Long-term Potentiation of Intrinsic Excitability in LV Visual Cortical Neurons

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Abstract

Neuronal excitability has a large impact on network behavior, and plasticity in intrinsic excitability could serve as an important information storage mechanism. Here we ask whether postsynaptic excitability of layer V pyramidal neurons from primary visual cortex can be rapidly regulated by activity. Whole-cell current clamp recordings were obtained from visual cortical slices and intrinsic excitability was measured by recording the firing response to small depolarizing test pulses. Inducing neurons to fire at high frequency (30-40 Hz) in bursts for 5 minutes in the presence of synaptic blockers increased the firing rate evoked by the test pulse. This long-term potentiation of intrinsic excitability (LTP-IE) lasted for as long as we held the recording (>60 min). LTP-IE was accompanied by a leftward shift in the entire frequency vs. current (FI) curve and a decrease in threshold current and voltage. Passive neuronal properties were unaffected by the induction protocol, indicating that LTP-IE occurred through modification in voltage-gated conductances. Reducing extracellular calcium during the induction protocol, or buffering intracellular calcium with BAPTA, prevented LTP-IE. Finally, blocking PKA activation prevented, while pharmacological activation of PKA both mimicked and occluded, LTP-IE. This suggests that LTP-IE occurs through postsynaptic calcium influx and subsequent activation of PKA. Activity-dependent plasticity in intrinsic excitability could greatly expand the computational power of individual neurons.
**Introduction**

Activity-dependent changes in neuronal circuits underlie the ability of organisms to learn. Much emphasis has been placed on activity-dependent changes in synaptic strength as a mechanism for information storage (Malenka and Nicoll 1999; Abbott and Nelson 2000). Yet the precise way a neuron integrates its synaptic inputs to generate action potentials (APs) is also of great importance. The amount of input necessary to evoke an AP, and the number and pattern of action potentials generated in response to a given input, will strongly effect network behavior. Plasticity in intrinsic excitability could thus have a major impact on network dynamics, and could serve as an important information storage mechanism (Marder 1998; Golowasch et al. 1999a, 1999b; Marder and Prinz 2002).

There is mounting evidence that intrinsic excitability can be regulated by activity (Zhang and Linden 2003; Daoudal and Debanne 2003). Chronically lowered activity in invertebrate systems and in cultured cortical neurons induces homeostatic changes in intrinsic excitability that tend to restore firing properties to their original values (Turrigiano et al. 1994, 1995; Thoby-Brisson and Simmers 1998; Desai et al. 1999b). A similar process appears to operate in the tadpole optic tectum *in vivo*, where hours of persistent visual stimulation decreases synaptic drive to tectal neurons (Aizenman et al. 2002), and this in turn leads to an increase in intrinsic excitability (Aizenman et. al. 2003). Synaptic activity can modulate presynaptic excitability on both long and short time scales (Nick and Ribera 2000; Ganguly and Poo 2000), and the intrinsic excitability of deep cerebellar nuclei neurons can be rapidly modulated by postsynaptic activity (Aizenman and Linden 2000). In addition, both metabotropic (Sourdet et al. 2003) and
inhibitory (Nelson et al. 2003) receptor activation can trigger long-term increases in excitability. These experiments demonstrate that many of the same manipulations that induce synaptic plasticity can also cause plasticity in intrinsic excitability.

Here we ask whether short periods of AP firing alter the intrinsic excitability of layer V (LV) pyramidal neurons from visual cortical slices. We found that a brief period of repetitive firing led to a long-lasting potentiation of intrinsic excitability (LTP-IE), characterized by a leftward shift in the frequency vs. current (FI) curve and a reduction in the threshold current for AP generation. LTP-IE was dependent on calcium influx, but not on activation of PKC or CaMKII. PKA inhibitors blocked LTP-IE, and activation of PKA with forskolin both mimicked and occluded LTP-IE. These data suggest that brief periods of high frequency firing alter intrinsic neuronal excitability through the activation of PKA. By altering the responsiveness of neurons to synaptic inputs, these changes in intrinsic excitability could serve as important modulators of circuit function.

