RAPID REPORT

Requirement of dendritic calcium spikes for induction of spike-timing-dependent synaptic plasticity

Björn M. Kampa, Johannes J. Letzkus and Greg J. Stuart

Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, Australia

Spike-timing-dependent synaptic plasticity (STDP) by definition requires the temporal association of pre- and postsynaptic action potentials (APs). Yet, in cortical pyramidal neurons pairing unitary EPSPs with single APs at low frequencies is ineffective at generating plasticity. Using recordings from synaptically coupled layer 5 pyramidal neurons, we show here that high-frequency (200 Hz) postsynaptic AP bursts, rather than single APs, are required for both long-term potentiation (LTP) induction and NMDA channel activation during EPSP–AP pairing at low frequencies. Furthermore, we find that AP bursts can lead to LTP induction and NMDA channel activation during EPSP–AP pairing at both positive and negative times. High-frequency AP bursts generated supralinear calcium signals in basal dendrites suggesting the generation of dendritic calcium spikes, as has been observed previously in apical dendrites during AP burst firing at frequencies greater than 100 Hz. Consistent with a role of these dendritic calcium spikes in LTP induction, pairing EPSPs with low frequency (50 Hz) AP bursts was ineffective in generating LTP. Furthermore, supralinear calcium signals in basal dendrites during AP bursts were blocked by low concentrations of the T- and R-type calcium channel antagonist nickel, which also blocked LTP and NMDA channel activation. These data suggest an important role of dendritic calcium spikes during AP bursts in determining both the efficacy and time window for STDP induction.

(Resubmitted 5 April 2006; accepted after revision 2 May 2006; first published online 4 May 2006)
Corresponding authors B. M. Kampa: Department of Neurophysiology, Brain Research Institute, University of Zurich, Switzerland. Email: kampa@hifo.unizh.ch; G. J. Stuart: Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, Australia. Email: greg.stuart@anu.edu.au

Previous work in pyramidal neurons in the cortex and hippocampus indicates that the magnitude and sign of changes in synaptic strength depend critically on the precise timing of pre- and postsynaptic action potentials (APs), with postsynaptic APs preceding EPSPs typically leading to long-term depression (LTD), while APs evoked just after EPSP onset typically lead to long-term potentiation (LTP) (Markram *et al.* 1997*b*; Bi & Poo, 1998; Sjöström *et al.* 2001). This bi-directional spike-timing-dependent plasticity (STDP) extends the original ideas of Donald Hebb, who postulated that synaptic transmission between two neurons could be altered if both neurons repeatedly fire coincident APs (Hebb, 1949). While the cellular mechanisms underlying STDP are not well understood, most studies agree there is an important role of NMDA receptors (Markram *et al.* 1997*b*; Bi & Poo, 1998; Sjöström *et al.* 2001), presumably due to their ability to detect coincident pre- and postsynaptic activity via relief of voltage-dependent magnesium block (Mayer *et al.* 1984; Nowak *et al.* 1984). During STDP the voltage driving unblock of NMDA receptors is supplied by postsynaptic APs, which actively propagate back into the dendrites of many neuronal types (Stuart *et al.* 1997*b*). However, single APs attenuate and can fail to backpropagate into some dendrites of pyramidal neurons (Stuart *et al.* 1997*b*). In cortical layer 5 pyramidal neurons, this failure of AP backpropagation can be rescued during high-frequency AP bursts, a typical firing mode of these neurons (Connors *et al.* 1982), due to boosting of AP backpropagation following AP summation and the generation of dendritic calcium spikes (Larkum *et al.* 1999; Kampa & Stuart, 2004). Consistent with the potential role of AP burst-evoked dendritic calcium spikes in synaptic plasticity, a number of studies indicate a role of AP burst firing in STDP (Pike *et al.* 1999; Meredith *et al.* 2003; Birtoli & Ulrich, 2004). These findings prompted us to investigate

 DOI: 10.1113/jphysiol.2006.111062

the dendritic mechanisms involved in induction of STDP between synaptically connected pairs of layer 5 pyramidal neurons during single APs and AP bursts.

Methods

Wistar rats (3–4 weeks old) were anaesthetized by inhalation of isoflurane, decapitated and 300 μm-thick sagittal slices of somatosensory cortex prepared, according to guidelines approved by the Animal Ethics Committee of the Australian National University. During recording, slices were perfused with an oxygenated extracellular solution containing 125 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 25 mM glucose, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ (pH 7.4 with 5% CO_2) at $35 \pm 1^{\circ}$C. Whole-cell current-clamp recordings were made from the soma of layer 5 pyramidal neurons using current-clamp amplifiers (Axon Instruments or Dagan Corporation) with pipettes containing 135 mM potassium gluconate, 7 mM NaCl, 10 mM Hepes, 2 mM $MgCl_2$ and 2 mM Na_2ATP (pH 7.2 with KOH), or in STDP experiments 130 mM potassium gluconate, 20 mM KCl, 10 mM Hepes, 4 mM Mg-ATP, 0.3 mM GTP and 10 mM sodium phosphocreatine (pH 7.4 with KOH, 290 mosmol l^{-1} with sucrose; Markram *et al.* 1997*b*; Sjöström *et al.* 2001). Unitary EPSPs between synaptically coupled layer 5 pyramidal neurons were evoked at low frequency (0.1 Hz) by somatic current injection into the presynaptic neuron. STDP was induced by 60 pairings of pre- and postsynaptic APs at 0.1 Hz. Pre- and postsynaptic APs were evoked by brief (2 ms; 4 nA) somatic current injections. During AP bursts the EPSP–AP time interval was defined as the time between the onset of the current pulse used to evoke the presynaptic AP and the onset of the current pulse used to evoke the middle AP in the postsynaptic burst. The amount of potentiation or depression was assessed by calculating the ratio of the average EPSP amplitude 20–30 min after induction relative to that during the 10 min preceding induction. In experiments using D-APV (D-2-Amino-5-phosphonopentanoic acid) (50 μM) or nickel (100 μM), these drugs were washed in just prior to STDP induction and washed out after STDP induction. Data were acquired at 20 kHz on a Macintosh computer. Axograph software (Axon Instruments, USA) was used for both acquisition and analysis. Statistical significance was determined using Student's *t* test at a significance level of 0.05. Statistically significant differences in the figures are indicated by asterisks. Pooled data represents mean $\pm$ S.E.M.

