, postsynaptic BAPTA had no effect on cLTD (Figure 4F; $p < 0.01$). Because Ca^{2+} influx through postsynaptic NMDARs is not required for cLTD, the NMDAR dependence of this LTD is likely to arise presynaptically.

Presynaptic NMDARs Modulate Neurotransmission

Presynaptic NMDARs have previously been found to enhance spontaneous release in entorhinal cortex (Berretta and Jones, 1996; MacDermott et al., 1999; Woodhall et al., 2001). We obtained similar results in visual cortical L5 neurons: APV reversibly reduced the frequency ($p < 0.0001$) but not the amplitude ($p = 0.85$) or rise time ($p = 0.49$) of miniature excitatory postsynaptic currents (mEPSCs; Figure 5). These data suggest that Ca^{2+} influx through presynaptic NMDARs elevate mEPSC frequency, perhaps by contributing to resting Ca^{2+} levels in the presynaptic terminal.

Presynaptic NMDARs have also been previously implicated in the regulation of evoked release in the cerebellum (Glitsch and Marty, 1999) and spinal cord (Liu et al., 1997) (see MacDermott et al., 1999, for review). We found a similar presynaptic regulatory function in visual cortical L5 pairs. APV dramatically and reversibly reduced the strength of individual connections during 30 Hz, but not 0.1 Hz, firing (Figure 6B; $p < 0.001$). This reduction is unlikely to reflect blockade of postsynaptic receptors, as identical results occurred during voltage clamp of the postsynaptic neuron at −90 mV (Figures 6A and 6B). A similar reduction of neurotransmission was obtained with MK801 wash-in during 30 Hz firing (55% ± 15%, n = 3; Figure 6D, triangles) but not at 0.1 Hz (102% ± 3.6%, n = 4, $p < 0.01$; data not shown). These results suggest that the presynaptic NMDARs are necessary for maintenance of neurotransmission during high-frequency firing.

It was previously reported that presynaptic NMDARs contain the NR2B subunit (Woodhall et al., 2001) at an age when postsynaptic NMDARs do not (Stocca and Vicini, 1998). We reproduced this finding by showing that, in P14–15 animals, ifenprodil had no effect on the NMDA:AMPA ratio (103% ± 6%, $p = 0.62$, n = 4; data not shown), whereas MK801 or APV wash-in reduced the ratio to 83% ± 6% of control values ($p < 0.05$, n = 15), as measured at resting membrane potential (cf. Markram et al., 1997a). At the same animal ages, the NR2B subunit-specific antagonist ifenprodil (Williams, 1993) reduced neurotransmission during 30 Hz, but not 0.1 Hz, firing (Figure 6C; $p < 0.005$). The locus of depression due to application of the NMDAR antagonists APV, MK801, or ifenprodil appeared presynaptic by CV and

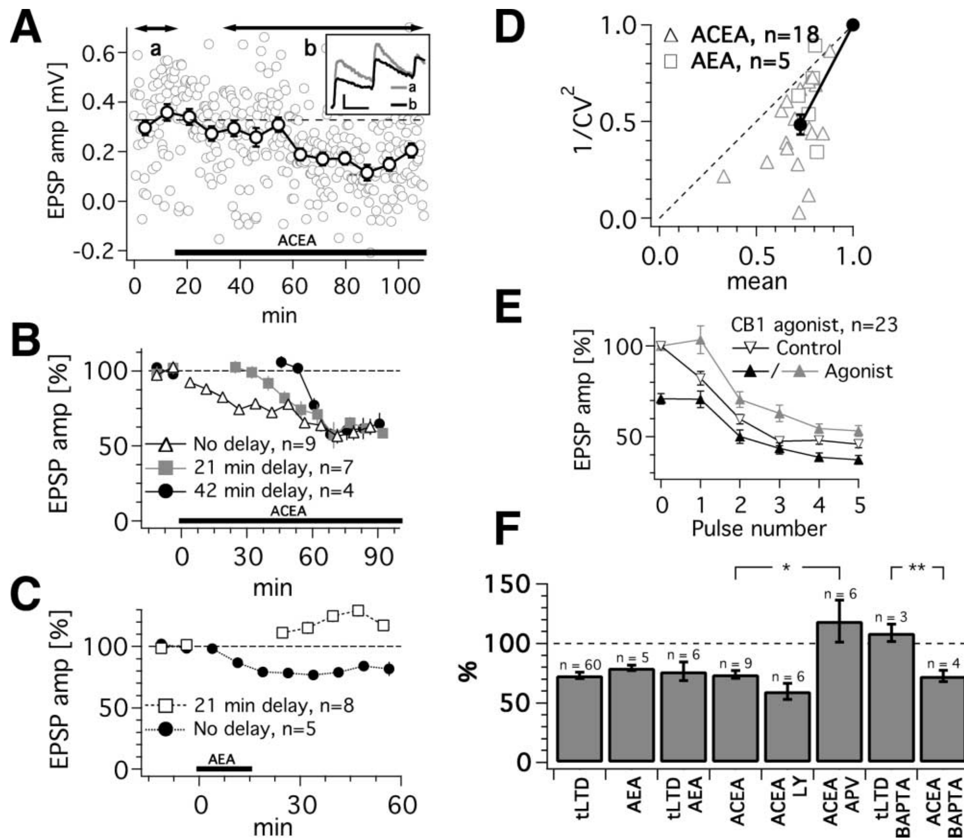

Figure 4. CB1 Receptor Agonists Mimic tLTD

(A) In the presence of presynaptic activity, the CB1 agonist ACEA produced depression in this paired recording. Inset: responses averaged over the horizontal arrows (a and b) indicate an accompanying change in STD. Scale bars: 0.1 mV, 20 ms.

(B) ACEA-induced depression is activity dependent because, in the presence of ACEA, depression occurs only after onset of presynaptic spiking (squares, 21 min delay; circles, 42 min delay).

(C) Brief wash-in of anandamide ("AEA") resulted in LTD in the presence (circles), but not the absence (squares), of presynaptic activity.

(D) CV analysis of CB1 agonist-induced depression suggests presynaptic expression.

(E) In agreement, CB1 agonist-induced depression consistently reduced STD (symbols as in Figure 1C).

(F) tLTD and anandamide ("AEA") produce comparable depression and occlude each other, because applying AEA after inducing tLTD ("tLTD & AEA") did not produce additional depression. AEA was applied for 15 min, as in Figure 3C. Depression induced by presynaptic activity in ACEA ("ACEA") is not blocked by LY341495 ("ACEA LY"), a broad-spectrum metabotropic glutamate receptor blocker, but is blocked by the NMDAR blocker APV ("ACEA APV"), suggesting that the activity dependence is due to presynaptic NMDARs. Consistent with this view, loading the postsynaptic cell with the Ca^{2+} chelator BAPTA blocked tLTD induction ("tLTD BAPTA") but not ACEA-induced LTD ("ACEA BAPTA"). These results argue strongly that the NMDAR dependence of CB1 agonist-induced depression does not arise postsynaptically.