Methods

Slices and physiological recordings. Long-Evans rats, p14-p19, were anesthetized with isoflurane and decapitated. The brain was rapidly removed and placed into ice cold artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 3 KCl, 2 MgCl₂, 1 NaH₂PO₄, 2 CaCl₂, 25 NaHCO₃, 25 dextrose. The osmolarity was adjusted to 310-320 mOsm with dextrose, and ACSF was continuously bubbled with 95% O₂/5% CO₂, to maintain pH 7.4. Three hundred micron thick coronal slices of the visual cortex were cut using a Series 1000 Vibratome (Technical Products International Inc., O'Fallon, MI). Slices were warmed to 36 °C for ten minutes and then allowed to return to room temperature. Slices were used after at least one hour
of incubation and not more than nine hours after slicing; all recordings were done at room temperature.

Thick tufted LV neurons were visually identified at 400X magnification using infrared DIC optics on an upright Olympus BX-50WI (Olympus, Melville, New York) microscope. Neuronal morphology and location within the slice were later verified using biocytin histochemistry. Recordings were discarded if the morphology or location indicated a cell type other than thick tufted LV. Glass micropipettes (5–10 MΩ, 1–2 μm tip diameter) were pulled from 1.0 mm thick-walled glass on a P-97 Flaming-Brown Micropipette Puller (Sutter Instruments Co., Novato, California) and filled with (in mM): 20 KCl, 100 (K)Gluconate, 10 (K)HEPES, 4 (Mg)ATP, 0.4 (Na)GTP, 10 (Na)Phosphocreatine, 0.5 EGTA and 0.1% w/v Biocytin, adjusted with KOH to pH 7.4, and with sucrose to 290–300 mOsm. Whole-cell current clamp recordings were performed using AxoClamp 2B, AxoPatch 1D, AxoPatch 200B or MultiClamp 700A amplifiers (Axon Instruments, Cuperton, Ca). Recordings were analog filtered at 3-5 kHz and digitized at 10 kHz. All acquisition and analysis was done using IgorPro (Wavemetrics, Oswego, OR). Recordings were discarded if the membrane potential changed by more than 6 mV or the resting input resistance (measured with a 300 ms hyperpolarizing 25 pA pulse) changed by more than 30%. Changing the inclusion criterion to < 10% changes in resting input resistance did not alter the results. Series resistance was calculated offline and recordings were discarded if it was > 40 MΩ, or changed by more than 10% during the course of the recording. In general series resistance was < 20 MΩ and was not compensated.

Pharmacology. All drugs were bath applied using a gravity perfusion system unless otherwise specified. All recordings were performed in the presence of antagonists of NMDA, AMPA/Kainate, and GABA_A receptors (D-APV 50 μM, CNQX 20 μM, and Bicuculline 20 μM,
respectively). The following drugs were used (all from Calbiochem, La Jolla, CA): the broad-spectrum protein kinase inhibitor H7 (100 µM bath applied or 200 µM intracellular), PKA inhibitor H89 (100 µM intracellular), PKC inhibitor calphostin-C (Calph-C, 100 µM intracellular), CaMKII inhibitor Ala peptide and scrambled Ala peptide (both 2-4 mM intracellular, gift of Leslie Griffith), adenyly cyclase activator forskolin (50 µm), and an inactive analog of forskolin, 1,9-dideoxyforskolin (50 µm).

**Experimental Protocol, Analysis and Statistics.** Following the formation of a > G seal, whole-cell access was achieved by rupturing the membrane with negative pressure. A waiting period of 5-10 min. followed while the cell was dialyzed with the pipette solution. Throughout the recording, intrinsic excitability was measured every 15 sec using a constant amplitude small depolarizing pulse (500 ms, 10-80 pA), the amplitude of which was selected to evoke 2-4 APs during the baseline period, and then remained constant throughout the recording. To construct FI curves and calculate the current threshold for AP generation, a range of current injection amplitudes were delivered (10-200 pA in 5-10 pA increments). For every neuron, each amplitude was presented 3 times in a pseudo-random order and the results averaged.

