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Stimulus-Evoked Modulation of Sensorimotor Pyramidal Neuron EPSPs

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Kohn, Adam, Carol Metz, Mark A. Tommerdahl, and Barry L. Whitsel. Stimulus-evoked modulation of sensorimotor pyramidal neuron EPSPs. *J Neurophysiol* 88: 3331–3347, 2002; 10.1152/jn.01012.2001. Sensory cortical neurons display substantial receptive field dynamics during and after persistent sensory drive. Because a cell's response properties are determined by the inputs it receives, receptive field dynamics are likely to involve changes in the relative efficacy of different inputs to the cell. To test this hypothesis, we have investigated if brief repetitive stimulus drive in vitro alters the efficacy of two types of corticocortical inputs to layer V pyramidal cells. Specifically, we have used whole cell recordings to measure the effect of repetitive electrical stimulation at the layer VI/white matter (WM) border on the synaptic response of layer V pyramidal cells to corticocortical input evoked by electrical stimulation of layer I or layer II/III and emulated by local application of glutamate. Repetitive stimulation (10 Hz for 3 s) at the layer VI/WM border transiently potentiated excitatory postsynaptic potentials (EPSPs) evoked by electrical stimulation of layer II/III by 97 ± 12% (mean ± SE). The recovery of EPSP amplitude to its preconditioning value was well-described by a single-term decaying exponential with a time constant of 7.2 s. The same layer VI/WM conditioning train that evoked layer II/III EPSP potentiation frequently caused an attenuation of layer I EPSPs. Similarly, subthreshold postsynaptic responses to local glutamate application in layers II/III and I were potentiated and attenuated, respectively, by the conditioning stimulus. Potentiation and attenuation could be evoked in the same cell by repositioning the glutamate puffer pipette in the appropriate layer. The conditioning stimulus that led to the transient modification of upper layer EPSP efficacy also evoked a slow depolarization in glial cells. The membrane potential of glial cells recovered with a time course similar to the dissipation of the potentiation effect, suggesting that stimulus-evoked changes in extracellular potassium (ECK) play a role in layer II/III EPSP potentiation. Consistent with this proposal, increasing the bath concentration of ECK caused a substantial increase of layer II/III EPSP amplitude. EPSP potentiation was sensitive to postsynaptic membrane potential and, more importantly, was significantly weaker for synaptic currents than for synaptic potentials, suggesting that it involves the recruitment of a postsynaptic voltage-dependent mechanism. Two observations suggest that layer II/III EPSP potentiation may involve the recruitment of postsynaptic sodium channels: EPSP potentiation was strongly reduced by intracellular application of *N*-(2,6-dimethyl-phenylcarbamoylmethyl) triethylammonium bromide (QX-314) and responses to local glutamate application were potentiated by high ECK in the presence of cadmium but not in the presence of tetrodotoxin. The results demonstrate a novel way in which brief periods of repetitive stimulus drive are accompanied by rapid, transient, and specific alterations in the functional connectivity and information processing characteristics of sensorimotor cortex.

INTRODUCTION

There is growing evidence that the response properties and receptive field (RF) characteristics of neurons in primary sensory cortex are dynamic, undergoing substantial transient modification during and after stimulus drive (Gilbert 1998). Cortical response dynamics occur on a broad time scale, ranging from hundreds of milliseconds to many minutes and include changes in stimulus selectivity (Dragoi et al. 2000; Movshon and Lennie 1979; Saul and Cynader 1989a,b), in RF size (Cavanaugh et al. 1999; Dinse et al. 1990), and in the influence of stimuli outside the classical RF (Das and Gilbert 1995). Because the RF properties of a sensory cortical neuron are determined, in part, by its inputs, the dynamics cited above are likely to involve changes in the efficacy of different sources of input to cortical pyramidal cells during and after sensory stimulation. For instance, the finding that the influence of stimuli outside the classical RF develops over time (Cavanaugh et al. 1999; Dinse et al. 1990) and depends on the level of current (Sceniak et al. 1999) and recent input drive (Das and Gilbert 1995) suggests that the efficacy of inputs from the surround, thought to be mediated either by long-range horizontal connections or by feedback connections (Gilbert 1998), changes substantially during and after the presentation of a visual stimulus. Currently, there is little information concerning how different types of input to a cortical cell are affected by the recent history of stimulus drive.

We have evaluated how repetitive stimulus drive can affect subsequent input to a layer V pyramidal cell in slices of rat sensorimotor cortex. The effect of repetitive sensory drive was mimicked by a relatively brief period (3 s) of repetitive electrical stimulation delivered to the layer VI/white matter border. Stimuli at this location provide input relayed via thalamocortical and intracolumnar pathways, activating a localized column-shaped region of cortex, similar to the pattern of excitation provided by a sensory stimulus (Kohn et al. 2000; Langdon and Sur 1990; Yuste et al. 1997). We evaluated the effect of this brief period of stimulus drive on the efficacy of corticortical inputs that, for layer V pyramidal cells in rat sensorimotor cortex, derive from neurons in the same cortical area, in motor and secondary somatosensory cortex, and in the contralateral hemisphere (Keller 1995). Specifically, excitatory postsynaptic potentials (EPSPs) conveyed by axons in layer

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II/III, a site at which long-range horizontal connections are made (Chapin et al. 1987), and by axons in layer I, the primary termination site for feedback inputs from the second somatosensory area (Cauller et al. 1998) were compared before and after the delivery of the repetitive stimuli to the layer VI/WM border. The results suggest that even a brief period of repetitive stimulus drive differentially and substantially alters the efficacy of the inputs that reach the apical dendrite of a layer V pyramidal cell via corticocortical axons in layers I–III.

METHODS

Coronal slices (450 μm) were prepared from sensorimotor cortex of young adult rats (21–35 days; Sprague-Dawley; Charles River). Following decapitation the brain was rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF). Slices were cut in cold, modified (NaCl was replaced by sucrose) ACSF using an oscillating tissue slicer (OTS-4000, Electron Microscopy Sciences) and then stored in warmed (30°C), oxygenated (95% O_2-5% CO_2) ACSF for $\geq$1 h. The composition of the ACSF (in mM) was 118 NaCl, 3.0 KCl, 2.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.2 NaH_2PO_4, and 10 glucose. In the experiments in which cadmium was added to the ACSF, NaH_2PO_4 was omitted to prevent precipitation.

Whole cell recordings

Slices were transferred from the reservoir to a recording chamber mounted to the stage of an inverted microscope (Diaphot 200, Nikon). The slice was submerged and held in place by a fine mesh, and the chamber perfused continuously (1.5–2 ml/min.) with warmed (26–29°C) oxygenated ACSF. Blind whole cell recordings were made using the method of Blanton et al. (1989). Patch pipettes were pulled from 1.5 mm OD glass on a standard electrode puller (Narishige PP-83), fire polished under visual control, and filled with a solution containing (in mM) 130 K-gluconate, 20 KCl, 10 HEPES, 10 glucose, 1 Mg Cl_2, 4 Mg-ATP, 0.2 GTP, 2 cAMP, 5 creatine phosphate, and 20 U/ml creatine phosphokinase. Pipettes were lowered into the slice and slowly advanced using a piezoelectric micropositioner (Burleigh). After seal formation ($>$1 GΩ), the cell membrane was ruptured and series resistance and whole cell capacitance adjusted. Recordings were made using an Axon Instruments amplifier (Axopatch 1A). Signals were low-pass filtered at 2 kHz, digitized using a A/D converter (Digidata 1200) and recorded with pClamp7 software (Axon Instruments). Recordings were not corrected for junction potential errors; full correction would make all membrane potential values ~12 mV more negative than reported. A neuron was regarded as acceptable for recording if it fired overshooting action potentials when depolarized by current injection, its resting membrane potential was less than $-$55 mV; and its input resistance was $>$50 MΩ. The mean duration of the recordings was 56 $\pm$ 3 min.