In the experiments using the activity-dependent NMDA antagonist MK801 to assess NMDA activation. The location of the synapses activated in these experiments is unknown, although it is likely to be within layer 5. NMDA EPSPs were isolated by addition of 20 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX; Tocris) and 5 μM bicuculline (Tocris) to the external solution and recorded at resting membrane potentials in the presence of 1 mM $MgCl_2$. Seventy EPSPs were evoked at 0.3 Hz and the last 10 EPSPs averaged to give the control response. MK801 (5 μM) was then added to the bath and after waiting 7 min for the concentration of MK801 to equilibrate 60 EPSPs were evoked at 0.3 Hz either alone or paired with post-synaptic APs. The average of the next 10 EPSPs evoked alone was used to evaluate EPSP amplitude after MK801 block. In these experiments postsynaptic APs were elicited antidromically or by brief somatic current injection (2 ms; 4 nA). During antidromic stimulation, just-subthreshold responses were examined at high gain to ensure that they were not contaminated by excitatory or inhibitory synaptic responses. During AP bursts the EPSP–AP time interval was defined as the time between extracellular stimulation and the middle AP in the burst. Induction of plasticity in the presence of MK801 can presumably be excluded as MK801 is an NMDA antagonist. In addition, these experiments were performed in the presence of AMPA receptor antagonists. Modelling of MK801 block, as described in Supplemental Fig. 1 (see Supplemental material), was performed using Axograph software (Axon Instruments).

Intracellular calcium was imaged using a confocal microscope (LSM 510; Zeiss) after loading cells with the calcium-sensitive fluorescent dye Oregon Green BAPTA-1 (200 μM; Molecular Probes) via the patch-pipette. Calcium-sensitive dyes were excited using the 488 line of an Argon laser, and emitted fluorescence detected via a dichroic mirror (510 nm) and long-pass filter (510 nm). Changes in fluorescence were measured with line scans at 0.3–1 kHz (average of 3–5 sweeps) across dendritic shafts with spines, and expressed as the percentage change in fluorescence relative to baseline ($\Delta F/F$) after background subtraction. Calcium signals in spines and the adjacent dendrite were pooled. APs were evoked by brief somatic current pulses (2 ms; 4 nA).

Results

Spike-timing-dependent plasticity

The classical view of STDP is that pairing a single AP with a single EPSP induces LTP at positive spike timings (EPSP before postsynaptic AP) and LTD at negative spike timings (EPSP after postsynaptic AP). To test the ability of single APs to generate STDP we made recordings from synaptically coupled layer 5 pyramidal neurons. Pairing unitary EPSPs (uEPSPs) with single postsynaptic APs (at 0.1 Hz) at a spike-timing interval of +10 ms failed to cause a change in uEPSP amplitude (Fig. 1*A*, *D* and *E*). This finding is consistent with previous work during low-frequency pairing of uEPSPs and APs in layer 5 pyramidal neurons (Markram *et al.* 1997*b*; Sjöström *et al.* 2001). In contrast, pairing uEPSPs with AP bursts (3 APs at 200 Hz) at the same spike-timing interval (+10 ms)

induced robust LTP (Fig. 1*B*, *D* and *E*; 173 ± 16% of control, $n = 5$, $P = 0.03$). Surprisingly, AP bursts also induced LTP (140 ± 11% of control, $n = 7$, $P = 0.01$) when paired at negative time intervals of −15 ms (Fig. 1*C*, *D* and *E*), a timing which typically generates LTD (Markram *et al.* 1997*b*; Bi & Poo, 1998; Sjöström *et al.* 2001). Pairing uEPSPs at more negative times (−40 ms) induced LTD (Fig. 1*E*; 81 ± 4.3% of control, $n = 11$, $P = 0.05$). Unitary EPSPs evoked in the absence of APs did not cause a change in uEPSP amplitude (Fig. 1*D*; 98 ± 6.1% of control, $n = 6$). Induction of LTP at both positive (+10 ms) and negative (−15 ms) spike timing intervals was blocked by the NMDA receptor antagonist D-APV (50 μM; +10 ms: 102.4 ± 7.1% of control, $n = 4$, $P = 0.88$; −15 ms: 106.4 ± 7.1% of control, $n = 4$, $P = 0.86$), indicating an NMDA-dependent mechanism. These data show that in the cortex induction of STDP during pairing of uEPSPs with APs in layer 5 pyramidal neurons requires AP bursts, as seen in the hippocampus (Pike *et al.* 1999; Meredith *et al.* 2003). Furthermore, we show that during AP bursts the STDP time window extends to negative spike timings.