The induction stimulus consisted of 500 ms depolarizing pulses delivered 60 times at intervals of 4 s. The induction stimulus amplitude was selected to evoke sustained action potential firing at approximately 30 Hz throughout the entire pulse (40-70 pA). The parameters of the evoked response during the induction stimulus (mean frequency, number of spikes, average depolarization, time of induction) were similar across all induction conditions. In addition, variations in these parameters did not correlate significantly with the magnitude of the change in excitability.
Measures of intrinsic excitability in response to a depolarizing test pulse included: spike rate, first spike latency, mean inter-spike-interval (ISI), spike voltage threshold (the interpolated membrane potential at which dV/dt equals 20V/sec (Bekkers and Delaney 2001)) and the rate of rise in $V_m$ (dV/dt) before the first action potential (the slope of the membrane potential calculated in a 5 ms window 10-15 ms before the first AP). Resting input resistance was calculated by measuring the steady state voltage deflection in response to a hyperpolarizing pulse (-25 pA, 300 ms). In addition, current versus voltage (IV) curves in the subthreshold range were constructed by measuring the steady state voltage deflection to a range of subthreshold hyperpolarizing and depolarizing pulses (500 ms injections, starting at -60 pA, in 10 pA steps, up to spike threshold). No difference in the IV relationship was found for any condition when the after-induction time window was compared to the before-induction time window.

Within and between cell comparisons were done as follows: each measurement of excitability was extracted from each test pulse and the average calculated over a 5 min. period (20-30 repetitions) both immediately before and 30 min. after induction. The same time points were used for control cells. A two-tailed paired Student’s t test for equal means was run to compare the response 30 minutes after the induction stimulus to the response before the induction stimulus for individual neurons. To compare statistics across a condition, an unpaired two-tailed Student’s t test for equal means was run comparing the mean response after to the mean response before across each population of cells. All means are expressed +/- SEM.

Results

Whole-cell current clamp recordings were obtained from thick tufted LV neurons from slices of the rat visual cortex (p14-p18). This preparation was used to determine if a brief period
of high frequency AP firing (Induction) could modulate intrinsic excitability. To prevent synaptic activation, excitatory and inhibitory ionotrophic transmission was blocked using bath applied CNQX to block AMPA, APV to block NMDA, and bicuculline to block GABA_A receptors.

**Induction caused a long-lasting increase in intrinsic excitability.** In order to characterize intrinsic excitability, the response to a 500 ms DC test pulse selected to evoke 2-4 APs was recorded with an interstimulus-interval of 15 s. In control recordings the response to this test pulse remained constant for the duration of the recording (Fig. 1A). In contrast, inducing neurons to fire at higher frequencies for 5 minutes (30 Hz for 500 ms every 4 sec, for an average firing rate of about 7-8 Hz) increased the number of APs elicited by the test pulse (Fig. 1B). This increase in intrinsic excitability lasted for as long as we held the recordings, indicating that brief periods of high frequency firing induced a long-term potentiation of intrinsic excitability (LTP-IE). Visually driven cortical responses are well within the 30 Hz range, suggesting that visual cortical pyramidal neurons are likely to experience this kind of activity *in vivo* (Steriade 2000, 2001). The induction protocol did not cause changes in resting V_m or R_in, indicating that the passive properties of the neuron were not affected (Fig. 1C).

To compare the time-course of LTP-IE across recordings, firing rates during the test pulse were normalized to the initial rate for each neuron, and averaged across 5 minute time bins for each neuron. These values were then averaged across all neurons for each condition (Fig. 2A). Spike rate for control neurons remained relatively stable throughout the 75 minute recording period, whereas the induction protocol produced a robust increase in spike frequency, to approximately 145% of control values (Fig. 2A, Induction p<0.0001, Control p>0.3). LTP-IE
was successfully induced as long as 35 min. after break-through, the longest delay that was
tested, suggesting that it is relatively resistant to wash-out from dialysis with the pipette solution.