STD analysis (Figures 6D and 6E; pooled STD index: -0.73 ± 0.40, n = 17). These results argue strongly that presynaptic NR2B-containing NMDARs modulate cortical synaptic transmission.

Activation of Presynaptic NMDARs Is Necessary for tLTD, but not for tLTP

Our data demonstrate that NMDAR blockers both suppress evoked neurotransmission (Figure 6) and abolish LTD induced by post-before-pre spiking (Sjöström et al., 2001) or by cannbinoid application (Figure 4F). To rule out that this apparent block of tLTD is due to suppression of evoked synaptic transmission by APV, we washed in and washed out APV and AEA in sequence, in the continuous presence of presynaptic 30 Hz firing. Note that the effects of both AEA and APV readily wash out (cf. Figures 4C and 6B). Combined wash-in of APV-AEA (Figures 7A and 7B) reproduced the presynaptic suppressive effect of APV (Figures 6A and 6B), but the responses recuperated after both drugs had washed out (Figures 7A and 7B; $p < 0.001$ for APV-AEA versus AEA-induced LTD; cf. Figure 4C), arguing that APV abolished cannabinoid-induced LTD by blocking induction of LTD, rather than by masking LTD through suppression of neurotransmission. This experiment furthermore underscores that presynaptic NMDARs are sensors for presynaptic activity: persistent effects of AEA wash-in in the presence of presynaptic spiking and NMDAR blockade (Figure 7B) are equivalent to those of AEA wash-in in the absence of presynaptic firing (Figure 4C; $p = 0.17$), since no LTD is produced in either case. In conclusion, these data suggest that presynaptic activity, as detected by presynaptic NMDARs, is a prerequisite for this form of LTD.

As mentioned above, presynaptic NMDARs are ifenprodil sensitive (Figures 6B and 6C; Woodhall et al.,

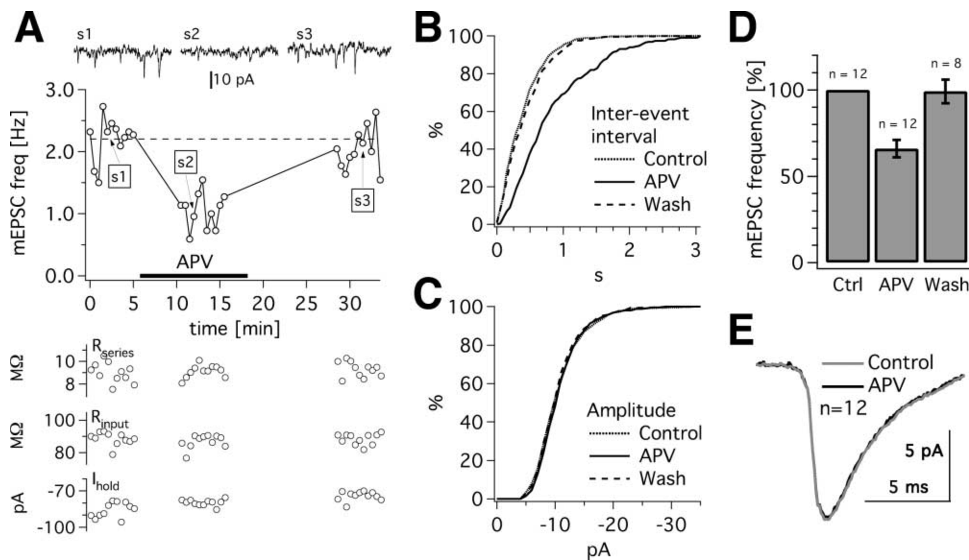

Figure 5. There Are Presynaptic NMDARs at Excitatory Synapses onto Neocortical L5 Neurons

(A) Sample voltage-clamp recording in which wash-in of the NMDAR blocker APV (horizontal bar) reversibly reduced mEPSC frequency. Sample traces (top) before (s1), during (s2), and after (s3) APV wash-in are 0.5 s long. R_{series}, R_{input}, and I_{hold} did not change significantly.

(B) Cumulative histogram of interevent interval of the sample experiment in (A), showing reversible reduction in mEPSC frequency due to APV wash-in.

(C) APV had no effect on mEPSC amplitude in the same experiment.

(D) APV robustly reduced mEPSC frequency reversibly. This observation strongly argues for the existence of presynaptic NMDARs. Control mEPSC frequency was 1.9 ± 0.3 Hz.

(E) The amplitude and kinetics of mEPSCs were not affected, arguing against a postsynaptic effect of APV.

2001), presumably because they contain the NR2B subunit (Williams, 1993), even though it has previously been demonstrated that postsynaptic NMDARs in visual cortical L5 neurons age P13–15 are not blocked by the ifenprodil analog CP101,606 (Stocca and Vicini, 1998). Since LTP depends critically on the activation of postsynaptic NMDARs, these observations suggest that tLTD, but not LTP, is ifenprodil sensitive. Indeed, Figure 7C illustrates a paired recording in which tLTD was abolished by ifenprodil, but LTP induced by pairing 200 ms long bursts (Markram and Tsodyks, 1996) was not. This LTP protocol produced potentiation in the presence of ifenprodil (192% ± 22%, n = 5) that was indistinguishable from control experiments (162% ± 7.4%, n = 31, p = 0.14; data not shown; ages P12–16), even though it is known to be blocked by APV (Markram and Tsodyks, 1996). Due to the short-lasting effects of ifenprodil during high-frequency firing (Figures 6B and 6C), the onset of LTP was delayed. This, together with the fact that LTP was induced late in the recordings, may have led to an underestimate of the magnitude of LTP in the presence of ifenprodil. Unlike LTP, tLTD was reliably blocked by ifenprodil (Figure 7D; $p < 0.001$).

Comparable results were obtained with timing-dependent LTP induced at 50 Hz (tLTP) (Markram et al., 1997b; Sjöström et al., 2001): the same amount of potentiation was obtained in the presence as in the absence of ifenprodil (Figure 7D; p = 0.85; ages P14–16). tLTP is also blocked by APV (Figure 7D; Markram et al., 1997b; Sjöström et al., 2001). The most parsimonious explanation of these data is that tLTD and tLTP can be pharmacologically dissociated by ifenprodil (Figure 7D) because tLTD, but not tLTP, depends critically on presynaptic NR2B-containing NMDARs.