NMDA receptor activation

To investigate the dendritic mechanisms underlying these findings we investigated the extent of synaptic NMDA receptor activation during induction of STDP using the activity-dependent NMDA antagonist MK801 (Huettner & Bean, 1988). With this method, the extent of MK801 block can be used to estimate the extent of NMDA receptor activation (see Supplemental Fig. 1). In these

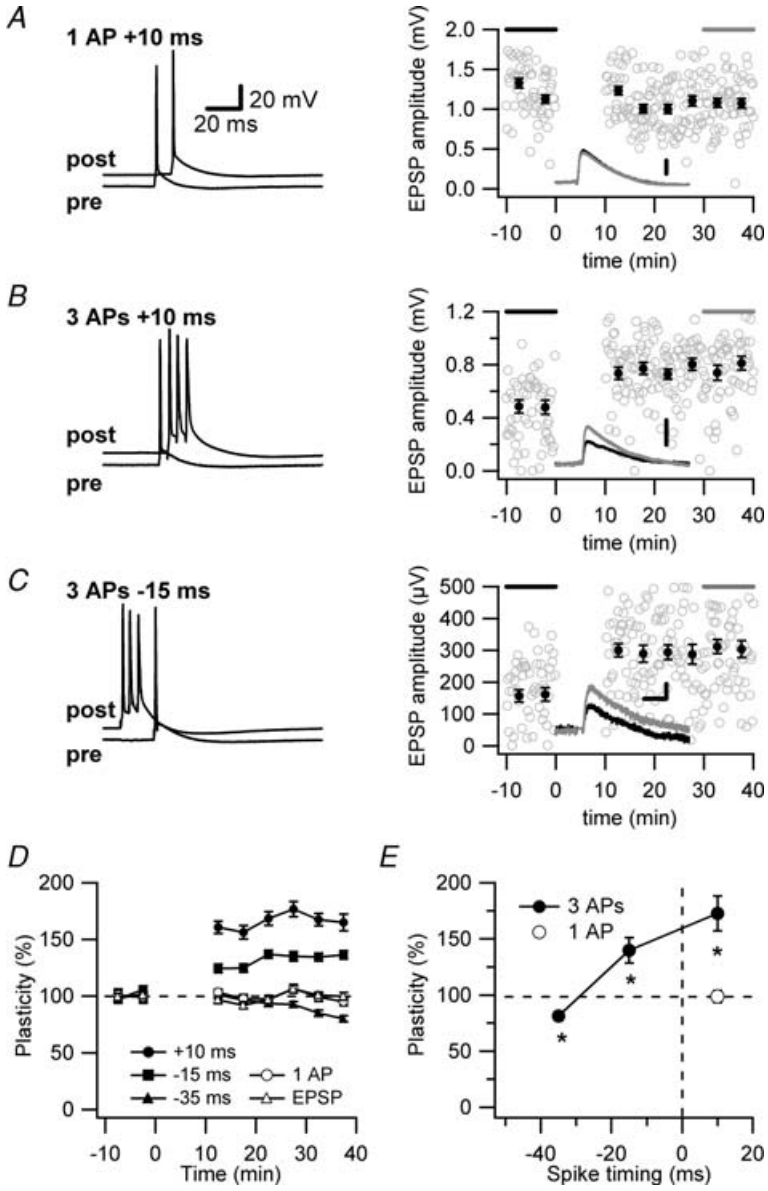

Figure 1. Spike-timing-dependent plasticity (STDP) in synaptically coupled layer 5 pyramidal neurons
A, pairing uEPSPs with single APs at low frequencies (0.1 Hz) fails to induce synaptic plasticity (+10 ms timing). Left panel shows induction protocol with pre- and postsynaptically generated APs. Right panel shows a plot of uEPSP amplitude *versus* time for a single experiment. Filled symbols show averages of 30 trials (± S.E.M.). STDP induction occurred at 0 min, and lasted for 10 min (60 pairings at 0.1 Hz). Inset, average uEPSPs before (black) and 20–30 min after (grey) STDP induction. Traces were averaged across the time span indicated by horizontal bars. Scale bar indicates 0.5 mV. *B* and *C*, same as *A*, showing examples of pairing uEPSPs with AP bursts (3 APs at 200 Hz) at positive (*B*; +10 ms) and negative (*C*; −15 ms) spike-timing intervals. Scale bars indicate 0.5 mV (*B*) and 0.1 mV and 10 ms (*C*). *D*, average time course of STDP across all cells for the indicated induction protocols. STDP induction occurred at 0 min, and lasted for 10 min (60 pairings at 0.1 Hz). *E*, summary of STDP induction with AP bursts at different timing intervals (−35 ms, −15 ms and +10 ms; $n = 11$, 6 and 5, respectively). Horizontal line indicates percentage change after stimulation of uEPSPs alone. Open symbol represents average result of pairing with single APs at +10 ms ($n = 5$).

experiments, NMDA EPSPs, evoked by extracellular stimulation in layer 5, were pharmacologically isolated by bath application of AMPA and $GABA_A$ receptor antagonists, and recorded at resting membrane potentials in the presence of 1 mM magnesium. NMDA EPSPs evoked on their own were reduced by $26 \pm 2\%$ ($n = 14$) of control amplitude after 60 trials in the presence of MK801 (Fig. 2*A* and *C*; 5 μM). Pairing NMDA EPSPs with single APs at +10 ms lead to similar MK801 block to that observed in the absence of APs (Fig. 2*C*; average reduction $23 \pm 5\%$, $n = 5$). In contrast, pairing NMDA EPSPs with AP bursts at +10 ms lead to an approximately twofold increase in MK801 block, with NMDA EPSP amplitude on average reduced by $54 \pm 2\%$ relative to control (Fig. 2*B* and *C*; $n = 9$). These data provide direct evidence that AP bursts are significantly more effective than single APs at activating synaptic NMDA receptors.