Neurons or glial cells were studied using a standard protocol (a "trial") consisting of a single repetitive stimulus train (the "conditioning stimulus") and $\geq$21 test stimuli (Fig. 1). Trials were repeated at 5-min intervals. The conditioning stimulus was delivered at the layer VI/WM border (S1 site; Fig. 1) through a concentric bipolar stimulating electrode (tip diameter, 50 μm) by a constant-current stimulator attached to a programmable TTL-pulse generator (Master 8, AMPI). Trains consisted of 0.2 ms square current pulses with a strength of 70–200 μA, delivered at a rate of 10 or 20 Hz for 3 s. Test stimuli were delivered at a rate of 0.2 Hz via a second bipolar stimulating electrode placed in either layer I or layers II/III (S2 site; Fig. 1) or by puffs of glutamate solution applied with a Picospritzer II system (General Valve). Electrical test stimuli were delivered at a tangential distance of 0.8–1.2 mm from the recorded cell. The stimulus strength used to elicit EPSPs was typically in the range of 40–70 μA; stimulus duration was 100 μs. Glutamate puffs were delivered through a glass micropipette placed in the superficial layers, radially above the recorded cell. Glutamic acid (300 μM) was dissolved in the standard ACSF or, when pharmacological manipulations were used, in a solution replicating that used for the "drug" condition. The spatial extent of the glutamate puff, estimated by visual inspection under high-power magnification ($\times$40) when Fast Green was included in the puffer pipette, was ~50 μm (puff strength of 20–30 psi, 5- to 150-ms duration). In some experiments, cyclothiazide (100 μM) dissolved in 1% DMSO was included in the puffer pipette to combat AMPA receptor desensitization (Yamada and Tang 1993). All drugs were purchased from Sigma (St. Louis, MO) except tetrodotoxin (TTX; Alamone, Jerusalem, Israel) and CGP35348 (kindly provided by Novartis; Basel).

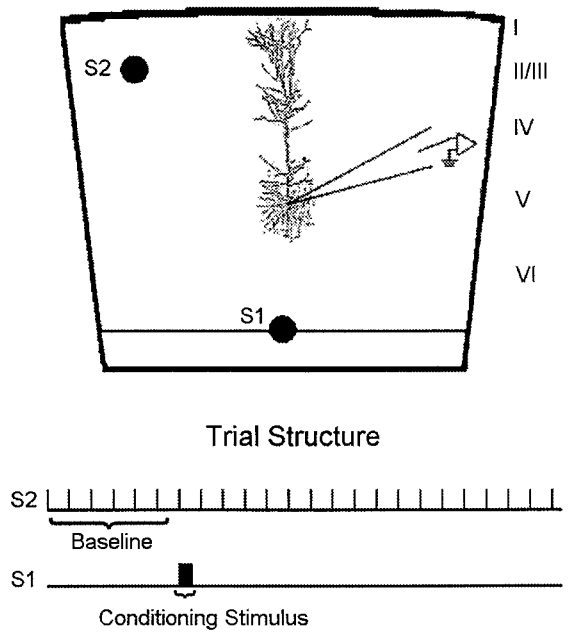

FIG. 1. Recording arrangement and trial structure. *A*: whole cell recordings were made from layer V pyramidal cells. A conditioning train was delivered via an electrode placed at the layer VI/white matter (WM) border (S1). Test stimuli were delivered in the upper layers (S2). *B*: trials began with the delivery of 6 test stimuli in the upper layers ("baseline" period), after which the conditioning train was delivered at the layer VI/WM border. Excitatory postsynaptic potential (EPSP) amplitude after conditioning was compared with that in the baseline period.

Data analysis

The effect of repetitive layer VI/WM stimulation on test EPSPs evoked from layers I and II/III was evaluated by comparing EPSP amplitude before ("baseline") and after conditioning. To enable a meaningful comparison among cells, the data were normalized on a trial-by-trial basis—that is, the amplitude of each EPSP is expressed in terms of the mean EPSP amplitude during the baseline period. When the effect of repetitive stimulation on EPSPs is reported as a single value, that value corresponds to the amplitude of the first EPSP evoked after conditioning stimulation. A paired *t*-test was used to assess, in each cell, whether the difference between the average amplitude of the first EPSP after the conditioning train and the baseline value of 100% was significant. Single cell differences associated with P values $<$0.1 were regarded as statistically significant. Variances are reported as $\pm$SE.

Histology

A subset of the recorded cells were filled with biocytin (0.5%). Slices containing biocytin-filled neurons were placed immediately

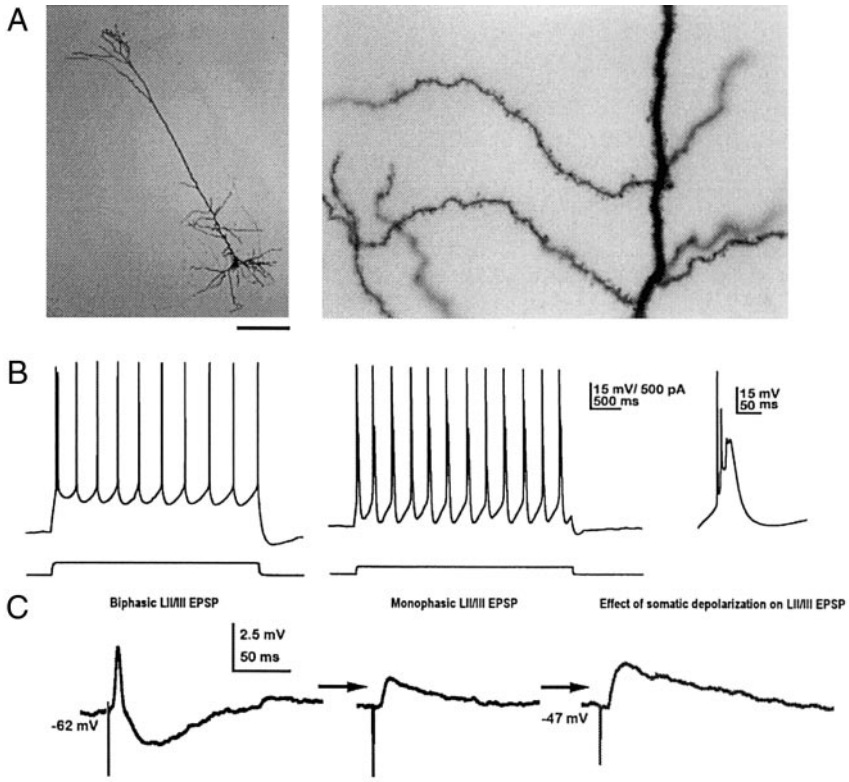

FIG. 2. Cell and EPSP characteristics. *A*: biocytin-filled layer V pyramidal cell at low (*left*) and high (*right*) magnification. Scale bar: *left,* 200 μm; *right,* 20 μm. *B*: spiking patterns of layer V pyramidal cells. Regular spiking (*left*) and bursting (*middle*; same cell at higher resolution on *right*) patterns evoked by depolarizing current injection (current steps shown under voltage trace). *C*: procedure for adjusting upper layer stimulus strength. Strong stimulation evoked a biphasic response (*left*); weaker stimuli elicited a pure EPSP (*middle*) uncontaminated by a hyperpolarizing component. The amplitude of EPSPs increased with somatic depolarization (*right*). Cell membrane potential: *middle* and *left,* was −62 mV; *right,* −47 mV.

after recording in a cold (4°C) phosphate-buffered 4% paraformaldehyde/4% sucrose (pH 7.4) solution and thereafter in 30% sucrose in 0.1 M phosphate buffer (pH 7.35) at 4°C for ≥24 h. Slices were frozen-sectioned parallel to the slice surface at 60 μm, pretreated with ethanol, reacted with ABC complex (Vector Laboratories, PK-4000), and DAB-H_2O_2 according to established procedures, mounted onto glass slides, dehydrated, cleared, and coverslipped.

RESULTS

Cell and EPSP characteristics

Whole cell patch-clamp recordings were obtained from 189 layer V neurons and 56 glial cells in rat sensorimotor cortex. Cells were classified as pyramidal cells if the firing pattern evoked by depolarizing current injection was regular spiking or bursting (Fig. 2, *A* and *B; n* = 182) (Connors et al. 1982). Fast spiking cells were classified as inhibitory interneurons ($n = 7$). No decrease in action potential height was observed during spike trains elicited by depolarizing current injection, suggesting that the recording location was somatic (Fig. 2*B*) (Callaway and Ross 1995; Spruston et al. 1995). Whole cell recordings of glial cells were distinguishable from neuronal recordings on the basis of a significantly different resting membrane potential (−75 ± 1 mV for glia compared with −61.9 ± 0.4 mV for neurons; $P < 0.001$, paired *t*-test), a significantly different input resistance (123 ± 13 MΩ for glia compared with 206 ± 8 MΩ for neurons; $P < 0.001$, paired *t*-test), and the absence of action potentials during depolarizing current injection. In 40 cells, biocytin labeling revealed the morphological identity of the recorded cell that, in every case, was consistent with the classification assigned on the basis of electrophysiological characteristics (Figs. 2*A* and 10*A*).