CB1 Agonist-Mediated LTD Depends on Frequency

NMDARs must bind glutamate and depolarize to open. The frequency dependence of NMDAR antagonist-mediated suppression of synaptic transmission (Figures 6B and 6C) indicates that presynaptic NMDARs are more strongly activated by high-frequency firing. Presumably, during low-frequency firing (0.1 Hz), glutamate binds to presynaptic NMDARs only after the terminal has repolarized. However, with 30 Hz firing, the spikes succeeding the first in a high-frequency burst provide the necessary depolarization. This view argues that ACEA-induced LTD (Figures 4A and 4B) should be frequency dependent as well. In agreement, 0.1 and 5 Hz firing did not produce LTD in the presence of ACEA (100% ± 7%, n = 10), whereas 15 and 30 Hz firing did (71% ± 3%, n = 14, $p < 0.01$; Figure 8A). Similar results were obtained with the endogenous cannabinoid AEA: 0.1 Hz firing yielded no LTD (105% ± 5%, n = 3), whereas 30 Hz firing did (79% ± 2%, n = 5, $p < 0.01$; Figure 8A). Given the indistinguishable results obtained with ACEA and AEA and the fact that responses were stable at low frequencies, we conclude that these effects are probably not due to some nonspecific drug-induced response rundown.

As opposed to CB1 receptor agonist-induced LTD (Figure 8A), tLTD can be produced at frequencies ranging from 0.1 to 20 Hz at these synapses (Sjöström et al., 2001). We therefore investigated whether CB1 receptors

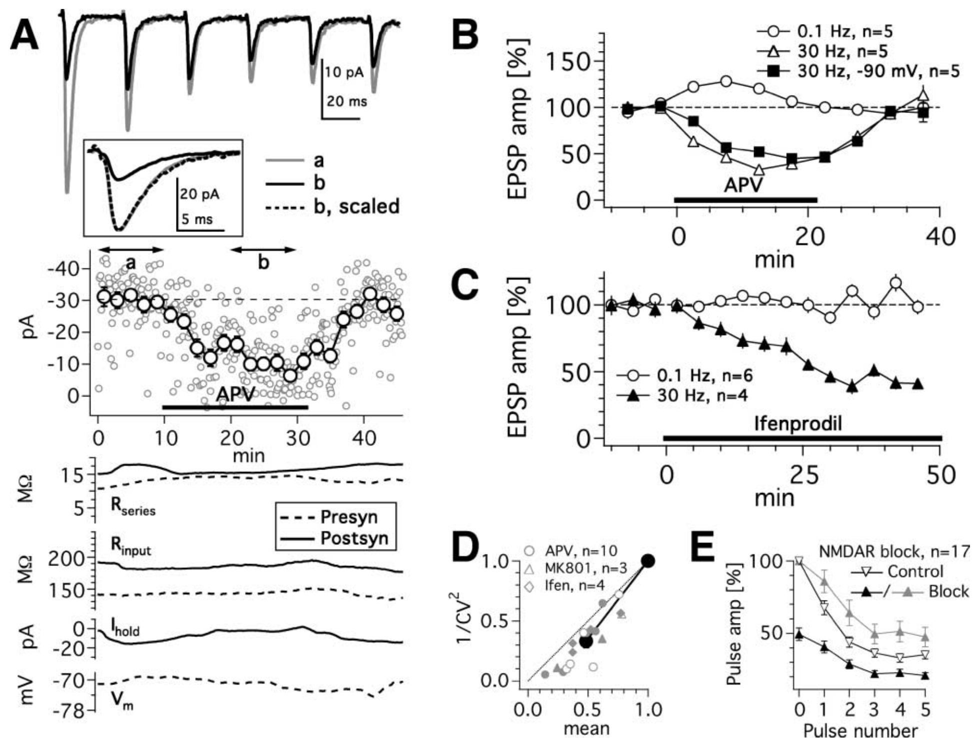

Figure 6. Presynaptic NR2B-Containing NMDARs Modulate Neocortical L5 Neurotransmission

(A) Sample paired recording illustrating the reversible suppressive effect of NMDAR blockade on neurotransmission. To minimize postsynaptic NMDAR-mediated responses, the postsynaptic cell was voltage clamped at −90 mV. Averaged responses before (a) and during (b) application of APV demonstrate a change in STD due to APV, and also the absence of a postsynaptic NMDAR-mediated component (inset, top, shows the first response in a train). R_{series}, R_{input}, presynaptic V_m, and postsynaptic I_{hold} did not change significantly.

(B) APV wash-in (horizontal bar) during 30 Hz spiking reversibly depressed unitary L5 connections recorded in current clamp (triangles) or in voltage clamp at −90 mV (squares, as described in A). Transmission was not depressed when spiking at 0.1 Hz (circles), indicating effects of presynaptic NMDARs are frequency dependent.

(C) The NR2B-specific NMDAR antagonist ifenprodil suppressed neurotransmission at 30 Hz (triangles), but not at 0.1 Hz (circles), suggesting presynaptic NMDARs contain the NR2B subunit.

(D) The suppressive effect of NMDAR blockers APV, MK801, and ifenprodil in the presence of 30 Hz spiking appeared presynaptic by CV analysis. Open symbols indicate experiments done with postsynaptic voltage clamp (as in A), while closed symbols were obtained in current clamp at resting V_m.

(E) Consistent with a presynaptic effect of NMDAR blockers during 30 Hz firing, STD was reduced. Data was pooled from the experiments with APV, ifenprodil, and MK801 described in (D). Symbols are as in Figure 1C.

are involved in tLTD at low frequencies (<10 Hz). Indeed, the CB1 antagonist AM251 abolished tLTD induced at 0.1 (101% ± 4%, n = 4; $p < 0.01$) and 20 Hz (102% ± 7%, n = 7, $p < 0.01$; Figure 8B). Endocannabinoid action on CB1 receptors is thus necessary but not sufficient for low-frequency tLTD. Some additional retrograde signal that we have not yet identified may transiently enhance NMDAR-mediated Ca^{2+} influx during low-frequency induction of tLTD. In agreement, low-frequency tLTD was abolished by NMDAR blockade (107% ± 4%, n = 7, $p < 0.01$; Figure 8B). In particular, low-frequency tLTD was blocked by ifenprodil (106% ± 6%, n = 5; data pooled in Figure 8B), supporting the idea that the NMDARs relevant to low-frequency tLTD are presynaptically located and contain the NR2B subunit (Figures 6C–6E; Woodhall et al., 2001). These data suggest that, during low-frequency post-before-pre spiking within the tLTD temporal window, presynaptic NMDARs are being activated (Figure 8B, squares). However, during unpaired low-frequency presynaptic spiking, they are not (Figure 8A). In conclusion, tLTD requires coincident activation of presynaptic NMDARs and CB1 receptors, but some unknown retrograde messenger is also needed for NMDAR activation at low frequencies.