Next, we investigated the dependence of MK801 block on the precise timing of AP bursts during EPSP–AP pairing. To calculate NMDA activation from MK801 block we corrected for the exponential, rather than linear, relationship between the amplitude of NMDA EPSPs in MK801 and the associated NMDA activation (see Supplemental material). NMDA receptor activation was found to be greatest when AP bursts were evoked just after EPSP onset (+10 ms, 2.6 ± 0.16 fold increase, $n = 9$), and was similar to that observed in the absence of APs when AP bursts were evoked well after (> 150 ms) or before (< 35 ms) EPSP onset (Fig. 2*D*). Consistent with data from STDP experiments (Fig. 1), significant NMDA receptor activation was observed when AP bursts were evoked just before EPSP onset (−15 ms, 2.11 ± 0.15 fold increase, $n = 7$). These findings indicate that activation of synaptic NMDA receptors is maximal at spike timings where LTP is observed (see Fig. 1*D*), whereas we could not detect activation of postsynaptic NMDA receptors during spike timings that generate LTD.

Role of dendritic calcium spikes in STDP

In the apical dendrites of layer 5 pyramidal neurons AP bursts at frequencies greater than 100 Hz lead to generation of dendritic calcium spikes and an associated supralinear increase in calcium (Larkum *et al.* 1999). Recent evidence suggests that AP bursts also lead to generation of dendritic calcium spikes in basal dendrites with a similar critical frequency (Kampa & Stuart, 2004). Consistent with this idea, high-frequency AP bursts (200 Hz) lead to a supralinear (4.6 ± 0.5 fold; $n = 5$) increase in intracellular calcium compared to single APs (Fig. 3*A* and *C*). The

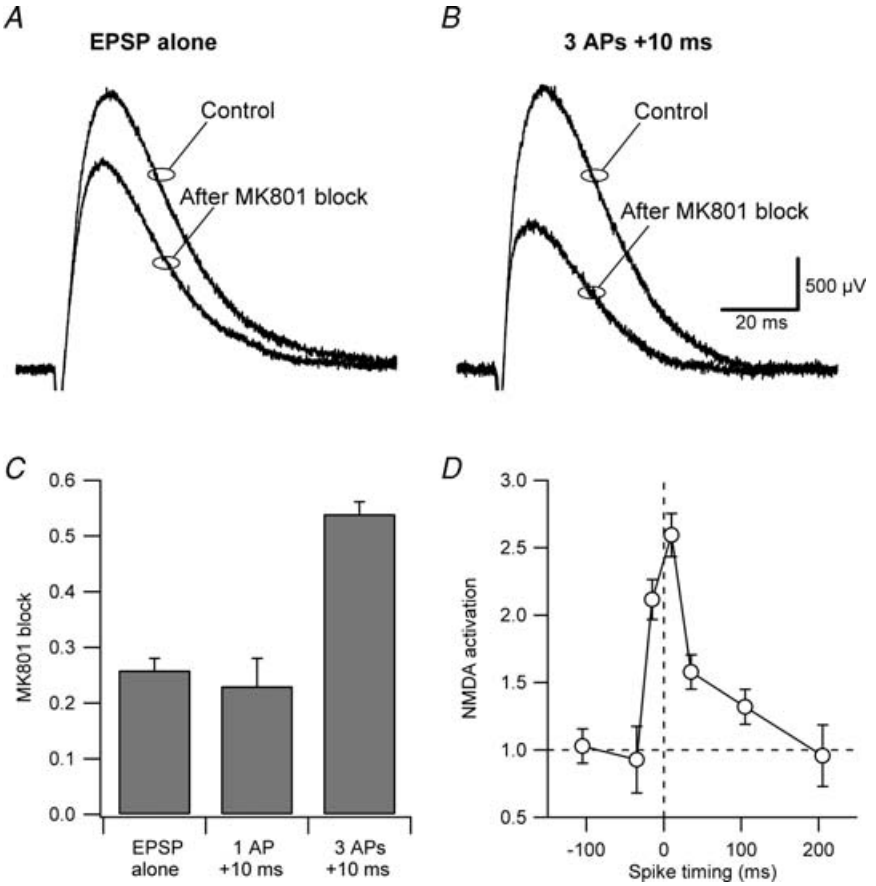

Figure 2. NMDA receptor activation during STDP induction
A, averaged NMDA EPSPs recorded at resting membrane potentials in the presence of 1 mM magnesium in control conditions and after stimulation of 60 EPSPs in the presence of MK801 (5 μM). *B*, average NMDA EPSPs in control and after stimulation of 60 EPSPs paired with AP bursts (3 APs at 200 Hz) at +10 ms timing in the presence of MK801 (5 μM). *C*, pooled data showing a twofold increase in MK801 block of NMDA EPSPs after pairing EPSPs with bursts of 3 APs relative to EPSP alone (MK801 block defined as: $1 - EPSP_{MK801}/EPSP_{control}$). Pairing NMDA EPSPs with single APs lead to a similar degree of MK801 block as that seen during EPSPs alone. *D*, NMDA receptor activation depends on EPSP–AP time interval. NMDA receptor activation during pairing of NMDA EPSPs with AP bursts (3 APs at 200 Hz) relative to that during EPSPs alone for different EPSP–AP time intervals (in ms): −105, −35, −15, +10, +35, +105, +205 (see Supplemental Fig. 1).