Subthreshold EPSPs were evoked by stimulating electrodes placed in layers I and II/III under visual control. The characteristics of EPSPs evoked by electrical stimulation of layers I and II/III are shown in Table 1. Layer I EPSPs had a longer 20–80% rise time and time to peak than layer II/III EPSPs, but these differences were not statistically significant (*t*-test; $P > 0.05$). Consistent with the findings of others, strong layer II/III stimuli typically elicited a biphasic response consisting of an EPSP followed by an inhibitory postsynaptic potential (IPSP) (Hirsch and Gilbert 1991). IPSPs were rarely observed after layer I stimulation (Cauller and Connors 1994; Shao and Burkhalter 1996). The strength of upper layer stimulation was adjusted so that small-amplitude monophasic EPSPs were evoked, uncontaminated by a hyperpolarizing component (Fig. 2*C*). The amplitude of these EPSPs often increased with somatic depolarization, an effect observed previously by others and attributed to the recruitment of somatic voltage-gated sodium channels (Fig. 2*C*) (Deisz et al. 1991, Hirsch and Gilbert 1991; Stuart and Sakmann 1995). Current-voltage curves of the synaptic responses evoked by layer II/III stimulation had an average extrapolated reversal potential of 0 ± 4.2 mV ($n = 10$ cells), suggesting that the procedure for isolating EPSPs in current-clamp was effective. The reliability and onset latency of the evoked EPSPs (Table 1) is consistent with a

TABLE 1. *Layer I and II/III EPSP characteristics*

	Layer I EPSPs	Layer II/III EPSPs
EPSP amplitude, mV	3.02 ± 0.2	2.89 ± 0.09
20–80% rise time, ms	9.14 ± 0.7	8.4 ± 0.04
Full width at half amplitude, ms	50.2 ± 2.3	52 ± 1.5
Time to peak, ms	28 ± 1.2	26 ± 0.8
Onset latency, ms	6.5 ± 0.3	6.3 ± 0.2

Values are means ± SE. EPSP, excitatory postsynaptic potential.

monosynaptically relayed response evoked at distance of 1 mm from the recorded cell (Gonzalez-Burgos 2000; Lohman and Rorig 1994). Potential contributions of polysynaptic contamination of evoked responses are considered in the discussion section.

Effect of layer VI/WM conditioning stimulation on layer II/III EPSPs

Each trial began with the recording of six EPSPs evoked by stimulation at the layer II/III site, defined as the "baseline" period (Fig. 3*A*). Layer II/III EPSPs were elicited at a rate of 0.2 Hz to avoid synaptic depression (Abbott et al. 1997; Markram and Tsodyks 1997). After the baseline period, a stimulus train—the conditioning stimulus—was delivered by an electrode at the layer VI/WM border. The conditioning train consisted of 3 s of stimulation at 10 Hz (84% of trials) or 20 Hz (16% of trials). In most cases, the cell fired an action potential in response to each stimulus in the conditioning train. The amplitude of layer II/III EPSPs was strongly potentiated after the conditioning stimulus (Fig. 3, *A* and *B*). To compute the strength of EPSP potentiation across the sample population of cells ($n = 126$), the average EPSP amplitude in the baseline period of each trial (402 trials) was normalized to 100%. The average EPSP amplitude after the conditioning train was 306 ± 40% of its baseline value (Fig. 3*C*, black squares). In 23 of the 402 trials, the potentiation was sufficient to convert a previously subthreshold EPSP to a response that triggered an action potential. To avoid overestimation of the potentiation effect, the average potentiation was recalculated after excluding trials in which EPSPs triggered regenerative events. When these trials were removed from the data set, the average layer II/III EPSP amplitude after a conditioning train was 197 ± 12% of its baseline value (Fig. 3*C*, gray circles). EPSP potentiation gradually diminished over time, with EPSP amplitude returning to baseline values within 20–40 s after conditioning. For the 379 trials that provided the data shown in Fig. 3*C* (gray trace), the decay of the potentiation was well described by a single-term decaying exponential with a time constant of 7.2 s.

To investigate the conditioning stimulus necessary for evoking a potentiation of layer II/III EPSPs, we evaluated the effect

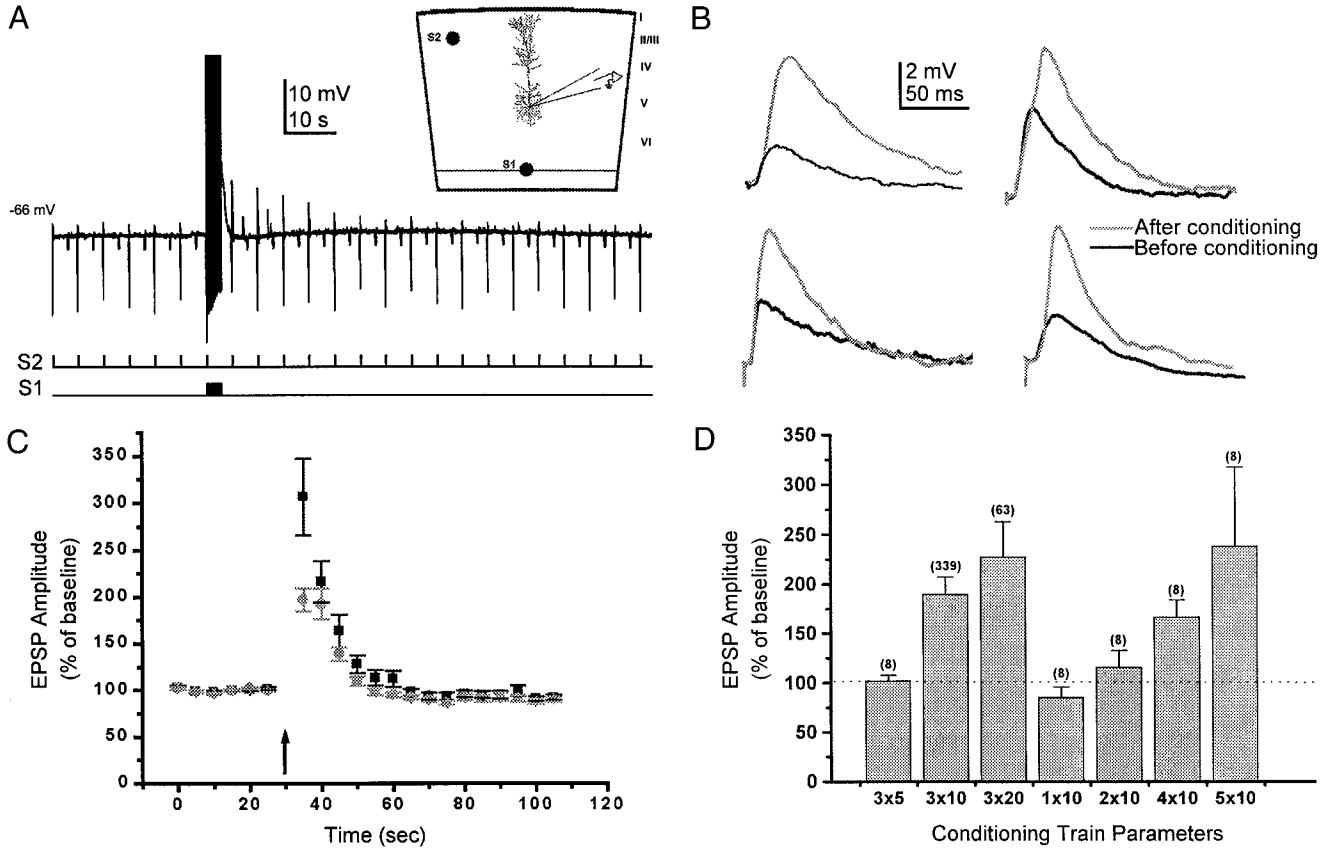

FIG. 3. Repetitive layer VI/WM stimulation potentiates EPSPs evoked from layer II/III. *A*: current-clamp recording from a layer V pyramidal cell. *Inset*: stimulating arrangement; stimulus markers (S1, S2) are shown under the voltage trace. Each test stimulus (S2) elicited a small EPSP. The amplitude of these responses transiently increased following repetitive stimulation at the layer VI/WM border. Axon potentials evoked by the conditioning train are truncated. Downward deflections in voltage trace reflect both stimulus artifact (coincident with stimulus markers) and the hyperpolarizing current injection used to measure input resistance. *B*: examples of layer II/III EPSPs recorded before (black trace) and immediately after the conditioning stimulus (gray traces). Data from 4 different cells. *C*: average effect of layer VI/WM stimulation on layer II/III EPSPs (black dots). EPSP amplitude in the baseline period is defined as 100%. EPSPs after conditioning are potentiated approximately threefold. Average effect after removing trials in which potentiated EPSPs triggered action potentials shown in gray (circles). Upward arrow marks the delivery of the conditioning stimulus at the layer VI/WM border. *D*: dependence of layer II/III EPSP potentiation on the parameters of the layer VI/WM conditioning train. Numbers in parentheses indicate the number of trials recorded for each condition. Short-duration and low-frequency conditioning trains fail to evoke layer II/III EPSP potentiation.