A Model for Neocortical L5 tLTD

Taken together, our findings suggest a simple model of tLTD induction: postsynaptic firing causes release of an endogenous cannabinoid, which activates presynaptic CB1 receptors; presynaptic firing activates presynaptic NMDARs; and the coincidence of CB1 and NMDA receptor activation produces a lasting depression of subsequent transmitter release (Figure 9A). An additional, unknown signal arising from the postsynaptic side may be involved in activating presynaptic NMDARs during low-frequency tLTD (cf. Figure 8). Regardless of the nature of this unknown signal (see Discussion), the model suggests the possibility that the temporal window over which pre- and postsynaptic firing induces tLTD reflects the time during which the endogenous cannabinoid is available at the presynaptic terminal. To test this idea, we inhibited the fatty acid amide hydrolase that normally

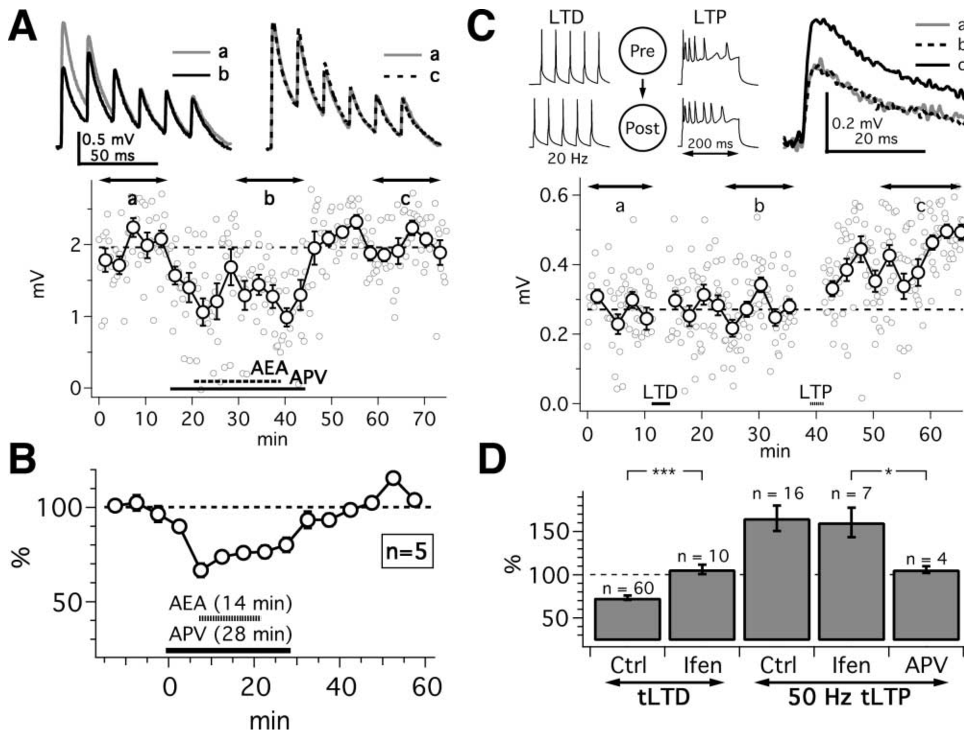

Figure 7. Activation of Presynaptic NMDARs Is Necessary for tLTD, but not for tLTP

(A) LTD induced by anandamide wash-in (dashed horizontal bar, "AEA") was abolished by NMDAR blockade (continuous horizontal bar, "APV") in this sample recording. Averaged response in the presence of APV/AEA (b) had significantly less STD as compared to control trace (a), and this effect washed out (c), consistent with the reversible suppressive presynaptic effect of APV (Figure 6). In addition, AEA-induced LTD (Figure 4C) was blocked by APV (cf. traces a and c). R_{series}, R_{input}, and V_m did not change significantly (not shown).

(B) APV wash-in reversibly suppressed neurotransmission (as described in Figure 6) and also abolished AEA-induced LTD (circles), suggesting activation of presynaptic NMDARs is required for this form of LTD.

(C) LTD induction by post-before-pre firing (−25 ms) at 20 Hz (continuous horizontal bar, "LTD"; APs inset top left) was abolished by the NR2B-specific NMDAR antagonist ifenprodil (present throughout). However, LTP induction (dashed horizontal bar, "LTP"; APs inset top middle) by pairing pre- and postsynaptic 200 ms long strong depolarizations was not abolished by ifenprodil. Inset top right: averaged traces from control (a), LTD (b), and LTP (c) periods (illustrated by horizontal arrows). R_{series}, R_{input}, and V_m did not change significantly (not shown). Baseline was at 0.1 Hz.

(D) Although ifenprodil abolished tLTD induction ("tLTD" induced at 0.1–20 Hz, −5 to −25 ms interval), similar tLTP (induced at 50 Hz) was obtained in the absence and presence of the NR2B subunit-specific NMDAR antagonist ifenprodil. The subunit-nonspecific NMDAR blocker APV, however, abolished tLTP, suggesting that tLTD, but not tLTP, depends critically on presynaptic NR2B-containing NMDARs.

terminates cannabinoid action (Kreitzer and Regehr, 2002; Wilson and Nicoll, 2002) with the inhibitor AA-5-HT (Bisogno et al., 1998), reasoning that this should prolong cannabinoid availability and hence increase the timing window for tLTD. Prior work has demonstrated that when the time between postsynaptic and presynaptic firing (Δt) is longer than 100 ms, no tLTD is observed at these synapses (Markram et al., 1997b; Sjöström et al., 2001). Indeed, under control conditions, no tLTD was obtained for Δt of −120 and −200 ms. In the presence of AA-5-HT, however, Δt of −120, −200, and −400 ms produced robust tLTD, indicating that the window had significantly widened (Figure 9B). It is important to note that AA-5-HT did not result in nonspecific EPSP rundown, because Δt of more than +25 ms (outside both the LTP and the LTD timing windows; see Sjöström et al., 2001) did not result in LTD (108% ± 3%, n = 4; Figure 9B). Furthermore, the entire timing curve was not shifted down by AA-5-HT, since Δt of −25 ms resulted in indistinguishable amounts of depression in the presence as in the absence of AA-5-HT (p = 0.9; Figures 9B and 9C).