supralinear increase during AP bursts was significantly reduced (to 3.5 ± 0.6 fold; $n = 5$) by bath application of low concentrations (100 μM) of the calcium channel antagonist nickel (Fig. 3*A*–*C*). These data support the idea that activation of dendritic T- or R-type calcium channels leads to the generation of dendritic calcium spikes in basal dendrites during AP bursts. To investigate the role of these dendritic calcium spikes in synaptic plasticity, we tested the impact of bath applications of nickel on both NMDA receptor activation and STDP. Pairing uEPSPs with high-frequency (200 Hz) AP bursts in the presence of nickel (100 μM) blocked LTP induction during pairing at +10 ms ($100 \pm 2\%$ of control; $n = 5$; $P = 0.5$), whereas during pairing at −15 ms LTP was converted into LTD (Fig. 3*D*; $83 \pm 3\%$ of control, $n = 5$, $P = 0.03$). Bath application of nickel (100 μM) also blocked activation of NMDA receptors during AP bursts, as assessed using MK801 (Fig. 3*E*; $n = 10$, $P = 0.0003$). Together, these data suggest that during STDP dendritic calcium spikes generated during AP bursts are required for both LTP induction and NMDA receptor activation.

To further test the hypothesis that dendritic calcium spikes are required for LTP induction we investigated the frequency dependence of STDP during AP bursts. If LTP requires the generation of dendritic calcium spikes, one would expect that pairing uEPSP with AP bursts at frequencies below critical frequency (∼100 Hz) should abolish STDP. We therefore compared STDP protocols during the pairing of uEPSPs and AP bursts at 200 Hz with that observed during AP bursts at 50 Hz (Fig. 4). To avoid potential problems due to the introduction of both positive and negative spike timings in these experiments, we kept the timing of the first AP constant during pairing at positive times, whereas the timing of the last AP was kept constant during pairing at negative times. These experiments indicated that pairing uEPSPs with AP bursts below the critical frequency for generation of dendritic calcium spikes is ineffective in generating synaptic plasticity. On average, during pairing with AP bursts at 50 Hz for positive spike timings uEPSPs were $97 \pm 5\%$ of control (Fig. 4*A*; $n = 4$, $P = 0.98$), whereas for negative spike timings uEPSPs were $102 \pm 7\%$ of control (Fig. 4*B*; $n = 5$, $P = 0.3$). These data indicate that STDP

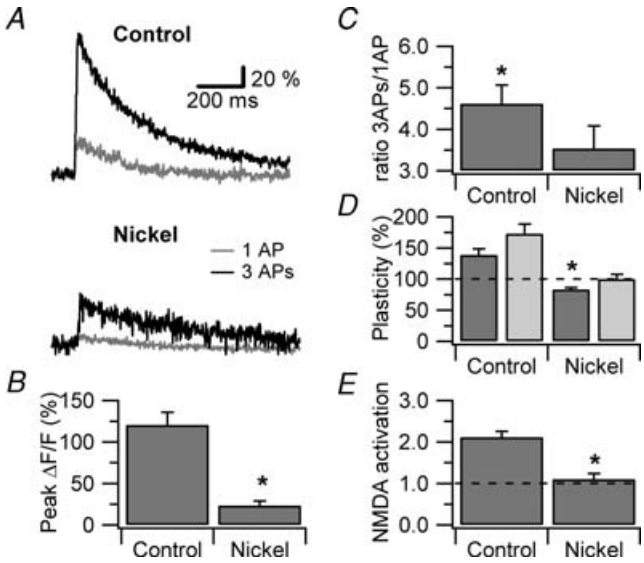

Figure 3. Generation of dendritic calcium spikes by AP bursts induces LTP
A, dendritic calcium influx during single APs (grey traces) and bursts of 3 APs (200 Hz; black traces) in control and following bath application of nickel (100 μM). Changes in intracellular calcium were imaged in basal dendrites (>150 μm from soma) filled with calcium-sensitive fluorescent dye Oregon Green BAPTA-1 (200 μM). *B*, average calcium influx in basal dendrites during AP bursts in control (left) and in the presence of nickel (100 μM; right). *C*, bursts of 3 APs cause a supralinear increase in intracellular calcium compared to single APs ($P < 0.05$; $n = 5$) that is significantly reduced in the presence of nickel. *D*, average percentage change in uEPSP amplitude relative to control (Plasticity) after pairing uEPSPs with AP bursts (3 APs at 200 Hz) at positive (+10 ms; light grey columns) and negative timing intervals (−15 ms; dark grey columns) in control (left) and in the presence of nickel (100 μM; right). Dashed line indicates baseline EPSP amplitude. *E*, average NMDA receptor activation during pairing of NMDA EPSPs with AP bursts at negative timing intervals (−15 ms) in control (left; $n = 7$) and in the presence of nickel (100 μM; right; $n = 10$). NMDA receptor activation was assessed with MK801 (see Fig. 2).

