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Neuropharmacology 52 (2007) 176–184
NEURO PHARMACOLOGY
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Multiple forms of long-term plasticity at unitary neocortical layer 5 synapses

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Received 9 June 2006; received in revised form 11 July 2006; accepted 12 July 2006

Abstract

Long-term potentiation and depression (LTP and LTD) are cellular plasticity phenomena expressed at a variety of central synapses, and are thought to contribute to learning and developmental changes in circuitry. Recurrent neocortical layer-5 synapses are thought to express a presynaptic form of LTP that influences the short-term plasticity of the synapse. Here we show that changes in synaptic strength elicited by pairing high frequency pre- and postsynaptic firing at this synapse result from a mixture of presynaptic and postsynaptic forms of plasticity, as assessed by the analysis of changes in coefficient of variation, short-term plasticity, and NMDA:AMPA current ratios. Pharmacological dissection of this plasticity revealed that block of presynaptic LTD with an endocannabinoid inhibitor enhanced LTP, while the apparently presynaptic component of LTP could be prevented by induction in the presence of blockers of nitric oxide. These data suggest that correlated high-frequency firing at layer-5 synapses simultaneously induces a mixture of presynaptic LTD, presynaptic LTP, and postsynaptic LTP.
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Keywords: Long-term potentiation; Long-term depression; Nitric oxide; Endocannabinoid; Neocortical layer 5

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1. Introduction

There is a tremendous diversity of cellular synaptic plasticity mechanisms expressed within the vertebrate central nervous system (Malenka and Bear, 2004; Turrigiano and Nelson, 2004). This diversity is presumably due to differences in the cellular and molecular machinery present at different classes of synapse, and/or at different times during development (see for example Palmer et al., 2004; Wierenga et al., 2006). Here we investigate the expression and induction mechanism(s) underlying neocortical long-term potentiation (LTP) at Layer 5 (L5) recurrent excitatory synapses. Previous work has suggested that LTP at this synapse is presynaptically expressed, and results in a redistribution of synaptic efficacy through changes in short-term depression (STD) (Markram and Tsodyks, 1996). This synapse also expresses a presynaptic form of long-term depression (LTD) that depends on endocannabinoid signaling (Sjöström et al., 2003, 2004). We examine long-term plasticity induced by correlated high-frequency pre- and postsynaptic firing, to determine whether there is also evidence for a postsynaptically expressed form of LTP at this synapse.

It is widely believed that the major locus of expression of a canonical form of LTP (NMDA receptor-dependent hippocampal CA1 LTP), as well as LTP at a number of other synapses, is through changes in the number and/or properties of postsynaptic AMPA receptors (Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Malenka and Bear, 2004). Nonetheless, a presynaptic contribution to these forms of LTP has remained a source of debate, and there is now considerable evidence at many CNS synapses for presynaptic forms of LTP that alter neurotransmitter release properties (Choi et al., 2000; Zakharenko et al., 2001, 2002; Markram and Tsodyks, 1996; Gerdeman et al., 2002; Stanton et al., 2005).

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doi:10.1016/j.neuropharm.2006.07.021

Determining the locus of expression is an important step in understanding the functional relevance of any given form of long-lasting plasticity for several reasons. First, once it is known, the molecular mechanisms of the plasticity can be studied (Malinow and Malenka, 2002), and this potentially allows links to be forged between plasticity and function. Second, the site of expression affects how information is transmitted across the synapse. Postsynaptic expression of LTP, for example, decreases the signal-to-noise ratio (Otmakhov et al., 1993). Presynaptic LTP, on the other hand, produces more STD, which leads to increased dynamic gain control (Abbott et al., 1997), and makes the potentiated synapse more sensitive to temporal coherence rather than to absolute firing rates (Tsodyks and Markram, 1997). Finally, the locus of expression will determine whether LTP and LTD can function as inverses of each other.

Postsynaptic induction, but presynaptic expression, of long-term plasticity implies the involvement of a retrograde (or transsynaptic) messenger that can signal from the postsynapse to the presynapse (Malenka and Bear, 2004). Recently, endocannabinoid-mediated retrograde signaling (Kreitzer and Regehr, 2002) has become well established as a mechanism underlying long-term depression (LTD) at many CNS synapses (Gerdeman and Lovinger, 2003; Duguid and Sjöström, 2006). The involvement of nitric oxide (NO)-mediated retrograde signaling in LTP has had a rockier history, because of the difficulty in reproducing early reports that NO-mediated signaling contributed to hippocampal CA1 LTP induction (Hölscher, 1997). More recently there has been a resurgence of interest in NO-mediated signaling in synaptic plasticity, and a number of recent reports have again suggested a role for NO in the generation of LTP at a variety of central synapses (Hawkins et al., 1998; Hölscher, 1999; Stanton et al., 2005; Wang et al., 2005).

Here we report that visual cortical L5 LTP induced by high-frequency correlated firing occurs through combined pre- and postsynaptic mechanisms, and that the relative contribution of pre- and postsynaptic changes varies at different unitary connections. Furthermore, LTP could be enhanced by a blocker of endocannabinoid signaling (which abolishes LTD at this synapse; Sjöström et al., 2003, 2004), and the presynaptic component of LTP could be abolished by blockers of NO signaling. These data demonstrate that the same induction protocol concurrently generates several forms of plasticity at neocortical L5 synapses: a presynaptic form of LTD, and a mixture of presynaptically and postsynaptically expressed LTP. The net change in synaptic strength thus arises through the integration of multiple signaling pathways and through multiple changes in synaptic function.