Inactivation of cannabinoids also requires a specific transporter (Kreitzer and Regehr, 2002; Wilson and Nicoll, 2002), which is inhibited by the drug AM404 (Beltramo et al., 1997). Like AA-5-HT, AM404 enabled LTD at timings previously outside the tLTD temporal window (Figure 9C). Cannabinoid availability is presumably determined not only by transport and hydrolysis, but by how much is produced. As with suppression of inhibition (Pitler and Alger, 1992), increased postsynaptic firing should enhance cannabinoid production, prolong its action, and therefore also widen the tLTD temporal window. Consistent with this, a burst of five postsynaptic APs at 20 Hz paired with a single presynaptic spike occurring 120 or 200 ms after the end of the burst produced robust tLTD (Figure 9C; see also Debanne et al., 1994). As expected, tLTD induced in the presence of AA-5-HT, AM404, and postsynaptic bursting were all expressed presynaptically as assessed by CV analysis

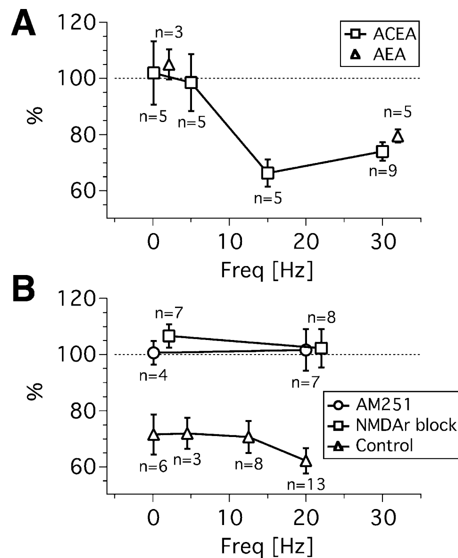

Figure 8. CB1 Agonist-Mediated LTD, but not tLTD, Depends on Frequency

(A) CB1 agonist-mediated LTD depends on frequency. Consistent with the involvement of presynaptic NMDARs (cf. Figures 6B and 6C), low-frequency firing in the presence of ACEA (squares) or AEA (triangles; displaced +2 Hz for visibility) produced no long-term plasticity. This implies that continuous CB1 receptor activation is sufficient for high-frequency, but not low-frequency, tLTD.

(B) The CB1 receptor antagonist AM251 (circles) as well as NMDAR blockade (squares; displaced +2 Hz for visibility) abolished tLTD over a wide range of frequencies. As a comparison, tLTD induction produced robust depression over the same frequency range (triangles, data pooled from Figure 1 and from previous experiments reported in Sjöström et al., 2001). These data suggest that simultaneous CB1 receptor and NMDAR activation is necessary for tLTD at all frequencies.

(Figure 9D), indicating that these manipulations did not produce some nonspecific postsynaptic EPSP rundown.

Discussion

Coincidence detection during Hebbian plasticity has traditionally been presumed to occur postsynaptically (Bi and Poo, 2001; Malenka and Nicoll, 1999). Experiments described here suggest that during induction of tLTD, coincidence detection can also occur at the presynaptic terminal. This novel form of presynaptic coincidence detection integrates signaling through NMDA autoreceptors and CB1 cannabinoid receptors and shapes the temporal window over which pre- and postsynaptic firing can induce tLTD.

L5 tLTD Is Presynaptically Expressed

Several observations suggested that L5 tLTD is expressed presynaptically. tLTD reduced STD during brief trains of high-frequency firing and produced a change in CV consistent with a presynaptic reduction in transmitter release. Control experiments using CNQX and lowered Ca^{2+} concentration verified that these two methods could indeed distinguish pre- from postsynaptic effects. Also, since pharmacological experiments argue for presynaptic induction via CB1 and NMDA receptors (see below), a postsynaptic expression mechanism would require an additional anterograde messenger. A more parsimonious interpretation is that induction and expression occur presynaptically.

Changes in CV (Faber and Korn, 1991) and in STD (Poncer and Malinow, 2001) can be subject to alternative interpretations. Specifically, a selective postsynaptic reduction in the quantal amplitude at the least variable synapses or at the most strongly depressing synapses could produce an apparently presynaptic reduction in transmission. It is important to note, however, that to explain the present results, tLTD would have to reliably occur only at the least variable and most depressing of the approximately five to eight synaptic contacts that comprise L5 unitary connections (Markram et al., 1997a). Not only did STD and $1/CV^2$ decrease on average, they decreased in each connection studied. In contrast, changes in paired-pulse facilitation (PPF) attributed to postsynaptic LTP are equally likely to increase as to decrease PPF (see Figure 2C in Poncer and Malinow, 2001).

In addition, some of the caveats relevant to the use of CV analysis in extracellular stimulation experiments do not apply. For example, with unitary connections, the synapse population is more homogenous and well defined and is not contaminated by inhibition or by multiple classes of excitatory synapses with widely differing properties. With extracellular stimulation, afferent fibers can be lost or gained, which is not possible with connected pairs.

Although cerebellar LTD (Wang and Linden, 2000) and NMDAR-dependent hippocampal CA1 LTD (Kemp et al., 2000; Oliet et al., 1997) are both postsynaptically expressed, a presynaptic locus has been demonstrated for LTD in the striatum (Gerdeman et al., 2002), nucleus accumbens (Robbe et al., 2002), and amygdala (Marsicano et al., 2002). There is also a presynaptically expressed mGluR-dependent form of LTD in CA1 (Kemp et al., 2000; Oliet et al., 1997; Zakharenko et al., 2002) and between neocortical L4 spiny stellate neurons (Egger et al., 1999). The induction mechanisms of tLTD at neocortical L5 and neocortical L4 spiny stellate synapses appear to be different, however, as mGluRs are involved in L4 tLTD (Egger et al., 1999) but not L5 tLTD (Figure 4F). In addition, induction of these two neocortical forms of tLTD exhibits distinct timing requirements (Egger et al., 1999; Sjöström et al., 2001). Induction and expression mechanisms of LTD are thus diverse and depend on brain region, synaptic identity, and experimental protocol.