We compared several additional measures of intrinsic excitability across neurons by
comparing firing properties during the baseline period to firing properties in a 5 min. bin 30 min.
after the induction protocol, or after a comparable period for control neurons (Fig. 2B). In
addition to increasing firing rates, induction produced a decrease in the latency to the first AP
(Induction, p<0.0001; Control, p>0.2), a decrease in the mean inter-spike-interval between
spikes (Induction, p<0.0001; Control, p>0.4) and an increase in the rate of rise of the membrane
potential before the first AP (Induction, p<0.005; Control, p>0.5). The rate of rise of the
membrane potential is the slope (dV/dt) measured in a 5 ms window 10 to 15 ms before the AP.
The inset shows an example trace before and after induction illustrating the decreased latency to
first spike and increased rate of rise of the membrane potential close to threshold. There were no
significant changes in any of these parameters for the control condition. There were no changes
in membrane potential or input resistance (calculated for a range of subthreshold current
injections) for either the induction or control conditions (Fig. 2C).

**LTP-IE resulted in a leftward shift in the FI curve.** Next, we wanted to determine if
induction caused a similar increase in excitability across a range of suprathreshold voltages. To
do this frequency vs. current (FI) curves were constructed by injecting a range of current
amplitudes before and after the induction protocol. Example FI curves from induction and
control recording are shown in Figure Fig. 3A. The insets show example traces before and 30
min. after induction and the same time points are shown for a control recording. Following
induction there was an increase in excitability over the whole range of suprathreshold current
amplitudes, whereas for control neurons there was no change (Fig. 3A, B). For both induction
and control, there was no significant change in the slope of the FI curve (Fig. 3B, linear fits), suggesting that this increase in excitability results from a simple shift of the FI curve to the left. This shift to the left was accompanied by a reduction in the threshold current needed to evoke a spike (Fig. 3C; Induction, p<0.0001; Control, p>0.7), determined by increasing the current injection amplitude in 5-10 pA steps until one AP was evoked. There was also a small but significant hyperpolarizing shift in the AP voltage threshold (from -41.04±0.7 mV to -42.05±0.7 mV; Induction, p<0.001; Control, p>0.5). This decrease in the threshold for AP generation suggests that following LTP-IE, neurons will become responsive to previously subthreshold inputs.

**LTP-IE is Calcium-dependent.** Many forms of synaptic plasticity are calcium-dependent. Since AP firing causes calcium influx, we asked whether LTP-IE is also a calcium-dependent form of plasticity. To determine whether calcium influx during the induction protocol is essential, we limited this influx by washing in ACSF with nominally 0 mM Ca$$^{++}$$ during the induction period. This prevented the long-lasting increase in intrinsic excitability (Fig. 4A, 0 Ca$$^{++}$$ Induction, p>0.5). An immediate effect of washing in nominally 0 Ca$$^{++}$$ ACSF was that neurons became temporarily more excitable, likely due to a reduction in calcium-dependent currents such as IK$$^{Ca}$$$$^{++}$$. This increase in excitability was transient and reversed as normal (2 mM Ca$$^{++}$$) ACSF was returned to the bath (Fig. 4A). To further characterize the dependence on calcium influx during the induction protocol, we buffered intracellular calcium by including the calcium chelator BAPTA in the recording pipette (10 mM). This blocked the increase in firing rate normally induced by the induction protocol, and prevented the shift to the left of the FI curve (Fig. 4B). For both the 0 calcium and BAPTA experiments the induction protocol induced no significant change in the threshold current for AP generation (Fig. 4C), firing rate (Fig. 4D), or
dV/dt before the first spike (Fig. 4E). These data suggest that a rise in intracellular calcium during the induction protocol is necessary for the induction of LTP-IE. Because many forms of synaptic plasticity are calcium dependent it is possible that LTP-IE could be induced concomitantly with changes in synaptic strength.

**LTP-IE is protein kinase-dependent.** AP firing and subsequent calcium influx have the effect of activating various 2nd messenger systems. Among the large number of potential mediators of calcium-dependent plasticity, we chose to examine the role of three different calcium-dependent kinases, cAMP-dependent protein kinase (PKA), protein kinase C (PKC), and calcium/calmodulin-dependent protein kinase II (CaMKII). We began by determining whether H7, a membrane-permeable broad-spectrum protein kinase inhibitor, was capable of blocking LTP-IE. Micromolar concentrations of H7 block PKA, PKC, CaMKII, and cGMP-dependent protein kinase (Hidaka et al. 1984; Malinow et al. 1989). When H7 was included in the recording pipette (200 µM) or was bath applied (100 µM) during the induction period, it prevented LTP-IE (Fig. 5A, H7 Induction, p>0.6). Bath application and intracellular dialysis with H7 had similar effects so the data were combined in Figure 5. H7 prevented the leftward shift in the FI curve normally produced by the induction protocol (Fig. 5B, compare to Fig. 3A, B, Induction) and the reduction in threshold current for AP generation (Fig. 5C, H7 Induction, p>0.3). These data strongly suggest that the increase in excitability following induction depends on protein kinase activation.