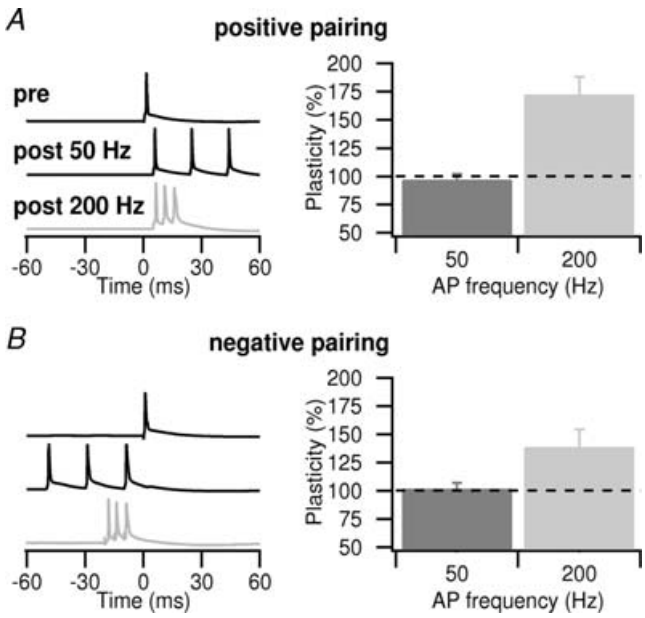

Figure 4. Frequency dependence of STDP
A, left, STDP induction protocol during EPSP–AP pairing with low-frequency (50 Hz) and high-frequency (200 Hz) 3 AP bursts at positive (*A*) and negative times (*B*). The timing of the first AP in the burst was kept constant during pairing at positive times, whereas the timing of the last AP was kept constant during pairing at negative times. Right, histograms of the percentage change in EPSP amplitude during positive (*A*) and negative pairing (*B*) with low-frequency (50 Hz; dark grey columns) and high-frequency (200 Hz; light grey columns) AP bursts.

induction requires AP bursts at supra-critical frequencies. Furthermore, they provide additional evidence supporting our conclusion that dendritic calcium spikes generated by high-frequency AP bursts are required for STDP induction.

Discussion

Classical models of synaptic plasticity indicate that induction is triggered by NMDA receptor-mediated calcium influx (Bliss & Collingridge, 1993). Consistent with this idea, we show that high-frequency AP bursts are effective triggers of both LTP and NMDA activation during EPSP–AP pairing at positive and negative times. In contrast, EPSP–AP pairing with single APs and low-frequency AP bursts was ineffective in generating LTP. We go on to show that both LTP and NMDA activation during high-frequency AP bursts were blocked by low concentrations of the calcium channel antagonist nickel, which also blocked supralinear increases in dendritic calcium influx during AP bursts. Together, these data suggest an important role of AP burst-evoked dendritic calcium spikes in STDP, and emphasize the importance of AP burst firing and active dendritic properties in STDP induction.

Comparisons with previous studies on STDP

Consistent with previous studies on STDP between synaptically coupled layer 5 pyramidal neurons (Markram *et al.* 1997*b*; Sjöström *et al.* 2001), low-frequency pairing of single APs with uEPSPs at positive times did not lead to LTP (Fig. 1). The most likely explanation for this finding is that low-frequency pairing with single APs does not generate sufficient dendritic depolarization at the site of synaptic input to remove the magnesium block of synaptic NMDA receptors. Consistent with this idea, we did not observe significant NMDA activation during EPSP–AP pairing with single APs (Fig. 2). Furthermore, a previous study has shown that LTP is only observed during low-frequency pairing of uEPSPs with single APs when it is associated with membrane potential depolarization supplied via additional synaptic input or somatic current injection (Sjöström *et al.* 2001). High-frequency AP bursts, on the other hand, can significantly depolarize the dendrites via generation of AP burst-evoked calcium spikes in the apical dendrites of pyramidal neurons (Stuart *et al.* 1997*a*; Larkum *et al.* 1999). Recent evidence indicates that high-frequency AP bursts can also generate calcium spikes in basal dendrites (Kampa & Stuart, 2004), consistent with the nickel-sensitive supra-linear increase in calcium in basal dendrites during AP bursts (Fig. 3). The increased amplitude and duration of dendritic depolarizations during dendritic calcium spikes would be expected to enhance removal of the voltage-dependent block of NMDA receptors by magnesium, and presumably explains why AP bursts are effective triggers of both NMDA activation (Fig. 2) and LTP induction (Fig. 1). The proposed role of dendritic calcium spikes in STDP during AP bursts may also explain the requirement for AP burst firing during LTP induction in hippocampal neurons (Pike *et al.* 1999; Meredith *et al.* 2003). Given that neighbouring layer 5 pyramidal neurons make synaptic connections onto both basal and apical dendrites of layer 5 pyramidal neurons (Markram *et al.* 1997*a*), it seems likely that AP burst-evoked dendritic calcium spikes in both basal and apical dendrites can play a role in the induction of STDP. Furthermore, it may be that only a subset of layer 5 to layer 5 inputs (approximately 6 per connection) are potentiated, and that potentiated inputs are those located on distal basal or oblique dendrites, or in the apical tuft.