2. Methods

2.1. Electrophysiology

All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Quadruple whole-cell recordings from thick-tufted L5 neurons in slices cut from visual cortex of Long–Evans rats age P14 to P21 were performed as previously described (Sjöström et al., 2001, 2003). ACSF contained (in mM): NaCl, 126; KCl, 3; $MgCl_2$, 1; NaH_2PO_4, 1; $CaCl_2$, 2; $NaHCO_3$, 25; dextrose, 25. Recordings were done at 32–34 °C, and slices were used up to 9 h after slicing. The identity of the neurons was verified by biocytin histochemistry (Vectastain ABC Elite kit, Vector Labs, Burlingame, CA).

Experiments were terminated if membrane potential changed more than 8 mV, input resistance changed more than 30%, or if the initial baseline period was unstable. In connected neurons, APs were either evoked once every 10 s, or six APs were fired at 30 Hz every 18 s (for the purpose of STD analysis, see below), throughout the entire experiment except the induction period. Individual presynaptic APs were triggered by 5-ms-long current injections, typically between 1.0 and 1.8 nA. LTP was induced approximately 15 min after breakthrough by pairing 200-ms-long current injections 30 times every 10 s (Markram and Tsodyks, 1996). Induction current injections ranged from 0.5 to 1.8 nA, with a mean of 1.2 ± 0.08 nA ($n = 31$). Individual spikes were not precisely timed during these current injections, but the firing frequency was such that LTP dominated over LTD (40–80 Hz) (Sjöström et al., 2001). We could find no correlation between the locus of LTP expression and firing frequency, amount of current injected, amount of depolarization, or various statistical parameters extracted from the induction firing patterns. After induction, responses were monitored for as long as possible or up to a total recording time of 80 min. Recordings were not included if shorter than 40 min. LTP magnitude was measured starting 15 min after the induction. With 30-Hz baseline firing, the amount of LTP was measured from the first response in each spike train. For determination of the NMDA:AMPA ratio, recordings were performed as described (Watt et al., 2004).

Whole-cell recording pipettes (4–10 MΩ, 1–2 μm diameter) were filled with (in mM): KCl, 20; (K)gluconate, 100; (K)HEPES, 10; (Mg)ATP, 4; (Na)GTP, 0.3; (Na)phosphocreatine, 10; and 0.1% w/v Biocytin, adjusted with KOH to pH 7.4, and with sucrose to 290–300 mOsm. Series resistance (typically 15 MΩ) was not compensated.

2.2. STD analysis

STD was measured from average responses (≥40 traces) to trains of six APs at 30 Hz. To account for temporal summation, an exponential was fit to the decaying portion of a preceding EPSP and subtracted from the EPSP that followed (Fig. 3A and B). The PPR was defined as

$$\frac{\mathrm{EPSP}_2 - \mathrm{EPSP}_1}{\mathrm{EPSP}_1}$$

where EPSP_i denotes the amplitude of the ith response. The change in PPR was defined as the PPR before LTP induction subtracted from the PPR after. The DTC ratio was defined as the ratio between the time constants, τ, of single exponentials fit to the peak of each response in a 30-Hz train after and before LTP induction, or

$$\frac{\tau_{\mathrm{before}}}{\tau_{\mathrm{after}}}$$

(Fig. 3C). We note that the change in PPR is relatively more sensitive to changes in short-term facilitation than to changes in STD as compared to the DTC ratio. In addition, since the extraction of decay time constants by exponential fits is notoriously sensitive to noise, we devised the STD index as a complementary approach to assessing changes in short-term plasticity. 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, or

$$\frac{\Delta\mathrm{EPSP}_1 - \frac{1}{5}\sum_{i=2}^{6}\Delta\mathrm{EPSP}_i}{\Delta\mathrm{EPSP}_1}$$

(Fig. 3D).

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2.3. CV analysis

With 30-Hz firing, only the first of the responses to a spike train was used. All post-induction responses were binned successively using the same bin size as for the single pre-pairing bin (40–60 responses per bin). The use of multiple CV bins provides a measure of the variability of the CV measurement (Fig. 2B) and enables the study of graded changes in CV over time, although none was found. The CV was corrected for the background noise, although it typically made little difference. The mean and $1/CV^2$ were normalized to the pre-pairing bin. Individual data points are the averages of all the post-pairing bins of a paired recording starting 15 min after induction, to allow for complete LTP expression. The measure φ was defined as the angle between the diagonal and the line formed by the CV analysis endpoint and the starting point at the coordinate (1,1).

2.4. NMDA:AMPA ratio

These experiments were carried out as previously described (Watt et al., 2004). Briefly, all experiments were carried out in low-magnesium ACSF (0.5 mM). During the baseline periods before and after induction, the postsynaptic neuron was voltage clamped to −65 or −50 mV. LTP induction, however, was performed in current clamp as described above. AMPA current was measured using a 1-ms-long window centered on the peak of the averaged response, while the NMDA component was extracted from a window ranging 30–60 ms after the presynaptic AP. Experiments with series resistance change >50% or initial EPSC peak <3 pA were excluded.

2.5. Statistics

Comparisons were made by Student's *t*-test for equal means (using unequal variances, if equality of variances *F*-test gave $P < 0.05$), unless stated otherwise. Means are reported as ±S.E.M. To determine whether correlations were significantly different from zero (Fig. 4) for the case where n is small, a two-sided Student's *t*-test of the expression

$$r\sqrt{\frac{n-2}{1-r^2}}$$

was evaluated (Press et al., 1992). Here, r and n are the correlation coefficient and the number of data points, respectively.

2.6. Pharmacology

N(ω)-Nitro-L-arginine methyl ester (L-NAME), cPTIO, and AM251 were used at 100 μM, 50 μM, and 900 nM final concentration, respectively, and were all purchased from Tocris. All drugs were bath applied.