To further characterize which protein kinases are necessary for LTP-IE, we tested selective inhibitors of PKA, PKC, and CaMKII. Inclusion of 100 µM H89, a specific PKA inhibitor (Chijiwa et al. 1990), in the pipette blocked the reduction in threshold current (Fig. 5C, H89 Induction), the increase in firing rate (Fig. 5D) and the change in dV/dt before the first spike.
(Fig. 5E) induced by the induction protocol. In contrast, including the PKC inhibitor calphostin-C in the pipette (Calph-C, 100 µM; Kobayashi 1989) did not prevent LTP-IE (Fig. 5C-D, Calph-C Induction). To determine if LTP-IE depends on CaMKII activation, additional experiments were performed in which the CaMKII inhibitor Ala peptide (Griffith et al. 1993) was included in the pipette at concentrations between 2 and 5 mM. The Ala peptide did not prevent LTP-IE (% change in spike rate: 120.06 ± 4.49, n=9, p<0.005, data not shown). Induction produced a similar magnitude LTP-IE when a scrambled version of Ala peptide was included in the pipette (% change in spike rate: 129.43 ± 8.19, p<0.05, n=6, data not shown). These data suggest that CaMKII activation is not essential for the induction of LTP-IE.

**PKA activation mimics and occludes LTP-IE.** To ask whether PKA activation is sufficient to induce LTP-IE, we used forskolin (forsk), an adenylyl cyclase activator, to directly elevated cAMP and activate PKA. A 10 min. bath application of forskolin (50 µM), caused a long-lasting increase in excitability that closely resembled that produced by our induction protocol (Fig. 6A, Forsk, p<0.005). Traces show example responses to the test pulse before and after bath application of forskolin. In addition, forskolin caused a shift to the left of the FI curve (Fig. 6B), a reduction of the threshold current for AP generation (data not shown, p<0.005), and an increase in dV/dt before the first spike (data not shown, p<0.03), just as is seen during LTP-IE. These data indicate that elevation of cAMP is sufficient to mimic the increase in excitability that follows the induction protocol. Forskolin application also occluded stimulation-induced LTP-IE. When the induction protocol was run after the forskolin-induced increase in excitability (Fig. 6C, n=5, Forsk) there was no additional increase in excitability (Fig. 6C; Forsk + Ind). The ability of PKA inhibitors to block, and forskolin to mimic and occlude LTP-IE, suggest that LTP-IE is induced via a PKA-dependent mechanism.
As a control for non-specific effects of forskolin (Harris-Warrick, 1989), we used an inactive analog of forskolin, 1,9-dideoxyforskolin. Bath application of this inactive analog did not cause an increase in excitability (Fig 6C, 1,9-Fors, n=8, p>0.09), and did not occlude stimulation-induced LTP-IE (Fig 6C, 1,9-Fors+Ind, p<0.002).

**DISCUSSION**

We have shown that a brief period of AP firing induces a long-lasting potentiation of intrinsic neuronal excitability (LTP-IE) in Layer V neocortical pyramidal neurons. This LTP-IE does not require synaptic activation, but is directly induced by postsynaptic depolarization, and requires calcium influx and activation of PKA. LTP-IE is characterized by a leftward shift in the FI curve and a reduction in threshold current, indicating that the sensitivity of the neuron to depolarizing current is increased. By increasing postsynaptic sensitivity, LTP-IE will tend to enhance spiking to previously subthreshold inputs. This could have long-lasting effects on information propagation through cortical networks, and could also modify the ease with which synaptic potentiation occurs.