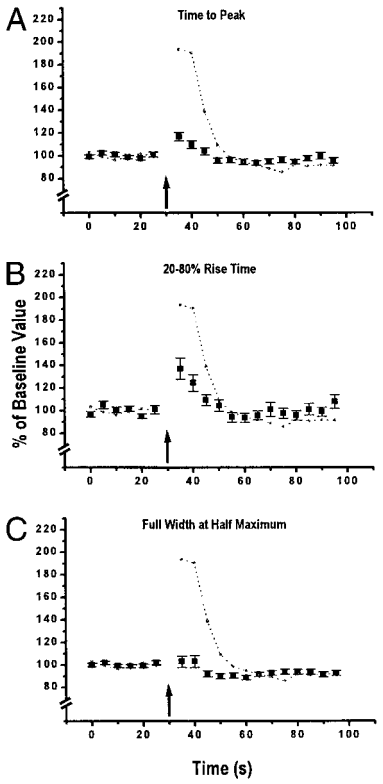

FIG. 4. Effect of repetitive layer VI/WM stimulation on layer II/III EPSP characteristics. *A*: effect of conditioning on layer II/III EPSP time to peak (●). Magnitude and time course of EPSP amplitude potentiation (· · ·) shown for comparison. ↑, time of conditioning stimulation. *B* and *C*: effect of conditioning on EPSP 20–80% rise time and full width at half-maximum, respectively. Conventions as in *A*.

on the magnitude of potentiation of varying both the duration and the frequency of the stimuli in the conditioning train (Fig. 3*D*). The data show that the strength of potentiation did not depend solely on the frequency or the duration of conditioning stimulation. For instance, 10 Hz trains lasting for either 1 or 2 s were ineffective, whereas a 10 Hz train of the same frequency that lasted 3–5 s did evoke potentiation. In general, the strength of potentiation increased with either longer-duration or higher-frequency conditioning trains. Because conditioning stimulation for 3 s, delivered at either 10 or 20 Hz, evoked a substantial potentiation that could be applied repeatedly in the same slice, we used these stimulus parameters in all subsequent experiments.

While a layer VI/WM conditioning train had a substantial effect on layer II/III EPSP amplitude, it had relatively little effect on other EPSP characteristics (see examples in Fig. 3*B*). To quantify the effects of conditioning on EPSP shape, we calculated the time to peak, 20–80% rise time, and full width at half-maximum for each EPSP. The effect of conditioning on each of these response indices is shown by the plots in Fig. 4 with the effect of conditioning on EPSP amplitude shown in outline on each plot for comparison. On average, conditioning led to slight increases in EPSP time to peak (117 ± 4%; Fig. 4*A*) and 20–80% rise time (137 ± 9%; Fig. 4*B*), but had no effect on EPSP full width at half-maximum (103 ± 5%; Fig. 4*C*). The increase in EPSP time to peak and rise time suggests that EPSP temporal summation might be enhanced after layer VI/WM conditioning stimulation.

During layer VI/WM conditioning stimulation, the membrane potential depolarized by an average of 10.1 ± 0.6 mV but recovered rapidly to baseline after termination of the conditioning train. The average membrane potential recorded in all 402 trials is shown in Fig. 5*A*. When the first EPSP after the conditioning train was elicited (at $t = 35$ s), membrane potential was, on average, depolarized by 0.95 mV; but when the next EPSP was evoked (5 s later; at $t = 40$ s), it was hyperpolarized by 0.57 mV. Because the EPSPs at both of these times were strongly potentiated, it is clear that EPSP potentiation does not depend directly on a stimulus-driven change in membrane potential at the presumed somatic recording site. The small but prolonged hyperpolarization that followed the conditioning stimulus was paralleled by a slight decrease in membrane resistance of similar duration (R_{IN} decreased to 83 + 2% of baseline; Fig. 5*B*). A similar membrane hyperpolarization and conductance increase was recently described by Sanchez-Vives et al. (2000) and ascribed to the activation of a sodium-gated potassium conductance.

While layer V pyramidal cells were the focus of this study, we were interested in determining whether the strong potentiation of layer II/III EPSPs after layer VI/WM stimulation also

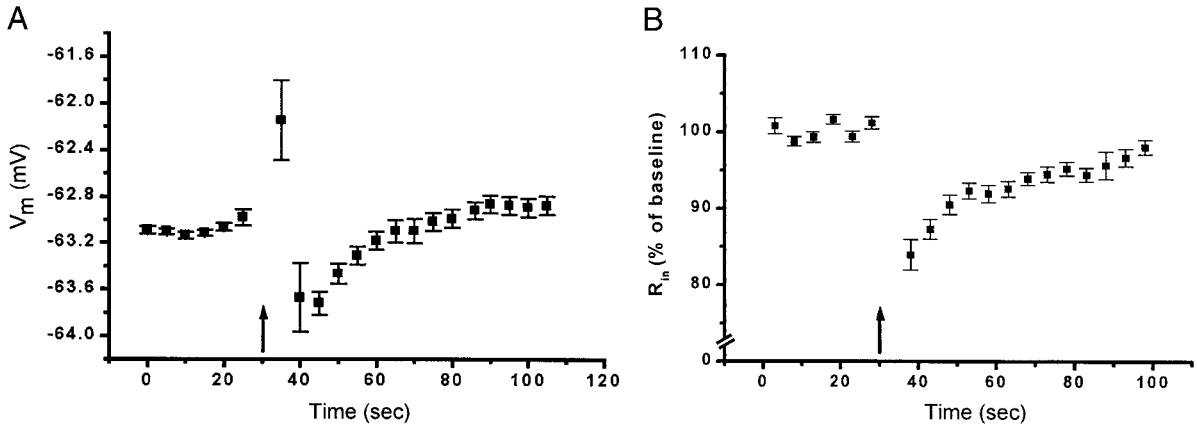

FIG. 5. Effect of repetitive layer VI/WM stimulation on somatic membrane potential and input resistance. *A*: average neuronal membrane potential during trial. Conditioning stimulation (indicated by ↑) evokes a short-lasting depolarization, followed by a more prolonged hyperpolarization. *B*: average normalized membrane resistance during trial. Conditioning stimulation (↑) causes long-lasting decrease in input resistance.