3. Results

We examined the locus of expression of LTP in monosynaptically connected thick tufted L5 neurons. Since the rate of connectivity between nearby L5 neurons is low (Song et al., 2005), quadruple recordings (Fig. 1, left panel) were used to

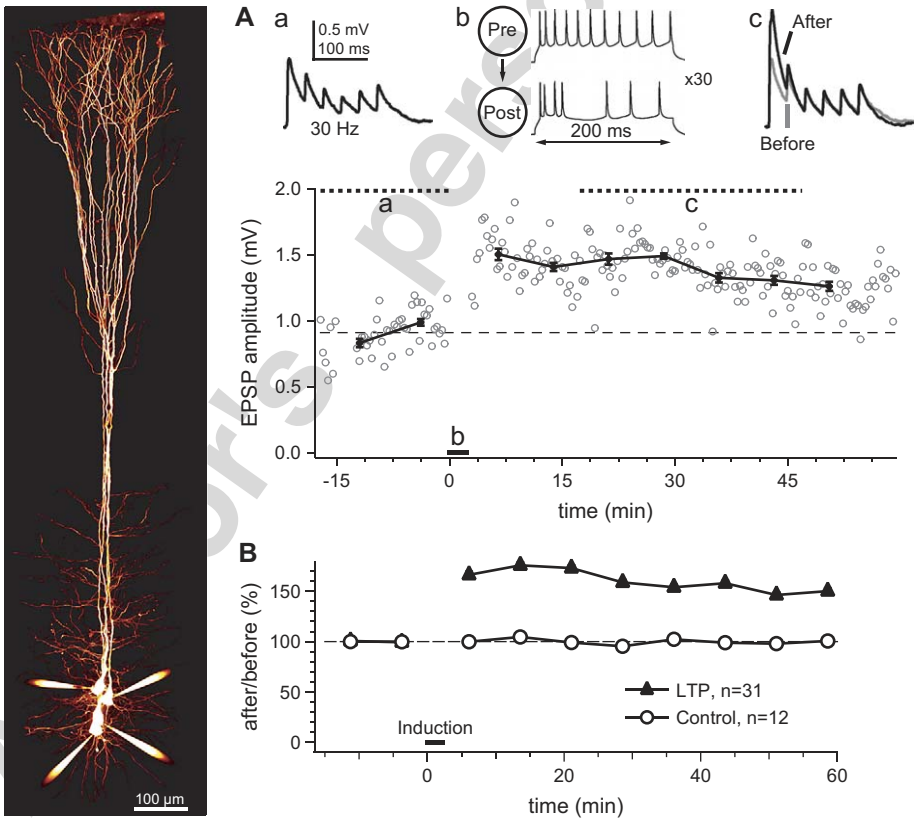

Fig. 1. Recordings of monosynaptically connected visual cortical L5 thick-tufted pyramidal neurons. (Left panel) Pseudo-colored maximum intensity projection of 2-photon laser-scanning microscopy stacks of Alexa-594-filled L5 pyramidal neurons, illustrating the quadruple whole-cell recording approach to finding monosynaptically connected L5-to-L5 pairs. (A) LTP of a unitary L5 pyramidal connection. Synaptic strength and STD were measured using a train of presynaptic APs precisely delivered at 30 Hz (a). After establishing a baseline response (a), plasticity was induced by eliciting pre- and postsynaptic spikes with paired 0.5-nA depolarizing current steps (b). Example traces before and after LTP induction (c) show the simultaneous potentiation and change in STD at this particular unitary connection. Bottom panel illustrates the time-course of change in synaptic strength following LTP induction (horizontal line, 'b') for this connected pair. (B) Normalized time-course of LTP averaged across unitary connections (closed triangles; error bars are too small to show up on plot). Recordings where only pre or only postsynaptic firing was elicited during the induction period did not exhibit plasticity (open circles).

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search for and identify monosynaptically connected pairs, as previously described (Sjöström et al., 2001; 2003). LTP was robustly produced by eliciting action potentials (APs) at 40–60 Hz from the pre- and the postsynaptic cells using paired 200-ms-long depolarizing current injections (Fig. 1Ab) (Markram and Tsodyks, 1996). During these current injections, pre- and postsynaptic APs were not precisely timed, yet LTP was invariably evoked (Fig. 1A, B; after/before = 162 ± 7%, $P < 0.01$ compared to control experiments), consistent with previously published results showing that LTP dominates over LTD at high frequencies at these (Sjöström et al., 2001) and other synapses (Froemke et al., 2006). This form of plasticity required both pre- and postsynaptic activity, since firing either the presynaptic or the postsynaptic neuron alone had no effect on synaptic transmission (Fig. 1B, open circles).

3.1. Variable changes in short-term depression following LTP induction

It was previously demonstrated that, at somatosensory cortical L5 synapses, LTP induction led to a dramatic increase in the amount of short-term depression (STD) (Markram and Tsodyks, 1996), suggesting a presynaptic expression mechanism (Zucker and Regehr, 2002). We looked for a similar effect at visual cortical L5 synapses by evoking precisely timed 30-Hz AP trains during the baseline periods before and after LTP induction (Fig. 1Ac). LTP induction increased the amount of STD in some connected pairs (Fig. 2A, top), but not in others (Fig. 2A, bottom). Averaged across paired recordings, the effect of LTP induction on STD was modest and mostly noticeable in the paired-pulse ratio (PPR) (Fig. 2B; $P < 0.05$ for change in PPR, paired t-test).