Learning paradigms alter neuronal excitability in a number of vertebrate and invertebrate systems. Increased excitability of photoreceptors contributes to the classical conditioning of visual responses in *Hermisenda* (Alkon 1984; Gandi and Matzel 2000). A long lasting potentiation of intrinsic excitability has been found in hippocampal CA1 and CA3 pyramidal neurons following trace eye blink conditioning (Moyer et al. 1996; Thompson et al. 1996) and water maze learning (Oh et al. 2003), and operant conditioning has similar effects in the olfactory (piriform) cortex (Saar et al. 1998; Saar et al. 2002; Saar and Barkai 2003 for a review). In addition, it has long been known that long-term potentiation (LTP) of hippocampal synapses
is accompanied by an increase in the ease with which spikes can be elicited in the postsynaptic neuron (E-S potentiation, Bliss and Lomo 1973; Abraham et al. 1987), a phenomenon that occurs in part through increased intrinsic excitability (Chavez-Noriega et al. 1990; Daoudal et al. 2002). Taken together, these studies indicate that LTP is only one of several plasticity phenomena that are associated with learning, and suggest that LTP-IE could be an important substrate for information storage or modulation of further plasticity. Our data demonstrate that brief periods of elevated firing, well within the physiological range for visual cortical neurons (Steriade 2000, 2001) is sufficient to induce LTP-IE of cortical pyramidal neurons. Similar effects have been demonstrated in deep cerebellar nuclear neurons (Aizenman and Linden 2000). This suggests that one mechanism by which learning paradigms could modify intrinsic excitability is through a simple increase in postsynaptic calcium influx induced by relatively brief periods of high frequency firing.

Postsynaptic calcium influx is a critical trigger for many forms of synaptic plasticity, including LTP and LTD (Malenka and Nicoll 1999; Abbott and Nelson 2000). LTP-IE is also calcium dependent, as limiting influx with nominally-zero calcium ACSF, or buffering intracellular calcium with BAPTA, both prevent LTP-IE. These manipulations are also likely to lower basal calcium, so we cannot rule out the possibility that it is lower basal calcium, rather than lack of calcium influx, that prevents LTP-IE. This calcium dependence suggests that traditional stimulation paradigms for inducing synaptic plasticity, such as tetanic stimulation, could concomitantly trigger intrinsic plasticity. Spike-timing dependent LTP (STDP) at unitary LV neocortical synapses is frequency-dependent, and requires bursts of 20-50 Hz firing such as those we have used to induce LTP-IE (Markram et al. 1997; Sjostrom et al. 2001), again suggesting that STDP and LTP-IE could be triggered together by the same stimuli. In addition,
in hippocampal neurons, repetitive firing can induce an increase in the amplitude of repetitive back propagating action potentials that is calcium dependent (Tsubokawa et al. 2003). This suggests that changes in intrinsic excitability induced by repetitive firing may modulate back propagating action potentials, which could in turn influence the ease with which STDP is induced. The patterns of activity required to trigger synaptic and intrinsic plasticity in vivo remain unclear, so while these two forms of plasticity may often be induced together and could influence each other, there may be activity regimes in which one or both forms of plasticity can be generated in isolation.

A number of calcium-dependent kinases take part in the complex signal transduction cascades that lead to long-lasting changes in synaptic strength. These include CaMKII, PKC, and PKA (Lisman et al. 2002; Mons et al. 1999; Malenka and Nicoll 1999). PKA is activated when calcium/calmodulin activates adenylyl cyclase, and increases intracellular cAMP levels. CAMP/PKA is thought to play a number of roles in neuronal plasticity, including a necessary role in the intermediate-term, protein-synthesis-independent phase of hippocampal LTP (Blitzer et al. 1995), and in changes in gene expression that may ultimately underlie very long-term plasticity (Bailey et al. 1996). We found that LTP-IE, like LTP, is dependent upon protein kinase activation during the induction period, as it could be prevented by including the broad-spectrum kinase inhibitory H7 in the pipette, or by perfusing H7 during the induction period. In contrast to many forms of LTP, however, LTP-IE could still be induced in the presence of PKC and CaMKII inhibitors, suggesting that these kinases do not play a necessary role in its induction. Our data suggest that the critical kinase is PKA, because the specific PKA inhibitor H89 prevented LTP-IE, while activating adenylyl cyclase with forskolin both mimicked and occluded firing-induced LTP-IE. PKA is not the only effector of cAMP action in neurons.
(Kopperud et al. 2003), so directly raising cAMP with forskolin could have downstream effects on neuronal properties that are independent of PKA activation. The ability of H7 and H89 to completely block LTP-IE, however, suggests that PKA activation is a necessary component of this signal transduction cascade.