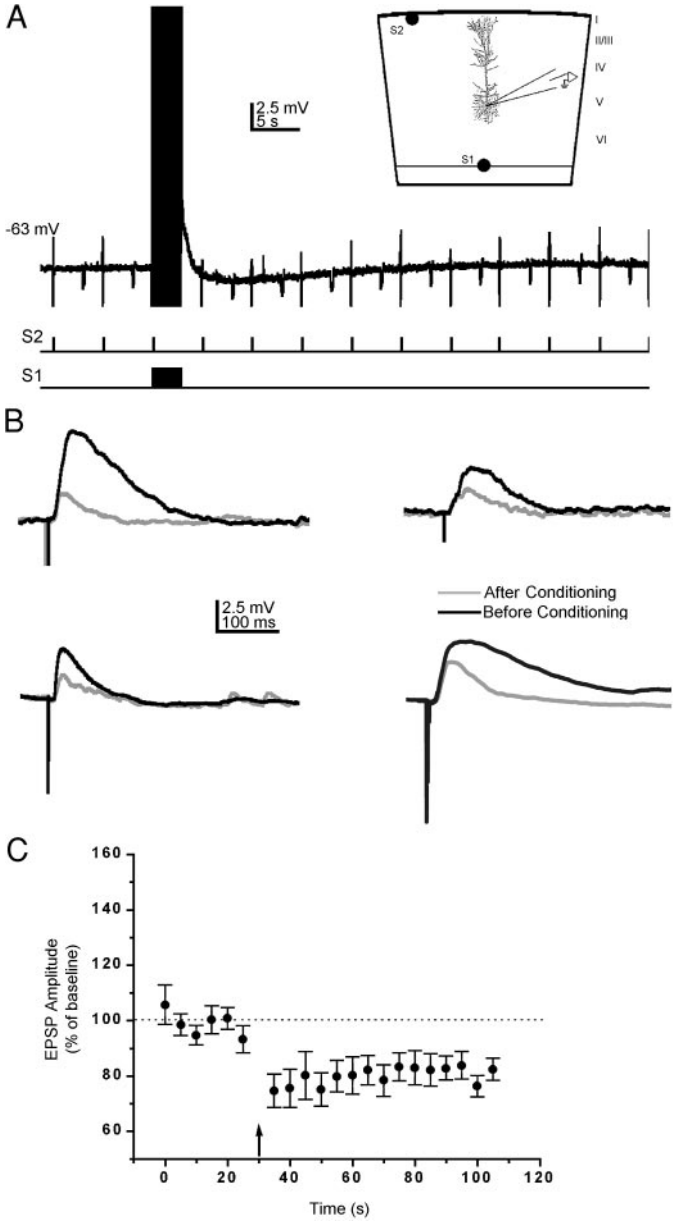

FIG. 6. Repetitive layer VI/WM stimulation attenuates layer I EPSPs. *A*: current-clamp recording from layer V pyramidal cell showing reduction of layer I EPSP amplitude after conditioning. Conventions and layout same as in Fig. 3. *B*: examples of layer I EPSPs before (black traces) and immediately after (gray traces) the conditioning train. Data are from 4 cells. *C*: average attenuating effect of layer VI/WM stimulation on layer I EPSPs after removing the minority of cells in which layer I EPSPs were potentiated (gray circles). Upward arrow, time of layer VI/WM conditioning stimulation.

occurred in other cell types. Although the data are limited, layer II/III EPSP potentiation was observed in layer II/III pyramidal neurons (42 trials recorded in 10 cells) and in layer V inhibitory interneurons (22 trials recorded in 7 cells). EPSP amplitude in these cells after conditioning was 144 ± 15 and 426 ± 80% of baseline, respectively.

Effect of layer VI/WM stimulation on layer I EPSPs

When EPSPs were evoked by stimulating layer I, layer VI/WM conditioning stimulation caused a *reduction* in EPSP amplitude in most cells (Fig. 6, *A* and *B;* 53 trials in 17 cells). Although, on average, layer VI/WM stimulation weakly potentiated layer I EPSPs (EPSPs after conditioning were 137 ± 15% of their baseline value) this across-cell average was strongly influenced by data obtained from a minority (35%) of the recorded cells. Discounting this minority of cells (see METHODS for criteria), the across-cell average effect of layer VI/WM stimulation was a significant *attenuation* of layer I EPSPs to 74 ± 6% of their baseline amplitude (Fig. 6*C*). Layer I EPSP attenuation had a more variable time course than the potentiation of layer II/III EPSPs, but, on average, it was longer lasting. Importantly, the effect of layer VI/WM conditioning on EPSP amplitude was consistent for a given cell. That is, in cells in which conditioning caused EPSP attenuation, the attenuation was observed on all trials after the delivery of the layer VI/WM conditioning stimulus.

In addition to its effect on layer I EPSP amplitude, the layer VI/WM conditioning train caused a decrease in EPSP time to peak (76 ± 7%; Fig. 7*A*), 20–80% rise time (67 ± 8%; Fig. 7*B*), and full width at half-maximum (66 ± 7%; Fig. 7*C*). The time course of the changes in EPSP shape closely follows the time course of the change in EPSP amplitude.

Effect of layer VI/WM stimulation on responses to glutamate application

The data obtained with electrical stimulation suggested that layer I and layer II/III EPSPs are differentially modulated by

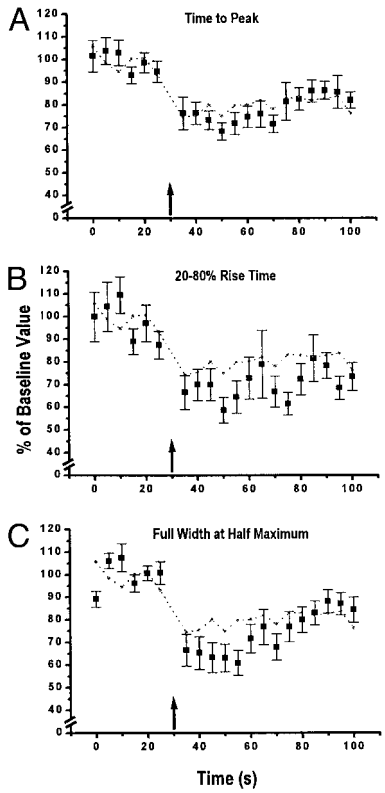

FIG. 7. The effect of repetitive layer VI/WM stimulation on layer I EPSP characteristics. *A*: effect of conditioning on EPSP time to peak (●). Magnitude and time course of layer I EPSP amplitude attenuation (· · ·) shown for comparison. ↑, time of the conditioning stimulus. *B* and *C*: effect of conditioning on EPSP 20–80% rise time and full width at half-maximum, respectively. Conventions as in *A*.

the conditioning train. However, due to the possibility that electrical stimulation evokes antidromic activity and activates interlaminar connections (Nowak and Bullier 1998), the actual locus of the synaptic input evoked by upper layer electrical stimulation is uncertain. In an attempt to provide more spatially localized synaptic input to the recorded cell, a series of experiments was carried out in which short-duration, pressure application of glutamate-containing ACSF (glutamate "puffs;" see METHODS) was used to provide subthreshold input directly to the recorded cell. By using low-pressure puffs of short duration, we were able to evoke small, symmetrically shaped postsynaptic responses that resembled the responses to glutamate observed in slices in which synaptic transmission had been blocked (see following text). Increasing the puff duration or pressure led to responses consisting of multiple components, each with a rapid onset resembling a synaptically mediated response, presumably due to the recruitment of activity in intermediary cells.

Repetitive layer VI/WM electrical stimulation also strongly potentiated the response of layer V pyramidal cells to glutamate puffs applied in layer II/III (Fig. 8*A;* 250–350 μm below the pial surface). The average response to glutamate application in layer II/III after conditioning was 171 ± 17% of the baseline value (Fig. 8*D;* 43 trials recorded from 14 neurons). In contrast, when the glutamate puff was applied in layer I (50–100 μm below the pial surface), a layer VI/WM conditioning train usually caused a substantial reduction in the amplitude of the response (12 of 14 cells; Fig. 8*B*). The average amplitude of the layer I response to glutamate after the conditioning train—discounting the two cells in which potentiation was observed—was 57 ± 8% of the response recorded during the baseline period (Fig. 8*D;* 36 trials recorded from 12 neurons). Thus the results obtained in experiments that used glutamate puffs in layers I and II/III to provide direct input to the recorded cell are consistent with the data obtained using electrical stimulation of those same layers.

Attenuation of the response to the layer I glutamate application was recorded in the same neurons in which layer VI/WM conditioning stimulation had resulted in a substantial potentiation of the response to layer II/III glutamate application (Fig. 8*C*). That is, both potentiation and attenuation were observed *in the same cell* if the site of glutamate application was moved from layer II/III to layer I (a distance of 200 μm). In 6/7 cells tested in this way, it was possible to move the puffer pipette back and forth between layers II/III and I, with the layer VI/WM conditioning train attenuating the layer I response and potentiating the response elicited from layer II/III. This result indicates conclusively that layer II/III EPSP