Recent work indicates that pairing AP bursts with large-amplitude EPSPs, evoked by extracellular synaptic stimulation in layer 2/3, leads to LTD in layer 5 pyramidal neurons (Birtoli & Ulrich, 2004). Similar to the AP burst-evoked LTP in our study, LTD in the study by Birtoli & Ulrich (2004) was observed during pairing EPSPs with AP bursts at positive and negative times, and was blocked by low concentrations of nickel, suggesting a role of dendritic calcium spikes during AP bursts. In contrast, the AP burst-evoked LTD observed by Birtoli & Ulrich (2004) was blocked by metabotropic glutamate receptor antagonists, and not by NMDA receptor antagonists. These data suggest that during synchronized activation of large numbers of inputs to layer 5 neurons, pairing AP bursts with EPSPs leads to a dominant form of LTD via a metabotropic glutamate receptor mechanism, whereas pairing AP bursts with uEPSPs leads to LTP via an NMDA receptor-dependent mechanism. One possible explanation for this difference is that during synchronized activation of large numbers of inputs glutamate can spill over, leading to activation of metabotropic glutamate receptors which reside outside the synaptic cleft (Baude *et al.* 1993). Under these conditions, metabotropic glutamate receptor activation may veto NMDA-dependent LTP induction.

NMDA receptor activation by backpropagating action potentials

Postsynaptic NMDA receptors are mainly located on dendritic spines (reviewed by Nusser, 1999). As it is impossible to record directly from these small structures, we used MK801, an activity-dependent NMDA receptor antagonist, to assess NMDA receptor activation during STDP. Pairing EPSPs with AP bursts in the presence of MK801 increased MK801 block, leading to a reduction in the amplitude of NMDA EPSPs. These data provide direct evidence that backpropagating APs are capable of removing the magnesium block of synaptic NMDA

receptor channels. Using this method we were not able to detect NMDA activation during EPSP pairing with single APs (Fig. 2*C*), as has been previously observed by others using calcium imaging (Koester & Sakmann, 1998). There are a number of possible explanations for this. One possibility is that the MK801 method is not sensitive enough to detect NMDA receptor activation during single EPSP–AP pairing. Alternatively, as backpropagating APs attenuate as they propagate into the apical (Stuart *et al.* 1997*a*) and basal dendrites (Kampa & Stuart, 2004), distal synaptic inputs may not be significantly depolarized by single APs. This may explain the apparent discrepancy with the work of Koester & Sakmann (1998), who only examined calcium influx during EPSP–AP pairing at very proximal dendritic locations (20–80 μm from the soma). In contrast, pairing high-frequency AP bursts with EPSPs significantly enhanced NMDA receptor activation at both positive and negative spike timings (Fig. 2*D*). This finding is consistent with recent studies showing that slow magnesium unblock (Vargas-Caballero & Robinson, 2003; Kampa *et al.* 2004) favours long-lasting depolarizations such as dendritic calcium spikes induced by AP bursts (Vargas-Caballero & Robinson, 2003).

Functional role of dendritic calcium spikes in synaptic plasticity

It is commonly assumed that the timing of a single back-propagating AP relative to an EPSP determines the sign and magnitude of synaptic modifications during STDP (e.g. Abbott & Nelson, 2000). Yet, as we show here, pairing single APs with uEPSPs at low frequencies fails to induce LTP between synaptically coupled layer 5 pyramidal neurons (see also Markram *et al.* 1997*b*; Sjöström *et al.* 2001). In contrast, pairing EPSPs with AP bursts successfully potentiated these synapses. Previous studies indicate that induction of LTP at cortical synapses during pairing with single APs requires large-amplitude, compound EPSPs (Sjöström *et al.* 2001), or pairing of both uEPSPs and APs at high frequencies (>10 Hz) (Markram *et al.* 1997*b*; Sjöström *et al.* 2001). As cortical neurons typically fire at low rates *in vivo* (~0.5 Hz) (Margrie *et al.* 2002), it is unclear whether these induction protocols will occur under physiological conditions. On the other hand, AP bursts are a physiologically relevant firing mode of cortical layer 5 pyramidal neurons, and have been observed *in vivo* during sensory input (Helmchen *et al.* 1999; Larkum & Zhu, 2002) or during the stimulation of layer 1 synaptic inputs mediating information from higher cortical areas (Larkum & Zhu, 2002). These data suggest that STDP *in vivo* may only take place during high-frequency AP burst firing. This notion is consistent with other studies suggesting that AP bursts play a critical role in information processing (Lisman, 1997; Snider *et al.* 1998).

Dendritic spikes can also be generated by correlated synaptic inputs in the absence of APs (Schiller *et al.* 1997), with recent work in pyramidal neurons in the hippocampus indicating a role of dendritic calcium spikes in the induction of non-Hebbian forms of synaptic plasticity that do not require backpropagating APs (Golding *et al.* 2002). Other studies show that induction of synaptic plasticity can lead to changes in active dendritic properties (Wang *et al.* 2003; Frick *et al.* 2004), which would be expected to influence the generation of future dendritic spikes. Together, these findings indicate that active dendritic mechanisms are likely to play an important role in determining both the efficacy, as well as the time window, for STDP induction (Lisman & Spruston, 2005).

References

Abbott LF & Nelson SB (2000). Synaptic plasticity: taming the beast. *Nat Neurosci* **3** (Suppl.), 1178–1183.

Baude A, Nusser Z, Roberts JD, Mulvihill E, McIlhinney RA & Somogyi P (1993). The metabotropic glutamate receptor (mGluR1 alpha) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron* **11**, 771–787.