We next examined the locus of LTP expression using coefficient of variation (CV) analysis (Fig. 2C). Assuming a binomial model of synaptic transmission, changes in release probability or number of functional release sites is accompanied by a change in the CV of the synaptic responses, such that plotting $1/CV^2$ against the change in efficacy gives rise to points above the diagonal. In contrast, purely postsynaptic changes (due to, for example, AMPAR insertion) should have little effect on $1/CV^2$, and generate points below the diagonal (Faber and Korn, 1991; Larkman et al., 1992). For the top example in Fig. 2A, LTP appeared presynaptic by CV analysis (Fig. 2C, top), whereas for the bottom example in Fig. 2A, CV analysis suggested a postsynaptic locus of LTP expression (Fig. 2C, bottom). In keeping with the variable change in STD (Fig. 2B), CV analysis of our entire data set indicated that, in individual connected pairs, LTP was expressed either presynaptically, postsynaptically, or both (Fig. 2D).

3.2. STD and CV analyses agree on the locus of LTP expression

For the example pairs in Fig. 2A, the change in CV and in STD suggested the same locus of expression. To determine if this was true across our data set, we searched for a correlation

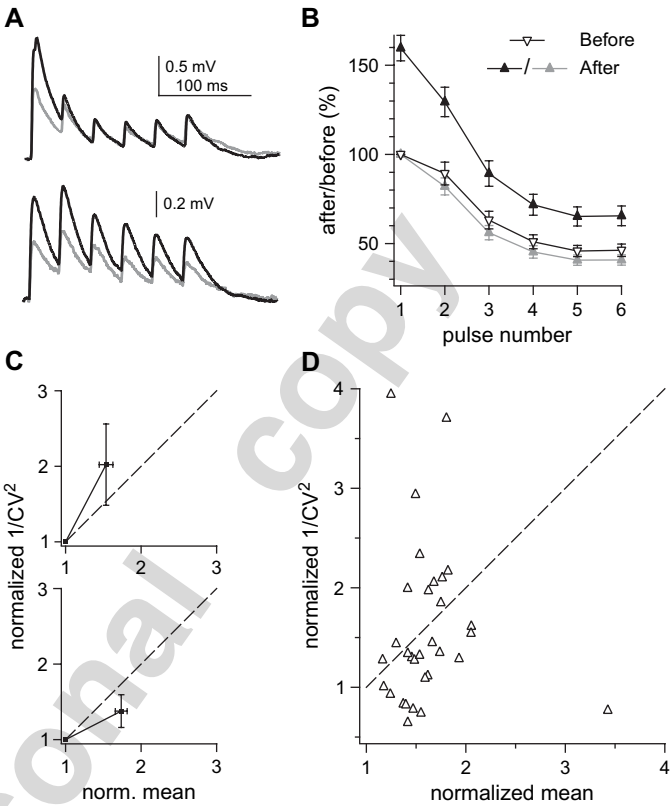

Fig. 2. Both STD and CV change following LTP induction. (A) Two example pairs showing a case where STD was affected by LTP induction (top) and a case where it was not (bottom). (B) Average STD before and after LTP induction ($n = 31$). Open triangles shows normalized STD before LTP induction; closed triangles shows STD after induction, and the gray triangles show normalized STD after induction to allow a comparison of the time course of depression before and after LTP. (C) CV analysis for the same two example pairs shown in A; top panel in A corresponds to top panel in B, and same for bottom panels. In this analysis, points above the diagonal indicate a presynaptic locus of plasticity, while points below the diagonal indicate a presynaptic locus. Error bars result from the use of several post-induction time bins to compute the average change in mean and $1/CV^2$ (see Section 2). (D) CV analysis for the entire LTP data set; each triangle indicates one connected pair of neurons. Error bars were removed for clarity.

between the effect of LTP induction on STD and CV. The change in short-term depression was quantified using three measures (Fig. 3, also see Section 2): the ratio of decay time constants (DTC ratio) of the EPSP trains before and after LTP induction; the normalized amount of LTP of the first response compared to the subsequent responses in a 30-Hz train (the STD index); and the change in PPR.

The site of LTP expression as assessed by CV analysis was quantified by the angle φ between the diagonal (dashed lines in Fig. 2C) and the line formed by connecting the unity point (1,1) and the point formed by plotting the normalized $1/CV^2$ and the normalized mean (solid lines in Fig. 2C). Assuming CV analysis works reliably at these synapses (Faber and Korn, 1991), a presynaptic mechanism is thus indicated by $\varphi \geq 0$, whereas $\varphi < 0$ indicates long-term plasticity is expressed postsynaptically. Plotting each of our three short-term depression measures versus φ generated significant correlations in all three cases (Fig. 4A–C).

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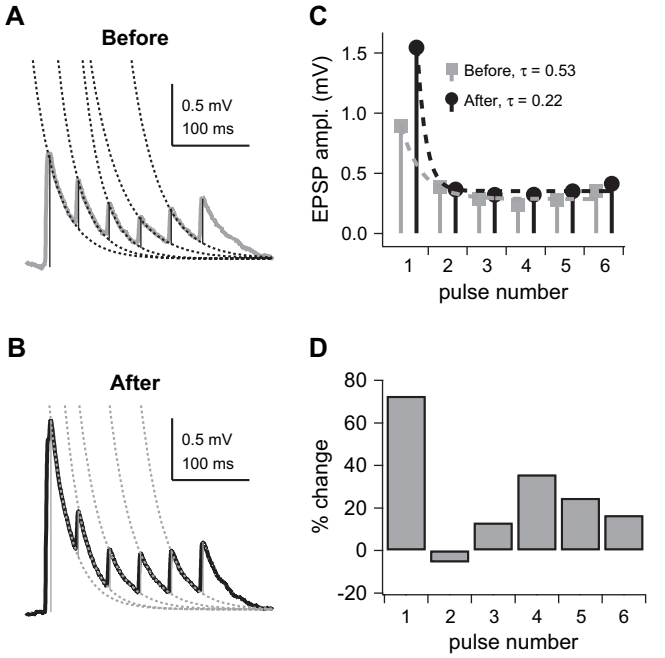