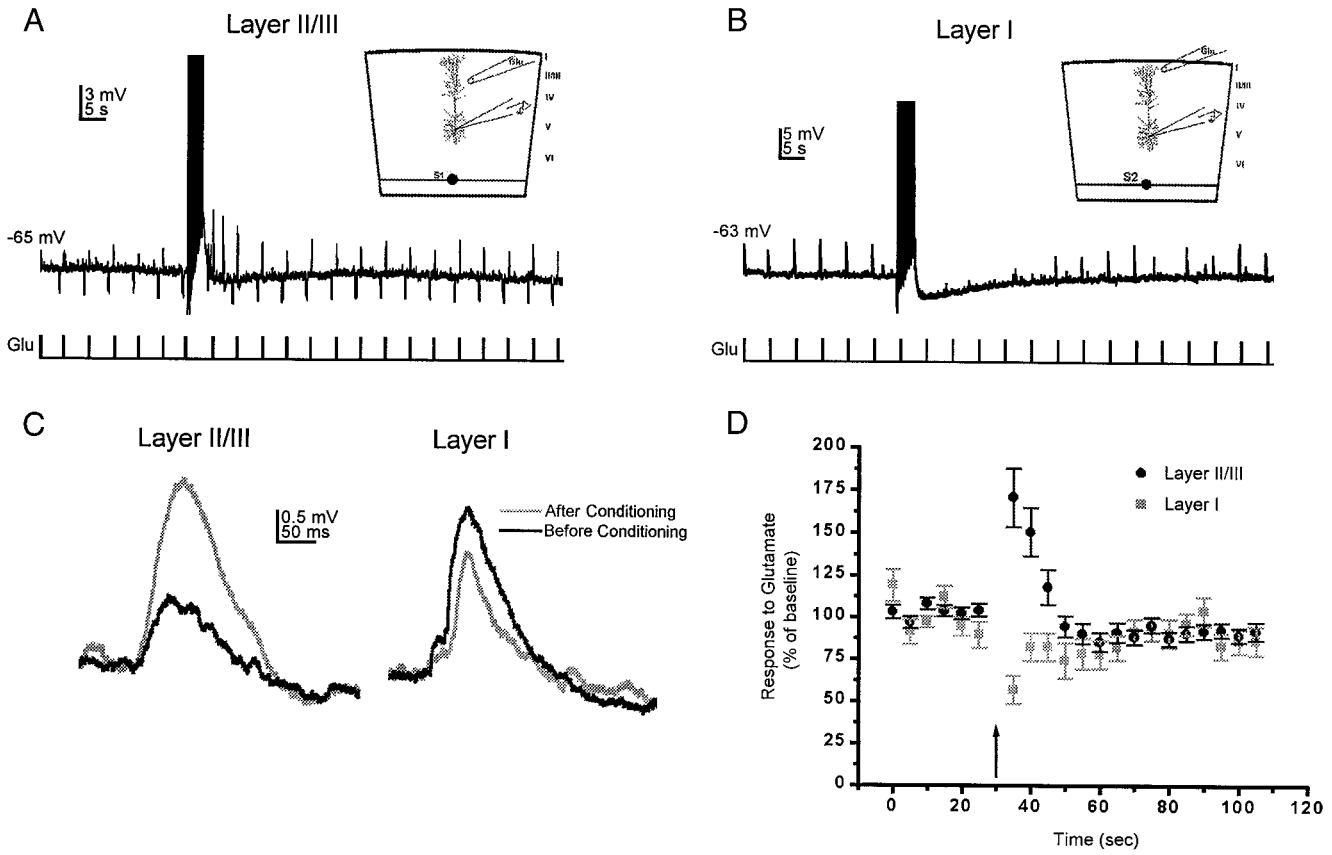

FIG. 8. The effect of repetitive layer VI/WM stimulation on the response to glutamate puffs in layers I and II/III. *A*: current-clamp recording from a layer V pyramidal cell. Stimulus markers for application of glutamate shown below voltage trace. Response to glutamate puff in layer II/III is potentiated after conditioning stimulation. *B*: response to layer I glutamate puff is attenuated by layer VI/WM conditioning stimulation. *C, left*: response to layer II/III glutamate puff before (black) and after (gray) the conditioning stimulus. *Right*: responses recorded in the same cell after moving the glutamate puffer pipette 200 μm to layer I. Responses before conditioning stimulation shown in black, after conditioning in gray. *D*: average effect of conditioning stimulation on the responses to glutamate application in layer I (gray squares) vs. II/III (black circles). Arrow indicates time at which conditioning stimulus was delivered.

potentiation and layer I EPSP attenuation can occur in the same cell. In addition, the strikingly different modulation of layer I and layer II/III glutamate responses (locations separated by only 200 μm) by layer VI/WM stimulation suggests that a glutamate puff provides spatially localized input to the dendrites of a layer V pyramidal neuron, consistent with the visualized spread of the glutamate solution (50 μm; see METHODS).

In summary, the EPSPs evoked by both electrical stimulation and glutamate puffs in layers I and II/III were influenced in opposite ways by repetitive stimulation applied at the layer VI/WM border. In 71% of the recorded cells, the responses to layer I stimulation were significantly attenuated, whereas in 69% of the cells the responses evoked by layer II/III stimulation were significantly potentiated (a significant difference $P < 0.01$, χ^2 test).

Effect of upper layer stimulation on layer VI/WM EPSPs

The results in the preceding sections showed that repetitive stimulation at the layer VI/WM border transiently strengthens layer II/III inputs and simultaneously weakens layer I inputs. To investigate whether repetitive stimulation in the upper layers was capable of modifying layer VI/WM input, experiments were carried out in which the roles of the two electrodes were reversed: the conditioning train was delivered either to layer I or to layer II/III and subthreshold EPSPs (mean amplitude 3.0 ± 0.2 mV) were evoked by the layer VI/WM electrode. Due to the recruitment of strong inhibitory input at higher stimulus strengths (described in a previous section), conditioning stimuli in layer II/III failed to evoke action potentials in layer V pyramidal cells. The inability of horizontal inputs to drive layer V cells is consistent with the view that input relayed via horizontal connections is weaker than that provided by thalamocortical feedforward inputs (Gilbert 1998). Nevertheless, the possibility remained that the recent history of activity in the network of horizontal axons would influence subsequent activity relayed via feedforward/columnar inputs.

We found, however, that repetitive layer II/III conditioning stimulation had no effect on the EPSPs evoked by test stimuli applied to the layer VI/WM border (Fig. 9*A*). The average EPSP amplitude evoked by a layer VI/WM stimulus after conditioning stimulation in layer II/III was 101 ± 7% of the baseline amplitude (14 trials recorded in 8 cells). In contrast, in the same cells the EPSP elicited by a layer II/III test stimulus was potentiated to 173 ± 24% (14 trials) of its baseline value after conditioning stimulation at the layer VI/WM site (Fig. 9*B*). Similarly, repetitive layer I stimulation had no effect on the EPSPs evoked from layer VI/WM (Fig. 9*B;* 10 trials in 6 cells); layer VI/WM EPSP amplitude after layer I conditioning was 92 ± 9% of that in the baseline period. In contrast, in 11 control trials conducted in the same cells, layer I EPSPs were