LTP-IE is most probably mediated by changes in voltage dependent ionic conductances as there were no accompanying changes in passive cell properties. LV neurons have a complex spatial expression pattern of voltage gated Na⁺, K⁺, Ca⁺⁺, and Ca⁺⁺ activated K⁺ channels (Huguenard et al. 1989; Bekkers 2000a, 2000b; Korngreen and Sakmann 2000; Zhu 2000; Sun et al. 2003). Pharmacological manipulation of ion channels alters intrinsic excitability (Bekker and Delaney 2001; Smith et al. 2002). PKA is known to phosphorylate and downregulate K⁺ channels, causing an increase in excitability (Hoffman and Johnston 1998). The decreased threshold for AP generation induced by LTP-IE suggests that calcium influx and subsequent PKA activation could act by altering voltage-dependent conductances that begin to activate around the AP threshold. Which conductances are affected, and whether this occurs through modulation of existing channels or insertion or removal of new channels, remains to be determined.

Like many forms of Hebbian synaptic plasticity, LTP-IE is likely to have a destabilizing influence on network function, because increased excitability will make it easier to fire the postsynaptic neuron, which should in turn generate further LTP-IE (Turrigiano 1999; Abbott and Nelson 2000). We have previously described a long-lasting regulation of intrinsic excitability in cortical pyramidal neurons that operates much more slowly (over days) and acts to adjust intrinsic excitability to compensate for altered activity (Desai et al. 1999a,b). This slow regulation of intrinsic excitability could help to mitigate the destabilizing effects of LTP-IE,
analogous to the way slow, homeostatic synaptic scaling has been proposed to counteract the destabilizing effects of Hebbian synaptic plasticity (Turrigiano et al. 1998; Turrigiano 1999).

Synaptic plasticity mechanisms such as LTP have been favorite candidate information storage mechanisms, in part because the ability to independently modify hundreds or thousands of synaptic inputs generates enormous computational power (Malenka and Nicoll 1999; Abbott and Nelson 2000). In contrast, LTP-IE will modify the sensitivity of the postsynaptic neuron to all of its inputs. This change in postsynaptic gain will tend to emphasize the contribution of that neuron to network activity. By lowering spike threshold, LTP-IE may also enhance the ability of synapses onto that neuron to undergo Hebbian plasticity. These considerations suggest that LTP-IE could play important roles in information storage and the modulation of synaptic plasticity.

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Figure Legends

**Figure 1.** A brief period of high frequency firing caused a long-lasting potentiation of intrinsic excitability (LTP-IE) in LV pyramidal neurons. (A) Example of control recording. Constant amplitude current pulses were delivered every 15 sec to measure intrinsic excitability. Firing properties remained stable throughout the duration of the recording. Example traces are from the time points indicated by the arrows. (B) Example of LTP-IE. Following a 10 min. baseline period (Before), the neuron was induced to fire at 30-40 Hz in 500 ms bursts every 4 sec for 5 minutes (Induction). Following the induction stimulus there was a rapid and long lasting increase in excitability (After). (C) Induction did not cause any changes in passive cell properties. Scale bars are 100 ms, 20 mV.

**Figure 2.** Characterization of LTP-IE. (A) Average time course of LTP-IE. For each neuron, spike rate was normalized to a 5 min. period (-10 to -5 min) at the beginning of the recording and values were averaged for all neurons in each condition. Induction (solid squares) produced a significant increase in spike rate evoked by the test pulse. There was no significant change in excitability for control neurons (open squares). (B) Induction decreased the latency to the first action potential (Spike Latency), decreased the mean inter-spike-interval (Mean ISI), and caused a significant increase in the slope of the voltage trajectory leading up to the first spike (Change in dV/dt). Inset shows an example neuron before (black trace) and after (grey trace) the induction stimulus. Note the decrease in the latency and the increase in the slope of the membrane potential leading up to the first spike. In control neurons there were no significant changes in
any of these parameters. Scale bars are 5 ms, 20 mV. (C) The passive properties remained stable in both induction and control neurons.