Fig. 3. Detailed description of the STD analysis methods. To account for temporal summation, response amplitudes (vertical continuous lines) before (A) and after LTP induction (B) were measured by subtracting exponential fits from averaged responses (thin dotted lines; averaged responses from same unitary connection as in Fig. 1A and 2A top panel). (C) The decay time constants before (τ_{before}) and after (τ_{after}) induction were extracted by fitting exponentials (thick dashed lines) to the responses extracted in (A) and (B), and the DTC ratio was defined as $\tau_{before}/\tau_{after}$. The PPR, $(EPSP_2 - EPSP_1)/EPSP_1$, was calculated from the first two responses extracted in this manner. Therefore, since there is a change in STD upon LTP induction for this particular unitary connection (cf. A and B), the DTC ratio is <1 and the change in PPR is <0. (D) By computing the amount of potentiation per response in C, the STD index, $(\Delta EPSP_1 - \overline{\Delta EPSP_{2-6}})/\Delta EPSP_1$, was calculated (see Section 2). This resulted in a value >0 for this paired recording, indicative of an increase in STD due to LTP induction.

At many central synapses, postsynaptic LTP is brought about by AMPA receptor insertion (Bredt and Nicoll, 2003; Malenka and Bear, 2004). At L5 synapses, we recently demonstrated that the NMDA-receptor-mediated component of synaptic transmission is also potentiated, but with a slower time-course; consequently, the NMDA:AMPA ratio is reduced immediately after the induction of postsynaptic LTP (Watt et al., 2004). After the induction of solely presynaptic LTP, however, there should be no reduction of the NMDA:AMPA ratio, since both the AMPA and the NMDA-receptor-mediated components are affected by an increase in presynaptic transmitter release. Consistent with the graded and dual expression of both pre- and postsynaptic forms of LTP at L5 synapses, we found that the locus of LTP expression (as measured by φ) determined the reduction of the NMDA:AMPA ratio (Fig. 4D). In other words, for relatively more presynaptic LTP expression ($\varphi > 0$), there was a smaller drop in the NMDA:AMPA ratio, whereas for relatively more postsynaptic LTP expression ($\varphi < 0$), the NMDA:AMPA reduction was more marked (Fig. 4D).

In conclusion, using three independent methods—based on CV, STD, and the NMDA:AMPA ratio—to assess the locus of

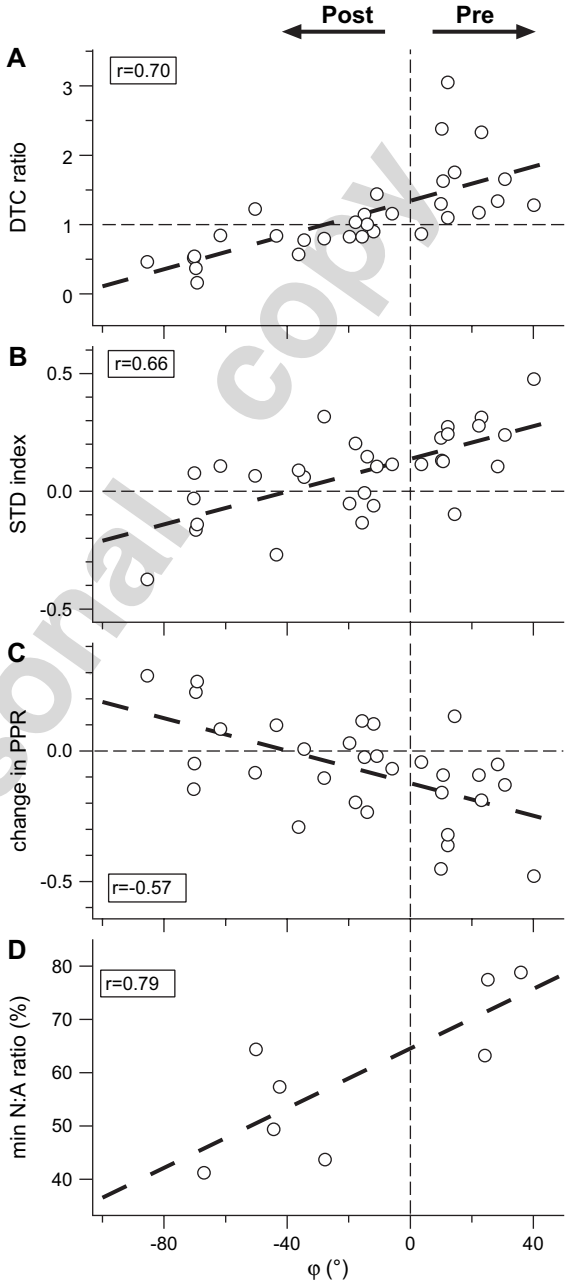

Fig. 4. Three independent measures suggest that LTP is expressed through a graded mix of presynaptic and postsynaptic changes. In each case, one of three measures of short-term plasticity (cf. Fig. 3) or the change in NMDA:AMPA ratio is plotted against the measure φ, derived from the CV analysis (see Section 2). If $\varphi > 0$, this indicates a presynaptic locus of LTP expression, while if $\varphi < 0$, this indicates a postsynaptic locus (arrows inset top). A plot of the DTC ratio of the EPSP trains before and after LTP versus φ (A) shows a significant correlation ($P < 0.0001$) between these two independent measures of the locus of expression. In agreement, the corresponding plots of the STD index versus φ (B) and the change in PPR versus φ (C) also indicate significant correlations ($P < 0.0001$ and $P < 0.001$, respectively). (D) The normalized minimal NMDA:AMPA current ratio immediately after LTP induction—another independent measure of the site of expression (see text)—was also significantly correlated with φ ($P < 0.05$).