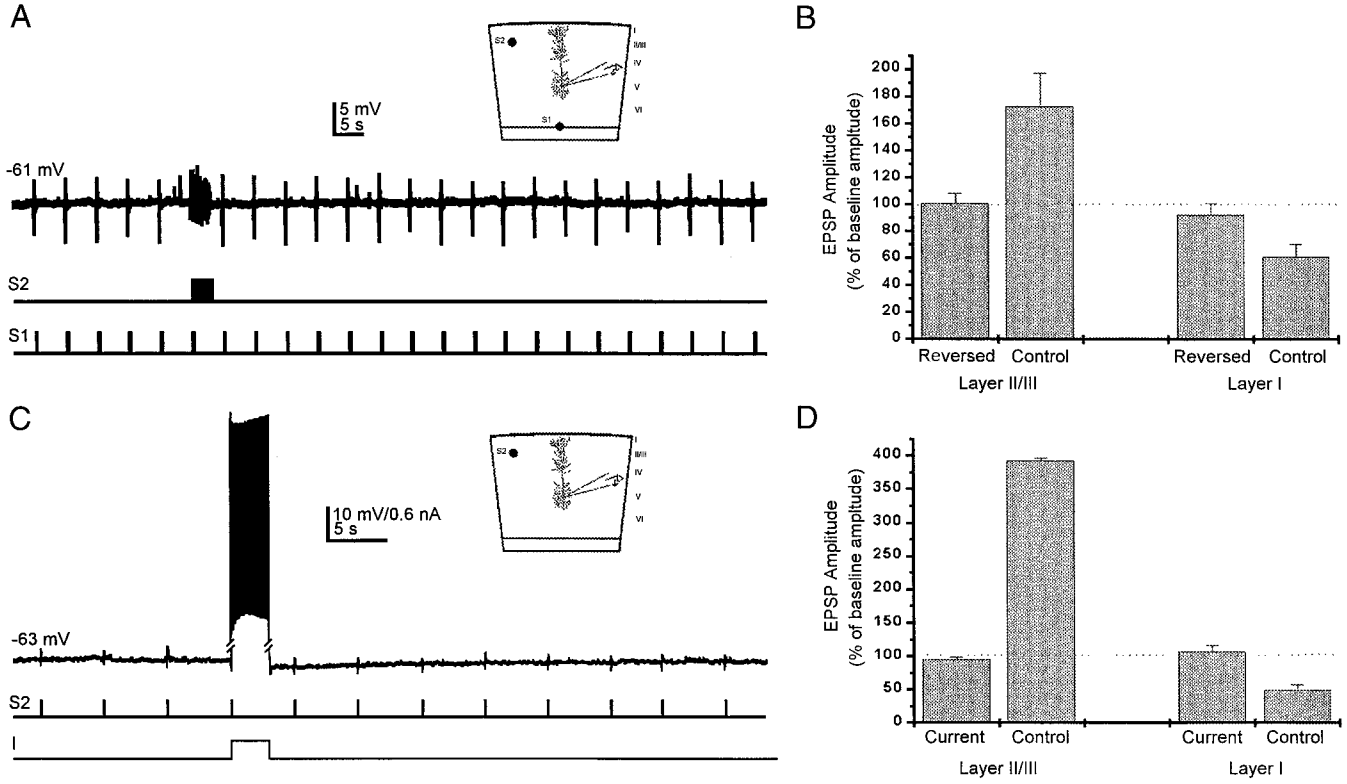

FIG. 9. The effect of upper layer stimulation on layer VI/WM EPSPs. *A*: current-clamp recording from a layer V pyramidal cell in which repetitive stimulation was applied in the top layers (S2) and test stimuli to layer VI/WM (S1). Top layer conditioning stimulation had no effect on layer VI/WM EPSPs. *B*: average effect of layer II/III conditioning (layer II/III-reversed) and layer I conditioning (layer I-reversed) on EPSPs evoked from layer VI/WM. Control bars indicate effect on amplitude of EPSPs evoked by top layer stimulation in trials in which the conditioning train was applied at the layer VI/WM border. *C*: current-clamp recording from a layer V pyramidal cell showing that postsynaptic firing, evoked by current injection, has no effect on layer II/III EPSPs. Markers indicating the delivery of test stimuli (S2) and the injection of current are shown under the voltage trace. *D*: average effect of depolarizing current injection. Labeling conventions as in *B*.

attenuated to 61 ± 9% of their baseline value by the standard conditioning stimulus at the layer VI/WM border (Fig. 9*B*). In summary, these experiments clearly demonstrate that the ability of repetitive activity elicited from the layer VI/WM border to modify upper layer EPSPs is *not* paralleled by an ability of repetitive upper layer drive to modify the EPSPs evoked by stimulation at the layer VI/WM border.

The inability of upper layer conditioning trains to affect layer VI/WM EPSPs, coupled with the frequent failure of upper layer stimuli to elicit firing in layer V neurons, suggested that the modification of layer I and layer II/III EPSP efficacy observed with layer VI/WM conditioning stimulation might be due to postsynaptic firing. To investigate this possibility, the layer VI/WM conditioning train was replaced by 3 s of suprathreshold depolarizing current injection (Fig. 9*C*). In none of the nine cells studied in this way (18 trials) did current injection lead to subsequent layer II/III EPSP potentiation; the average layer II/III EPSP amplitude after current injection was 95 ± 4% of the baseline amplitude (Fig. 9*D*). In contrast, in the same cells the conditioning stimulation at the layer VI/WM site elicited a strong potentiation of layer II/III EPSPs (393 ± 4%; 20 trials). Similarly, layer I EPSPs were unaffected by depolarizing current injection with EPSP amplitude after depolarization being 108 ± 8% of baseline. In nine control trials recorded in the same cells, the standard layer VI/WM conditioning train reduced the amplitude of the layer I EPSPs to 50 ± 7% of baseline amplitude (Fig. 9*D*). Finally, using our conventional layer VI/WM conditioning trains, we observed both layer II/III EPSP potentiation and layer I EPSP attenuation on trials in which the conditioning stimulus failed to evoke action potentials in the recorded cell (data not shown). Together these results show clearly that postsynaptic firing is neither necessary nor sufficient to cause the modification of synaptic efficacy observed after repetitive stimulus drive at the layer VI/WM border.

Effect of conditioning on glia

Having determined that EPSP modification is not due solely to postsynaptic spiking, we turned to other potential mechanisms for the observed effects. A fortuitous insight into the mechanism of layer II/III EPSP potentiation came from whole cell recordings of glial cells (5 layer II/III and 51 layer V glial cells; Fig. 10*A*) that were occasionally encountered during attempts to perform neuronal recordings. Specifically, recordings from glial cells revealed that the layer VI/WM conditioning train, which potentiates layer II/III input to pyramidal cells, is accompanied by a slow and substantial depolarization in glial cells (10.2 mV for the recording shown in Fig. 10*B*, indicated by ΔV_m). The average glial cell depolarization evoked by the standard 3 s, 10 Hz conditioning train was 12.1 ± 0.6 mV. Evidence reported by others suggests that this depolarization is due to a localized, stimulus-induced increase in extracellular potassium (ECK) (Ransom and Goldring 1973;

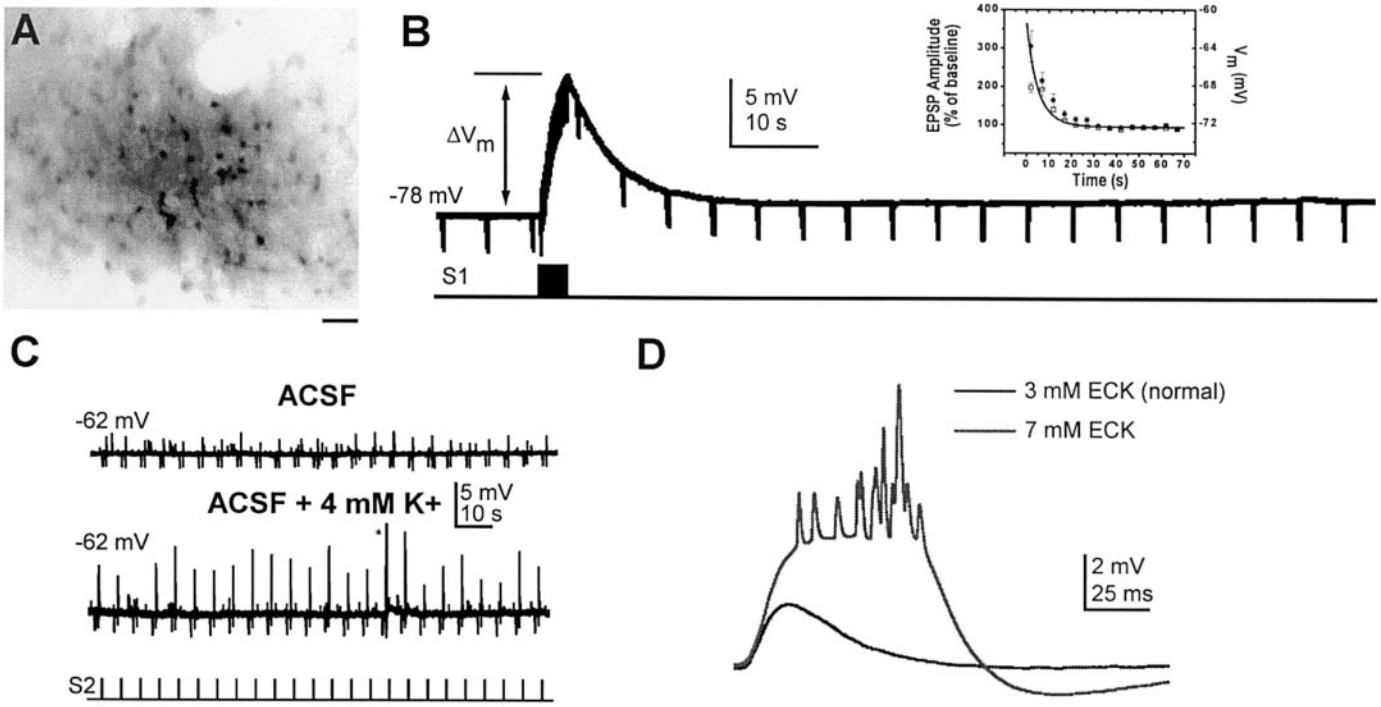

FIG. 10. Evidence that layer II/III EPSP potentiation is due to a stimulus-driven increase in ECK. *A*: biocytin stained synctium of astrocytes. Scale bar 100 μm. *B*: current-clamp recording from layer V glial cell. Repetitive layer VI/WM stimulation elicits small depolarization of amplitude ΔV_m. Glial membrane potential recovers over several tens of seconds. The downward deflections in the voltage trace are due to hyperpolarizing current injection used to measure input resistance. *Inset*: Time course for dissipation of EPSP potentiation (*filled circles* show data obtained in all trials; *open squares* show data after excluding trials in which subthreshold layer II/III input became suprathreshold); and time course of the repolarization of glial membrane potential (*solid line*). EPSP potentiation and glial membrane potential decay with a similar time course. *C*: layer II/III EPSPs recorded in standard ACSF (*top trace*), and after raising ECK by 4 mM (*middle trace*). Stimulus markers shown in bottom trace. Asterisk indicates truncated suprathreshold response. *D*: average layer II/III EPSP recorded in standard ACSF (containing 3 mM ECK, *black trace*) and after the addition of 4 mM KCl (*gray trace*).