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

Birtoli B & Ulrich D (2004). Firing mode-dependent synaptic plasticity in rat neocortical pyramidal neurons. *J Neurosci* **24**, 4935–4940.

Bliss TV & Collingridge GL (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31–39.

Connors BW, Gutnick MJ & Prince DA (1982). Electrophysiological properties of neocortical neurons in vitro. *J Neurophysiol* **48**, 1302–1320.

Frick A, Magee J & Johnston D (2004). LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. *Nat Neurosci* **7**, 126–135.

Golding NL, Staff NP & Spruston N (2002). Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* **418**, 326–331.

Hebb DO (1949). *The Organization of Behavior*. Wiley, New York.

Helmchen F, Svoboda K, Denk W & Tank DW (1999). In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons. *Nat Neurosci* **2**, 989–996.

Huettner JE & Bean BP (1988). Block of N-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. *Proc Natl Acad Sci U S A* **85**, 1307–1311.

Kampa BM, Clements J, Jonas P & Stuart GJ (2004). Kinetics of Mg^{2+} unblock of NMDA receptors: implications for spike-timing dependent synaptic plasticity. *J Physiol* **556**, 337–345.

Kampa BM & Stuart GJ (2004). Dendritic electrogenesis and action potential backpropagation in basal dendrites of cortical layer 5 pyramidal neurons: a calcium and voltage imaging study. *4th Forum of European Neuroscience (FENS)*, *July 2004*, *Congress Center*, *Lisbon*. A222.218.

Koester HJ & Sakmann B (1998). Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proc Natl Acad Sci U S A* **95**, 9596–9601.

Larkum ME, Kaiser KM & Sakmann B (1999). Calcium electrogenesis in distal apical dendrites of layer 5 pyramidal cells at a critical frequency of back-propagating action potentials. *Proc Natl Acad Sci U S A* **96**, 14600–14604.

Larkum ME & Zhu JJ (2002). Signaling of layer 1 and whisker-evoked Ca^{2+} and Na^{+} action potentials in distal and terminal dendrites of rat neocortical pyramidal neurons in vitro and in vivo. *J Neurosci* **22**, 6991–7005.

Lisman JE (1997). Bursts as a unit of neural information: making unreliable synapses reliable. *Trends Neurosci* **20**, 38–43.

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

Margrie TW, Brecht M & Sakmann B (2002). In vivo, low-resistance, whole-cell recordings from neurons in the anaesthetized and awake mammalian brain. *Pflugers Arch* **444**, 491–498.

Markram H, Lubke J, Frotscher M, Roth A & Sakmann B (1997*a*). Physiology and anatomy of synaptic connections between thick tufted pyramidal neurones in the developing rat neocortex. *J Physiol* **500**, 409–440.

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

Mayer ML, Westbrook GL & Guthrie PB (1984). Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* **309**, 261–263.

Meredith RM, Floyer-Lea AM & Paulsen O (2003). Maturation of long-term potentiation induction rules in rodent hippocampus: role of GABAergic inhibition. *J Neurosci* **23**, 11142–11146.

Nowak L, Bregestovski P, Ascher P, Herbet A & Prochiantz A (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* **307**, 462–465.

Nusser Z (1999). Subcellular distribution of neurotransmitter receptors and voltage-gated ion channels. In *Dendrites*, ed. Stuart G, Spruston N & Hausser M, pp. 85–113. Oxford University Press.

Pike FG, Meredith RM, Olding AW & Paulsen O (1999). Postsynaptic bursting is essential for 'Hebbian' induction of associative long-term potentiation at excitatory synapses in rat hippocampus. *J Physiol* **518**, 571–576.

Schiller J, Schiller Y, Stuart G & Sakmann B (1997). Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *J Physiol* **505**, 605–616.

Sjöström PJ, Turrigiano GG & Nelson SB (2001). Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. *Neuron* **32**, 1149–1164.

Snider RK, Kabara JF, Roig BR & Bonds AB (1998). Burst firing and modulation of functional connectivity in cat striate cortex. *J Neurophysiol* **80**, 730–744.

Stuart G, Schiller J & Sakmann B (1997*a*). Action potential initiation and propagation in rat neocortical pyramidal neurons. *J Physiol* **505**, 617–632.

Stuart G, Spruston N, Sakmann B & Häusser M (1997*b*). Action potential initiation and backpropagation in neurons of the mammalian central nervous system. *Trends Neurosci* **20**, 125–131.

Vargas-Caballero M & Robinson HP (2003). A slow fraction of Mg^{2+} unblock of NMDA receptors limits their contribution to spike generation in cortical pyramidal neurons. *J Neurophysiol* **89**, 2778–2783.

Wang Z, Xu NL, Wu CP, Duan S & Poo MM (2003). Bidirectional changes in spatial dendritic integration accompanying long-term synaptic modifications. *Neuron* **37**, 463–472.

Acknowledgements

We acknowledge the support of both the NHMRC of Australia, and the Alexander von Humboldt Foundation.

Author's present address

B. M. Kampa: Department of Neurophysiology, Brain Research Institute, University of Zurich, Switzerland.

Supplemental material

The online version of this paper can be accessed at:
DOI: 10.1113/jphysiol.2006.111062
http://jp.physoc.org/cgi/content/full/jphysiol.2006.111062/DC1
and contains supplemental material consisting of a figure and text entitled 'Quantification of MK801 block'.

This material can also be found as part of the full-text HTML version available from
http://www.blackwell-synergy.com