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LTP expression, we obtained concordant results. Our data argue that two apparently pre- and postsynaptic forms of LTP coexist at the same unitary connections, and are induced by similar activity patterns. In addition, the degree of presynaptic and postsynaptic LTP appears to vary in a graded manner from one paired recording to the next (Fig. 4).

3.3. NO as a retrograde signal at L5 synapses

It is possible that, at these synapses, LTP is always postsynaptically expressed, but that AMPA receptor insertion at postsynaptically silent synapses (Malinow, 1994; Malenka and Nicoll, 1997; Malinow et al., 2000) selectively "unsilences" high-p contacts so that changes in both CV and STD incorrectly suggest a presynaptic site of LTP expression. Since LTP is induced postsynaptically (Bliss and Collingridge, 1993; Mainen et al., 1995; Malenka and Nicoll, 1999; Sheng and Kim, 2002), a presynaptic locus of expression requires a retrograde signal, and makes the prediction that blocking this signal will selective block the presynaptic component of L5 LTP. Since NO may sometimes act as a retrograde messenger for LTP (Hölscher, 1997; Hawkins et al., 1998), we decided to test the role of NO in inducing LTP at L5-to-L5 synapses. Blocking NO retrograde signaling using the nitric oxide synthase (NOS) antagonist L-NAME (Nowicky and Bindman, 1993; Moore and Handy, 1997), or the non-membrane-permeable NO scavenger cPTIO (Ko and Kelly, 1999), reduced the amount of LTP relative to control (L-NAME, $130 \pm 12\%$, $n = 8$; cPTIO, $140 \pm 9.3\%$, $n = 6$;). Pooling the results from LTP induction in the presence of L-NAME and cPTIO showed that blocking NO signaling significantly reduced the amount of LTP (Fig. 5A; LTP in L-NAME/cPTIO was 136% of control; significantly different from LTP alone, $P < 0.05$).

In keeping with the view that only the presynaptic component of LTP was blocked by inhibitors of NO signaling, LTP induction in the presence of L-NAME did not lead to any change in short-term plasticity ($P = 0.25$ for change in PPR, paired t-test; cf. Fig. 5B, left panel, where data is pooled with cPTIO). Furthermore, the remaining LTP component was expressed postsynaptically according to CV analysis (Fig. 5C, D). Similarly, LTP expression in the presence of cPTIO appeared postsynaptic by analysis of short-term plasticity ($P = 0.86$ for change in PPR, paired t-test; cf. Fig. 5B, left panel, pooled with L-NAME) and CV (Fig. 5C, D). Taken together, these results lend strong support to the view that LTP at these synapses has a presynaptic component, and that presynaptic LTP is induced by a signaling pathway that requires release of NO.

In previous work, we suggested that, when pre- and postsynaptic neurons fire together above 40 Hz, the resulting plasticity might be a mixture of LTP and LTD (Sjöström et al., 2001, 2003). If so, then blockade of LTD during high-frequency correlated firing should result in increased amounts of LTP. LTD at this synapse is presynaptically expressed, and depends on endocannabinoid signaling so can be blocked with the CB1 antagonist AM251 (Sjöström et al., 2003, 2004). Indeed, induction in the presence of AM251 resulted in enhanced LTP (Fig. 5A, $213 \pm 22\%$, significantly different from control LTP levels, $P < 0.007$). In addition, the LTP in AM251 had a larger presynaptic component as assessed by analysis of short-term plasticity ($P < 0.05$ for change in PPR, paired t-test) and CV (Fig. 5B right panel, C, D). Finally, we tested whether CB1 receptor blockade by itself induced plasticity by washing in AM251, but found it had no effect on EPSP amplitude ($98 \pm 1.6\%$, $n = 4$; $P = 0.59$, two-tailed t-test for mean different from 100%).

Across all pairs that expressed LTD (post-before-presynaptic firing at 0.1–20 Hz, or presynaptic firing paired with postsynaptic subthreshold depolarization at 0.1–30 Hz; Sjöström et al., 2001, 2003, 2004; plus additional experiments presented herein), CV analysis indicated a purely presynaptic locus, as can be seen from the histogram of φ (Fig. 6). However, for experiments in which pairings induced LTP (0.1–100 Hz, pre-before-postsynaptic firing; Sjöström et al., 2001, 2003; Watt et al., 2004; plus additional experiments presented herein), expression was either presynaptic, postsynaptic, or both (Fig. 6).

The most parsimonious explanation for the results presented in Fig. 6 is that LTP is expressed through a combination of pre- and postsynaptic mechanisms, whereas LTD is only expressed presynaptically. Since blockade of LTD results in increased levels of LTP (Fig. 5A), our data furthermore suggest that correlated high frequency firing at L5 synapses simultaneously induces a mixture of presynaptic LTD, presynaptic LTP, and postsynaptic LTP.

4. Discussion

We have shown that L5 neocortical recurrent excitatory synapses express several forms of long-lasting plasticity that can be concurrently activated by pairing high-frequency pre- and postsynaptic firing. A combination of CV analysis, STD analysis, and an analysis of changes in the NMDA:AMPA ratio all suggest that LTP at this synapse has both a pre- and a postsynaptic component, and that the degree to which pre- and postsynaptic changes are induced varies in a graded manner from synapse to synapse. The presynaptic component depends on NO signaling, while the postsynaptic component does not. Taken together with our previous work (Sjöström et al., 2001, 2003, 2004; Watt et al., 2004), these data show that correlated high frequency firing activates a mixture of synaptic changes composed of a presynaptic endocannabinoid-dependent LTD, a presynaptic NO-dependent LTP, and an additional postsynaptic form of LTP. Our data therefore suggest that plasticity at these synapses is rather complex and is composed of several different mechanistically distinct processes. Recent results obtained at both neocortical layer-2/3 (Bender et al., 2006) and hippocampal synapses (O'Connor et al., 2005) corroborate the notion that LTP and LTD may be simultaneously induced by correlated firing. This suggests that the concurrent induction of LTP and LTD by coincident pre- and postsynaptic activity may be a general principle of plasticity, rather than the odd exception.