Endocannabinoids as Retrograde Messengers

A role for endocannabinoids as retrograde messengers in LTD has recently been demonstrated in the striatum (Gerdeman et al., 2002), nucleus accumbens (Robbe et al., 2002), amygdala (Marsicano et al., 2002), and hippocampus (Chevaleyre and Castillo, 2003). Unlike cortical tLTD, these forms of LTD require activation of mGluRs and in most cases do not require NMDARs (Calabresi et al., 1992; Chevaleyre and Castillo, 2003; Partridge et al., 2000; Robbe et al., 2002). Prior studies have revealed that cannabinoids can modulate the induction of LTP and LTD at hippocampal and neocortical

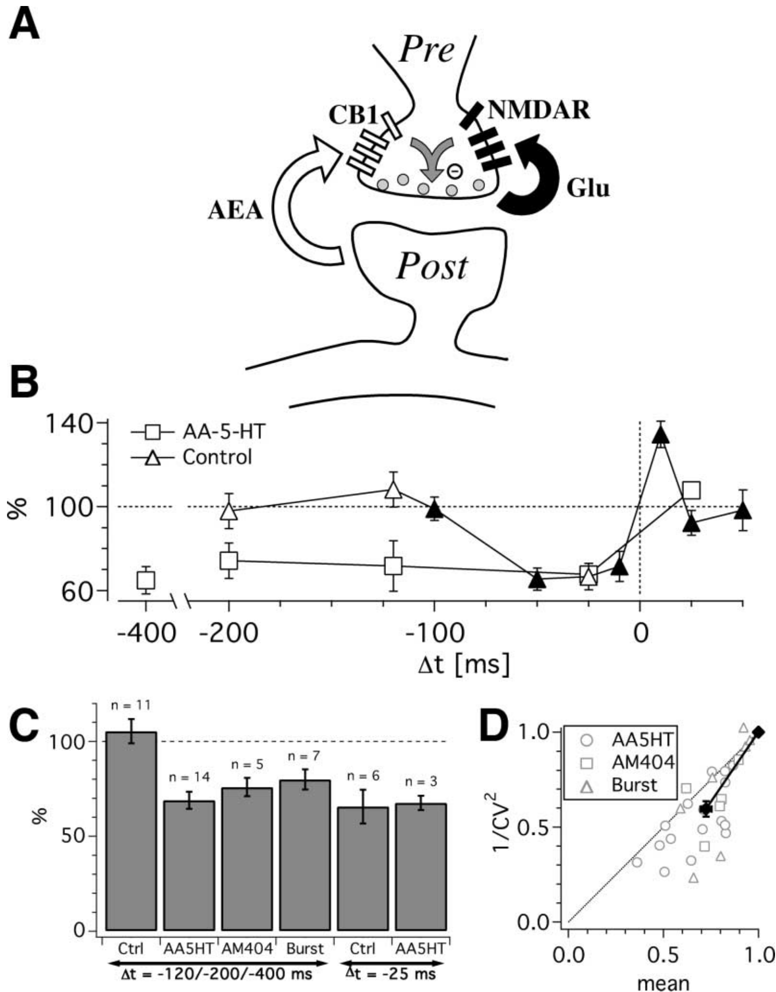

Figure 9. The tLTD Temporal Window Depends on Endocannabinoid Availability

(A) Proposed model for tLTD at L5 synapses. Postsynaptic activity produces release of AEA or other endocannabinoid (white arrow), which activates presynaptic CB1 receptors. Presynaptic release of glutamate (Glu, dark arrow) activates presynaptic NMDARs. Coincidence of both signals results in tLTD by reducing subsequent release (gray arrow). An additional, unknown signal arising from the postsynaptic side may be involved in activating presynaptic NMDARs during low-frequency tLTD (not shown).

(B) This model predicts that the tLTD window (illustrated by spike-timing curve, triangles) should broaden if termination of endocannabinoid response is slowed. Consistent with this, the FAAH antagonist AA-5-HT widens the tLTD temporal window (squares). Filled symbols represent data reproduced for comparison from prior experiments (Sjöström et al., 2001). Note that AA-5-HT does not cause nonspecific EPSP rundown, since positive timings (+25 through +400 ms pooled, n = 4) did not result in LTD. Induction and baseline were at 0.1 Hz.

(C) The FAAH blocker AA-5-HT and the AEA transporter antagonist AM404 both broaden the tLTD temporal window. Postsynaptic bursting (5 spikes at 20 Hz), which may increase endocannabinoid release, also widens the temporal window of tLTD. Importantly, AA-5-HT does not act by shifting the spike timing curve down, because it does not enhance tLTD for $\Delta t = -25$ ms. Induction and baseline were at 0.1 Hz.

(D) LTD produced in the presence of the FAAH blocker AA-5-HT (circles), the AEA transporter antagonist AM404 (squares), and postsynaptic bursting (triangles) appeared presynaptic by CV analysis.

synapses (Auclair et al., 2000; Carlson et al., 2002; Misner and Sullivan, 1999; Sullivan, 1999). Taken together, these studies suggest that cannabinoid signaling may play a more widespread role in synaptic plasticity than previously appreciated. In addition, the identification of cannabinoids as key mediators of spike timing-dependent plasticity in the neocortex may help explain the well-documented effects of cannabis on cognitive function (Solowij, 1998).

Several pieces of evidence suggest that, as at other synapses, the endogenous cannabinoid is produced postsynaptically and acts on the presynaptic terminal. The Ca^{2+} chelator BAPTA blocked tLTD when introduced into the postsynaptic neuron, presumably because it interferes with cannabinoid production (Di Marzo et al., 1994; Kreitzer and Regehr, 2001). However, it had no effect on depression produced by direct activation of cannabinoid receptors. The depression induced by CB1 activation, like that produced during tLTD, had the physiological hallmarks of a presynaptic reduction in transmitter release. This presynaptic depression is not akin to more rapid endocannabinoid-mediated modulatory effects observed at synapses in hippocampus (Wilson and Nicoll, 2001), cerebellum (Kreitzer and Regehr, 2001), and neocortex (Trettel and Levine, 2003). At these synapses, strong depolarization of the postsynaptic neuron suppresses inhibition (DSI) or excitation (DSE) within 2 s and for up to 60 s (Kreitzer and Regehr, 2001; Pitler and Alger, 1992; Wilson and Nicoll, 2001). The depression observed at L5-to-L5 synapses, however, expresses more slowly, lasts longer, requires presynaptic activity (Figure 1B), and, once induced, does not require continued activation of CB1 receptors (Figures 3A and 4C). In addition, excitatory L5-to-L5 synapses do not undergo DSE (P.J.S. and S.B.N., unpublished data).

Results presented here suggest that enhanced availability of an endogenous cannabinoid at the presynaptic terminal can lengthen the timing window for tLTD. Impeding endocannabinoid hydrolysis pharmacologically extended the timing window (Figures 9B and 9C), as did increasing postsynaptic activity (Figure 9C), which would be expected to enhance endocannabinoid production. Prior studies have found that increased postsynaptic firing lengthens DSI (Pitler and Alger, 1992) and can lengthen the temporal window for tLTD in hippocampal CA3 slice culture (Debanne et al., 1994). Variation in the rate of cannabinoid production or inactivation at different synapses may account for previously observed differences in the duration of the temporal win-

dow for tLTD (Bi and Poo, 1998; Debanne et al., 1998; Feldman, 2000; Sjöström et al., 2001; Zhang et al., 1998).