**Figure 3.** LTP-IE is characterized by a leftward shift in the FI curve. (A) Left panel shows an example FI curve before (open symbols) and after (closed symbols) induction. Right panel shows example FI curves from the same time points for a control neuron. Arrow indicates the current amplitude used for the example traces. (B) Average FI curves for each condition. For each neuron current was normalized to the current amplitude that evoked one spike during the baseline period. Induction caused a leftward shift in the average FI curve. For both induction and control there was no significant change in the slope of the FI curve (linear fits). (C) LTP-IE decreased the threshold current (minimum current needed to evoke one spike). Inset shows an example trace before and after induction, illustrating that a previously subthreshold stimulus was suprathreshold after induction. Scale bars are 100 ms, 20 mV.

**Figure 4.** LTP-IE is calcium dependent. (A) Bath application (grey bar) of nominally 0 Ca\(^{++}\) during the induction period prevented LTP-IE. Nominally 0 mM Ca\(^{++}\) ACSF produced a temporary increase in excitability that reversed as regular ACSF was returned to the bath. (B) Buffering intracellular calcium with BAPTA (10 mM intracellular) also prevented LTP-IE. When BAPTA was included in the pipette there was no change in the FI curve following induction. (C) There was no decrease in threshold current when induction was run in the presence of nominally 0 mM Ca\(^{++}\) or BAPTA. Changes in spike rate (D) and dV/dt (E) were indistinguishable from control when induction was run with nominal Ca\(^{++}\) or BAPTA.

**Figure 5.** LTP-IE depends on protein kinase activation. (A) H-7, a broad-spectrum protein kinase inhibitor (either 100 µM bath applied, n=6, or 200 µM intracellular, n=6), completely blocked the increase in excitability following induction. H7 was bath applied (grey bar) 5 min.
before the induction stimulus and terminated 10 min. later, always after the end of the induction
stimulus (solid bar). Similar results were obtained when H7 was included in the pipette. (B) H7
prevented the leftward shift in the FI curve normally produced by the induction protocol. (C,D,
E) H7 (H7 Induction) also prevented the decrease in threshold current, change in spike rate, and
change in dV/dt. The PKA inhibitor H89 (H89 Induction) also prevented the increase in
excitability produced by the induction protocol. In contrast, LTP-IE was still produced when
PKC activity was inhibited using calphostin-C (Calph-C Induction).

**Figure 6.** PKA activation mimicked and occluded LTP-IE. (A) A 10 min. bath application of
forskolin (black bar) produces a long-lasting increase in excitability that closely resembled LTP-
IE. Inset traces show example responses to the test pulse for the time points indicated by the
arrows. (B) Bath application of forskolin produced a leftward shift in the FI curve. (C) To
determine if the increased excitability induced by forskolin occluded firing-induced LTP-IE, an
induction stimulus was run (Forsk+Ind) following bath application of forskolin (Forsk). This did
not cause any further change in excitability. An inactive analog of forskolin (1,9-
dideoxyforskolin) did not increase intrinsic excitability (1,9-Fors) and did not occlude stimulus
induced LTP-IE (1,9-Fors+Ind).
Figure 1
Figure 2

A

![Graph showing normalized spike rate over time](image)

B

![Bar graphs showing induction and control spike latency and mean ISI](image)

C

![Bar graphs showing change in dV/dt and V_m, R_in](image)
Figure 3

A

B

C

Cudmore and Turrigiano: Long-term plasticity of intrinsic excitability
Figure 5

A

B

C

D

E

Cudmore and Turrigiano: Long-term plasticity of intrinsic excitability

Figure 5
Figure 6

A

Normalized Spike Rate

1.6

1.4

1.2

1.0

0.8

Time (min)

-20

-10

0

10

20

30

40

50

Forsk (n=10)

B

Spike Rate (Hz)

16

14

12

10

8

6

4

2

Normalized Current (pA)

50

60

70

80

90

100

110

Forsk

Before

After

C

% Change Spike Rate

160

140

120

100

80

(n=5)

(n=8)**

Forsk

Forsk+Ind

1.9-Fors

1.9-Fors+Ind