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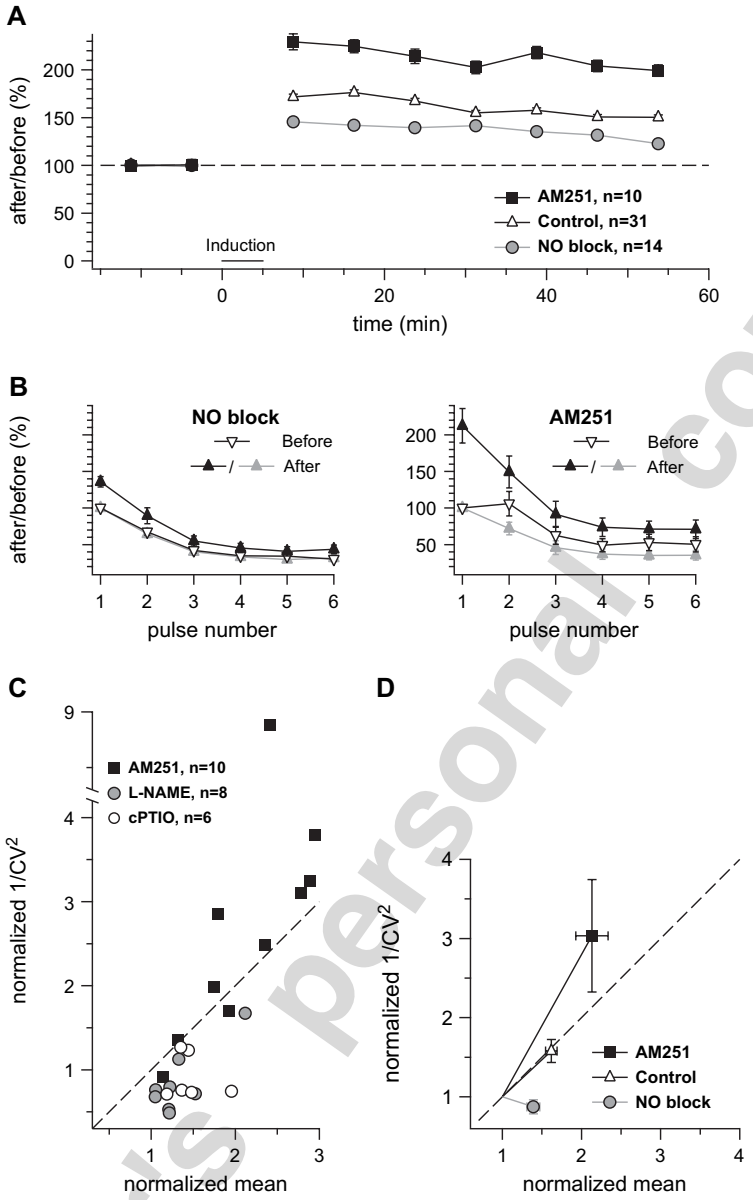

Fig. 5. Pharmacological dissection of LTP. (A) LTP induced under control conditions (open triangles; same data as in Fig. 1B), in the presence of the endocannabinoid inhibitor AM251 (closed squares), or during NO signaling blockade (closed gray circles; pooling results obtained using the NO synthase inhibitor L-NAME and the NO scavenger cPTIO). (B) Left panel: Short-term plasticity before and after LTP induction during NO blockade (L-NAME and cPTIO results pooled). Right panel: Short-term plasticity before and after LTP induction in the presence of AM251. (C) CV analysis of individual LTP experiments in the presence of AM251, L-NAME, or cPTIO. (D) Average change in $1/CV^2$ following LTP induction under the conditions indicated in C. Note that CB1 blockade (AM251) increases both the amount of LTP and the degree of presynaptic expression, whereas NO blockade reduces the amount of LTP while at the same time rendering the locus of expression completely postsynaptic.

One issue is how strongly the CV and STD analysis argue for a presynaptic locus of change. It is formally possible that, at these unitary L5 synapses, there is a population of postsynaptically ''silent'' release sites that can be unmasked and re-masked by ''presynaptic'' LTP and LTD, respectively (Malinow, 1994; Malenka and Nicoll, 1997; Malinow et al., 2000). Since there is an average change in short-term plasticity following LTP induction (Fig. 2B; Markram and Tsodyks, 1996), this would require that these sites have a significantly higher release probability than sites that are not subject to this silencing and unsilencing process (Poncer and Malinow, 2001). The evidence that LTD at this synapse is presynaptically induced is quite strong (Sjöström et al., 2003, 2004; Duguid and Sjöström, 2006), so if the locus of expression were purely postsynaptic, this would require an additional anterograde message to the postsynapse. Similarly, the putatively presynaptic component of LTP we document here requires both production of NO and release of NO into the extrasynaptic space (where it can be scavenged by cPTIO). This strongly suggests that NO serves as a retrograde signal at this synapse, as has been suggested at a number of other synapses (Hawkins et al., 1998; Hölscher, 1999; Stanton et al., 2005; Wang et al., 2005). Thus, while we cannot absolutely rule out a postsynaptic locus for these two processes, the simplest