Presynaptic NMDA Receptors

Activation of CB1 receptors produced LTD that required presynaptic activity and was still NMDAR dependent. CB1 agonist-induced LTD did not require an NMDAR-mediated rise in postsynaptic Ca^{2+}, however, because it was not blocked by postsynaptic BAPTA (Figure 4F). The simplest explanation for these observations is that the NMDARs are located on the presynaptic terminal. Presynaptic NMDARs have previously been proposed to modulate release in the cerebellum (Glitsch and Marty, 1999) and entorhinal cortex (Woodhall et al., 2001) and to play a role in cerebellar LTD (Casado et al., 2002). NR1 and NR2A/B subunits have been observed at cortical terminals using immunoelectron microscopy and light microscopy, and at least some of these are located on terminals made by L5 pyramidal neurons (Aoki et al., 1994; Charton et al., 1999; DeBiasi et al., 1996).

For DSI (Kreitzer et al., 2002; Wilson and Nicoll, 2001) and some forms of long-term plasticity that also depend on retrograde signaling (Engert and Bonhoeffer, 1997; Schuman and Madison, 1994), diffusion of the retrograde messenger can significantly reduce synapse specificity. The dual requirement for activation of presynaptic NMDA and CB1 receptors in L5 tLTD may impart synapse specificity to retrograde signaling by restricting depression to concurrently active synapses.

In addition to their role in tLTD induction, presynaptic NMDARs appear to strongly modulate transmission between L5 neurons. This was evident from the ability of APV to reversibly reduce the strength of evoked transmission during 30 Hz firing, an effect that was presynaptic by STD and CV analysis. This modulation persisted even when activation of postsynaptic NMDA receptors was abolished by voltage clamp to −90 mV (Figure 6A). The open channel blocker MK-801 also modulated transmission presynaptically, arguing against nonspecific effects of APV. Consistent with a presynaptic action, APV also reversibly reduced the frequency of mEPSCs without affecting their amplitude or kinetics. Further work is needed to determine whether or not this modulation is also present at excitatory synapses in other layers, in other cortical regions, and at other times in development. The ability of presynaptic NMDARs to modulate transmission implies that conclusions reached from prior antagonist studies implicating postsynaptic NMDARs in learning, experience-dependent plasticity, and sensory function may need to be reexamined.

Postsynaptic NMDARs in rat visual cortical L5 neurons, like those at many other synapses (Sheng et al., 1994; Williams et al., 1993), undergo a developmental switch in their subunit composition so that they are no longer sensitive to the NR2B-specific antagonist ifenprodil at the ages we studied (Stocca and Vicini, 1998). However, we found that presynaptic reduction of neurotransmission was still ifenprodil sensitive (Figures 6C and 6D), in agreement with a prior study in entorhinal cortex (Woodhall et al., 2001). Although each of the NMDAR antagonists studied—APV, MK-801, and ifenprodil—produced similar effects on evoked transmission and all blocked tLTD, only antagonists active at postsynaptic NMDARs—APV and MK-801—blocked tLTP. This pharmacological dissociation between tLTP and tLTD is consistent with the known requirement for postsynaptic NMDARs in LTP induction as well as the requirement for presynaptic NMDARs in tLTD induction proposed here. This does not, however, rule out a possible additional contribution of postsynaptic NMDARs in tLTD.

Frequency Dependence

The effects of blocking presynaptic NMDARs were frequency dependent. The effect of APV, MK-801, and ifenprodil was to potently depress transmission during 30 Hz firing, whereas transmission was not affected during 0.1 Hz firing (Figures 6B and 6C). The lack of depression during low-frequency firing may seem surprising in light of the dramatic reduction in mEPSC frequency observed after APV application (Figure 5). However, the mechanisms that generate evoked and spontaneous release are likely to differ. Presynaptic NMDAR activation by ambient glutamate may enhance spontaneous release by increasing Ca^{2+} levels in the terminal, but presumably this does not contribute significantly to evoked release. In addition to binding ambient glutamate, presynaptic NMDARs would be expected to bind glutamate originating from evoked release at the same terminal. During low-frequency firing, however, evoked glutamate binds only after the terminal has been depolarized by the AP. Assuming presynaptic NMDARs exhibit Mg^{2+} block, the resulting Ca^{2+} flux is small. On the other hand, during high-frequency firing, evoked glutamate and depolarization are present simultaneously. The resulting NMDAR-dependent Ca^{2+} influx is likely to be significantly greater under these conditions (cf. Casado et al., 2002).

We hypothesize that during baseline transmission, presynaptic NMDARs enhance the ability of the terminal to sustain high-frequency release. This could occur, for example, by enhancing Ca^{2+}-dependent mechanisms that replenish the readily releasable pool (Dittman and Regehr, 1998; Klingauf et al., 1998). We cannot completely rule out the possibility that the relevant NMDARs are located on cells other than the ones recorded from, and act on the presynaptic terminal via another unidentified messenger. However, given the demonstration of presynaptic NR1 (Aoki et al., 1994) and NR2A/B (Charton et al., 1999; DeBiasi et al., 1996) subunits, this is not the most parsimonious explanation.

CB1 agonist-induced LTD depended on frequency, consistent with the need for presynaptic NMDAR activation. However, as opposed to cerebellar NMDAR-dependent LTD (Casado et al., 2002), neocortical L5 tLTD is not frequency dependent (Figure 8B; Sjöström et al., 2001). These results indicate that there is some additional signal that transiently enhances NMDAR-mediated Ca^{2+} influx during low-frequency induction of L5 tLTD. This signal, which could be the same endocannabinoid, may transiently depolarize the presynaptic terminal to boost NMDAR Ca^{2+} flux during low-frequency post-before-pre firing. Alternatively, postsynaptic activity-dependent dendritic release of glutamate (Nedergaard et al., 2002) would ensure that presynaptic NMDARs are glutamate bound at the time of the presynaptic AP. Future experiments are needed to address these possibilities.

Functional Implications

The change in STD observed after tLTD extends prior findings that long-term plasticity can alter short-term synaptic dynamics (Markram and Tsodyks, 1996) by showing that these alterations are bidirectional. Strongly depressing synapses most efficiently transmit transient increases in presynaptic firing (Abbott et al., 1997; Tsodyks and Markram, 1997). By decreasing short-term depression, tLTD weakens synapses but also makes them better suited for transmitting more sustained presynaptic firing.