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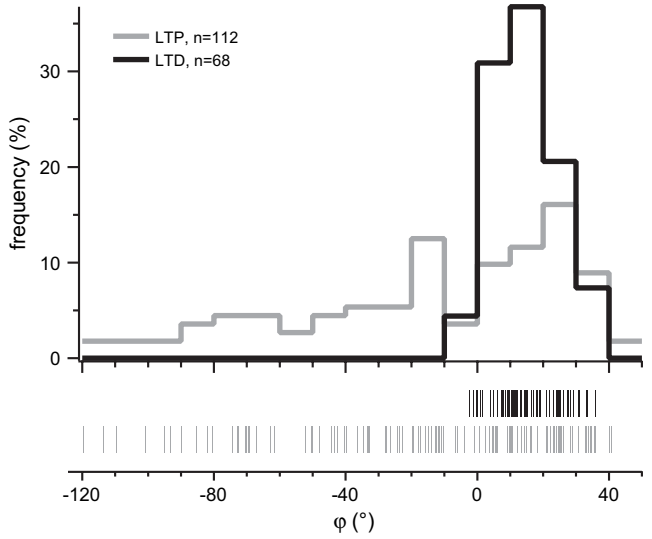

Fig. 6. A histogram of φ for our entire data set of LTP and LTD experiments (excluding those conducted in the presence of pharmacological agents). Each vertical line in bottom graph denotes an experiment carried out using one unitary L5-to-L5 connection. Note that this figure includes a re-analysis of previously published LTD and LTP experiments (Sjöström et al., 2001, 2003, 2004; Watt et al., 2004) in addition to the data included in this paper. LTD induction protocols included post-before-presynaptic firing at 0.1–20 Hz as well as pairing of presynaptic firing at 0.1–30 Hz with postsynaptic subthreshold depolarization. LTP induction protocols included pre-before-postsynaptic firing at 0.1–100 Hz, or pre- and postsynaptic depolarizing current pulses as in Fig. 1.

explanation for our data is that this synapse expresses two forms of presynaptic long-term plasticity: a depression process that requires retrograde endocannabinoid signaling, and a potentiation process that requires retrograde NO signaling. Importantly, these two processes appear to be functional inverses of each other in terms of their effects on the dynamics of synaptic transmission (Abbott et al., 1997; Tsodyks and Markram, 1997). Thus, the ultimate effect a particular activity pattern has on presynaptic transmission will depend on its relative efficacy at eliciting release of endocannabinoid and NO.

In addition to a presynaptic component to neocortical L5 LTP, we also report a postsynaptic component that is insensitive to NO inhibitors. Previous studies at this synapse in somatosensory cortex have suggested that LTP is purely presynaptically expressed (Markram and Tsodyks, 1996; Dodt et al., 1999; Eder et al., 2002). It is not clear why these investigators did not find evidence for a postsynaptic component; in one case the recording configuration and induction protocol were nearly identical (Markram and Tsodyks, 1996). Differences in age might contribute: our data spanned a larger age range (postnatal days 14–21) than the Markram and Tsodyks study (postnatal days 13–15); differences in brain area might also contribute (visual cortex versus somatosensory cortex).

The presence of a postsynaptic form of LTP at these synapses raises the issue of whether they also express a form of postsynaptic LTD. It would seem computationally important to be able to reverse the changes in synaptic strength induced by LTP through a mechanism that targets the same expression locus, yet our previous work at this synapse, using a variety of different induction protocols, has only evidenced presynaptic LTD which is fully blocked by endocannabinoid inhibitors (Sjöström et al., 2003, 2004). On the other hand, there are reports of postsynaptically expressed LTD at L5 synapses, using glutamate uncaging to induce and measure plasticity (Dodt et al., 1999; Eder et al., 2002; Holthoff et al., 2004) or strong synaptic stimulation to activate NMDA spikes (Holthoff et al., 2004). These reports demonstrate that—under certain conditions at least—a postsynaptic form of LTD can also be induced at these synapses. One possible reason for the differences in expression mechanism with different methods of stimulation is the degree of activation of extrasynaptic glutamate receptors. Our experiments generate presynaptic LTD of relatively weak unitary connections (Song et al., 2005). These other studies, however, employed either strong extracellular stimulation (Holthoff et al., 2004) or glutamate uncaging (Dodt et al., 1999; Eder et al., 2002; Holthoff et al., 2004) to evoke postsynaptic LTD, both of which should generate more glutamate spill-over than the activation unitary connections. If activation of extrasynaptic glutamate receptors is necessary for postsynaptic LTD (as some studies have suggested; Massey et al., 2004; Yang et al., 2005), then global correlations in a large number of presynaptic inputs may be required to trigger postsynaptic LTD, while relatively local correlations in a small number of inputs may generate predominantly presynaptic LTD.

Presynaptic LTD at L5 neocortical synapses requires presynaptic coincidence detection of endocannabinoid release and presynaptic NMDA receptor activation (Sjöström et al., 2003; Duguid and Sjöström, 2006). Given that presynaptic LTP appears to require NO signaling, our data suggest that the presynapse plays an important role in detecting and integrating competing signals for synaptic plasticity. Our data suggest that realistic patterns of activity at these synapses can elicit multiple forms of pre- and postsynaptically expressed plasticity, and the ultimate effect a pattern of activity has on synaptic transmission will depend on—among other things—its relative efficacy at inducing release of competing retrograde signals.

Acknowledgements

We thank Michael Häusser, Alanna Watt, and Arnd Roth for help and useful discussions, and Dan Feldman for suggesting the use of the NMDA:AMPA ratio as a measure of the locus of plasticity. This work was supported by EY 014439 (G.G.T.), MH 66338 (S.B.N.), and a Marie Curie Intra-European Fellowship (P.J.S.). P.J.S. also thanks the Wellcome Trust and the Gatsby Foundation for support.

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