It is widely believed that induction of spike timing-dependent LTP requires postsynaptic EPSP-AP coincidence (Bi and Poo, 2001; Magee and Johnston, 1997). Here, we show that, in addition, signals from both sides of the synapse converge on the presynaptic terminal during tLTD. Postsynaptic APs not only gate postsynaptic plasticity, but act presynaptically via a retrograde messenger. Presynaptic glutamate not only signals across the synapse, but locally via autoreceptors. The convergence of these signals permits tLTD to be driven by the temporal patterning of pre- and postsynaptic activity. This may enhance the flexibility of tLTD, because its timing requirements can be controlled by modulators or activity patterns that influence the breakdown or production of cannabinoids. Modeling studies have revealed that the timing dependence of long-term plasticity can endow neural networks with important computational features, such as causality detection, synaptic competition, and sequence learning (reviewed in Abbott and Nelson, 2000). Therefore, modulation of the tLTD temporal window could change the computations performed by cortical circuits. In particular, lengthening or shortening the temporal window of tLTD alters the timescale over which correlated firing produces plasticity.

Experimental Procedures

Electrophysiology

Paired whole-cell recordings from thick-tufted L5 neurons in slices cut from visual cortex of Long-Evans rats age P12 to P21 were performed as previously described (Sjöström et al., 2001). ACSF contained (in mM): 126 NaCl, 3 KCl, 1 $MgCl_2$, 1 NaH_2PO_4, 2 $CaCl_2$ (unless otherwise specified), 25 $NaHCO_3$, 25 Dextrose. Recordings were done at 32°C–34°C, and slices were used up to 10 hr after slicing. The identity of neurons was verified by biocytin histochemistry (Vectastain ABC Elite kit, Vector Labs, Burlingame, CA).

Connected neurons fired once every 10 s, or 6 APs at 30 Hz every 18 s (for the purpose of STD analysis, see below) throughout the entire experiment except the induction period. tLTD was induced by post-before-pre firing at 0.1–20 Hz (Sjöström et al., 2001). After induction, responses were monitored for as long as possible or up to a total recording time of 110 min. Recordings shorter than 40 min were excluded. LTD magnitude was measured starting 15 min after the induction. With 30 Hz firing, the amount of LTD was measured from the first response in each spike train.

Internal solution contained (in mM): 20 KCl, 100 (K)Gluconate, 10 (K)HEPES, 4 (Mg)ATP, 0.3 (Na)GTP, 10 (Na)Phosphocreatine, and 0.1% w/v Biocytin, adjusted with KOH to pH 7.4, and with sucrose to 290–300 mOsm.

Experiments in current clamp were terminated if V_m changed more than 8 mV, if R_{in} changed more than 30%, or if the initial baseline period was unstable. In typical recordings (n = 54), R_{in} (68.9 ± 5.3 MΩ) changed by 3% ± 1.6%, V_m (−64.8 ± 0.4 mV) changed by −0.14 ± 0.19 mV, and R_s (22 ± 1 MΩ, not compensated) increased by 14.8% ± 2.8%. R_s increases for tLTD experiments (Figure 1C, top graph) were not significantly different (12.5% ± 4.6%, n = 17) from those in control experiments (Figure 1C, bottom graph; 14.7% ± 5.7%, n = 13; p = 0.77), indicating that R_s increases did not produce depression. Connections weaker than 0.2 mV were excluded (based on a sample of 894 connections, less than 17% of all connections were <0.2 mV), as the low signal-to-noise ratio complicated STD and CV analysis (see below).

For the voltage-clamp experiments in Figure 5D (open symbols), R_s (14.1 ± 2.3 MΩ) changed by −3.2% ± 3.8% (p = 0.39, paired t test), R_{in} (98.2 ± 14 MΩ) by 4.4% ± 4% (p = 0.86), presynaptic V_m (−68 ± 0.9 mV) by −1.1 ± 0.25 mV (p = 0.39), and postsynaptic I_{hold} (−34 ± 14 pA) by 8.7 ± 9.5 pA (p = 0.66).

Statistics

Statistical significance was assessed by Student's t test for equal means at the 0.05 level (using unequal variances, if equality of variances F test gave p < 0.05), unless stated otherwise. Significance was corrected for multiple comparisons using the Bonferroni-Dunn method (in StatView, SAS Institute, NC). ANOVA was applied when the equality of variances F test permitted it at the 0.05 level. The nonparametric tests of Kruskal-Wallis and Mann-Whitney gave comparable significance levels in all cases. Means are reported as ±SEM, unless otherwise specified (Figure 2A). One, two, and three asterisks indicate p < 0.05, p < 0.01, and p < 0.001, respectively.

STD Analysis

STD was measured from average responses (≥40 traces) to trains of six APs at 30 Hz. To account for temporal summation, exponential fits to preceding EPSPs were subtracted. The STD index was defined as the amount of change of the first response in a 30 Hz train, minus the average of the changes of the subsequent responses, normalized to the change of the first response.

CV Analysis

With 30 Hz firing, only the first of the responses to a spike train was used. All postinduction responses were binned successively using the same bin size as for the single prepairing bin (Figure 2A; 40–60 responses per bin). The CV was corrected for the background noise, although it typically made little difference. The mean and $1/CV^2$ were normalized to the prepairing bin. Individual data points in Figures 2B, 4D, 6D, and 9D are the averages of all the postpairing bins of a paired recording starting 15 min after induction, i.e., when LTD is expressed (cf. Figure 2A). For clarity, only experiments with at least 1% LTD were included in these figures.

mEPSC Analysis

mEPSCs were recorded in L5 neurons clamped to −70 mV in the presence of TTX and bicuculline and analyzed using in-house software as previously described (Turrigiano et al., 1998). Detection criteria included amplitude >5 pA and rise time <3 ms. Overlapping events or events with poor baselines were excluded. Averaged mEPSCs were obtained by aligning events on the rising phase. At least 75 and up to 550 events were acquired from each cell in each condition (i.e., control, APV, wash). R_{in} (92 ± 2.4 MΩ) changed by 3.6% ± 1.1% (p = 0.38), I_{hold} (−63.7 ± 13.4 pA) by 10 ± 8.4 pA (p = 0.57), R_s (10 ± 1 MΩ) by 19% ± 14% (p = 0.24), and V_m (66 V ± 1 mV) by 1.0 ± 0.41 mV (p = 0.51).

Pharmacology

AM251, AM404, ACEA, LY341495, and AEA (Tocris Cookson, MO) were used at 900 μM, 10 μM, 125 nM, 100 μM, and 40 μM final concentrations, respectively. AA-5-HT (Cayman Chemical, MI) was used at 100 μM concentration. BAPTA, CNQX, TTX, Bicuculline, MK-801, ifenprodil, and D/L-APV (Sigma) were used at 10 mM, 0.3 μM, 0.1 μM, 20 μM, 2 μM, 4 μM, and 200 μM, respectively. We note that it was crucial to wash with ethanol or change the perfusion tubing after each use of lipofilic drugs such as AM404, AA-5-HT, and AM251, and that the effects of these drugs, as well as those of MK801, ifenprodil, and ACEA, do not readily wash out.

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