Schwartzkroin and Prince 1979). Given the approximately Nernstian relationship between ECK and glial membrane potential (Somjen 1995), the change in ECK elicited by the standard 3 s, 10 Hz conditioning train to the layer VI/WM border is calculated to be ~2 mM (for a depolarization of 12.1 mV). The largest depolarization we recorded (26.7 mV)—after a 3 s, 20 Hz layer VI/WM conditioning train—corresponds to a change in ECK of ~6 mM. This calculated estimate of the stimulus-induced change in ECK agrees well with previously reported direct measurements of ECK accumulation after similar patterns of repetitive electrical stimulation (Benninger et al. 1980; Fritz and Gardner-Medwin 1976; Holthoff and Witte 1996; Poolos et al. 1987).

After the end of the conditioning stimulus, glial cell membrane potential slowly recovered back to the preconditioning value (Fig. 10*B*). This recovery is well described by a single-term decaying exponential with a time constant of 5.8 ± 0.7 s (171 trials recorded in 35 glial cells, least squares minimization with goodness of fit of $P < 0.01$). The similar time course of glial repolarization and the dissipation of pyramidal cell EPSP potentiation (time constant of 7.2 s; see Fig. 10*B, inset*, for comparison) after the delivery of a conditioning stimulus train at the layer VI/WM border raised the possibility that a stimulus-driven change in ECK might be involved in layer II/III EPSP potentiation.

Mechanisms of EPSP potentiation

To test whether the layer II/III EPSP potentiation that follows a layer VI/WM conditioning train could be related to the increase in ECK evoked by such stimulation, experiments were carried out in which layer II/III EPSPs were recorded from layer V pyramidal cells while the potassium concentration in the bath was increased from 3 to 7 mM. Neurons depolarized slightly (4–6 mV) in the higher ECK, an effect that was offset by hyperpolarizing current injection. As illustrated in the recording shown in Fig. 10*C*, the elevation of the ECK concentration caused a substantial potentiation of the amplitude of layer II/III EPSPs ($n = 6$ cells). Average EPSPs calculated from responses in normal and elevated ECK are shown in Fig. 10*D*. Discounting stimuli that triggered action potentials, the average potentiation of layer II/III EPSPs by the elevation in ECK was 204 ± 30%. In those cells held for the entire protocol, the effect of raising ECK was entirely reversed by returning to standard ACSF ($n = 2$ cells). Layer II/III EPSPs were more strongly potentiated by the increase (4 mM) in bath ECK than by layer VI/WM conditioning stimulation, consistent with the calculated estimate of conditioning-evoked ECK accumulation (2–4 mM).

A stimulus-driven increase in ECK could potentiate layer II/III EPSPs in a number of ways, including effects at either a pre- or postsynaptic locus. We initially attempted to distinguish between these possibilities by manipulating postsynaptic membrane potential with current injection. In 14 cells, the membrane potential in each trial was set at the resting membrane potential or was depolarized or hyperpolarized by 10–15 mV by current injection. Layer II/III EPSPs were potentiated after layer VI/WM conditioning stimulation when the cell was hyperpolarized (Fig. 11*C*) or at rest (Fig. 11*B*), but when the cell was depolarized (Fig. 11*A*), the potentiation was substantially weaker. On average, layer II/III EPSP amplitude after conditioning was 175 ± 21% ($n = 24$ trials) of the baseline value when trials were conducted at resting membrane potential. When the cells were hyperpolarized (to −75 to −80 mV), the potentiation increased slightly to 201 ± 32% ($n = 23$ trials), and at depolarized membrane potentials (−50 to −55 mV), the potentiation was significantly smaller (106 ± 24%; 14 trials; $P < 0.05$, paired *t*-test), although it was still observed in some cells.

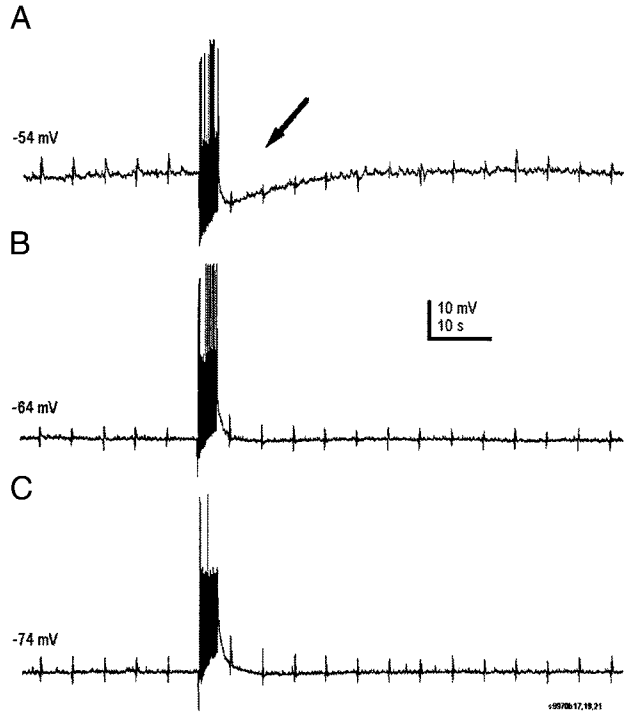

FIG. 11. Dependence of layer II/III EPSP potentiation on postsynaptic membrane potential. Current-clamp recordings performed at depolarized (*A*), resting (*B*), or hyperpolarized (*C*) membrane potential; values indicated to the left of voltage traces. EPSP potentiation occurs at resting membrane potential and when the cell is hyperpolarized but not when the cell is depolarized. ↓, strong afterhyperpolarization that follows the conditioning stimulus when the cell is depolarized.

The sensitivity of EPSP potentiation to postsynaptic membrane potential does not, however, prove that the mechanism generating the effect is postsynaptic. The weakness of the potentiation phenomenon at depolarized membrane potentials could be due to the recruitment of an unrelated postsynaptic mechanism—not active at resting or hyperpolarized membrane potentials—which counteracts the potentiation. For instance, depolarization of the presumed somal recording site by current injection would be expected to result in the recruitment of the somatic sodium channel and enhancement of EPSPs in the baseline period. The hyperpolarization observed after the conditioning train (see 11*A*, ↓) would result in a loss of this somatic enhancement, potentially counteracting the EPSP potentiation usually observed.

Because of the difficulty interpreting the results of current-clamp recording performed at different membrane potentials, we performed additional experiments in which we compared the effect of a layer VI/WM conditioning stimulus on layer II/III excitatory synaptic currents (EPSCs) and EPSPs recorded at nearly the same membrane potential. Given that potentiation occurred with essentially no change in somatic membrane potential (Fig. 5*A*), we performed both the voltage- and cur-

rent-clamp recordings at the resting membrane potential. We reasoned that if the mechanism responsible for the potentiation were postsynaptic and voltage-gated (e.g., the recruitment of a voltage-gated boosting conductance), the potentiation of EPSCs would be expected to be weaker than of the EPSPs. The experiments revealed that this was indeed the case: layer II/III EPSPs were potentiated more strongly than were the underlying synaptic currents. Data from an exemplary cell are shown in Fig. 12. In this case, the potentiation of the postsynaptic potential was substantial (Fig. 12*A*, *left*), but the postsynaptic current recorded on a trial conducted immediately after the current-clamp recording was unaffected by the conditioning stimulus (Fig. 12*A*, *right*). The scatter plot in Fig. 12*B* compares the potentiation on consecutive current- and voltage-clamp recordings in 78 trials recorded in 28 cells. The majority of the points lie above the identity line indicating a stronger potentiation of potentials than currents. On average, EPSCs were potentiated to 158 ± 10% of baseline amplitude after conditioning, while EPSP potentiation in the same cells was 246 ± 16% ($P < 0.001$, paired *t*-test).

Synaptic input, particularly when delivered to the distal dendrite, is unlikely to be effectively voltage clamped by a somatic recording pipette due to space-clamp limitations. Thus a failure of voltage-clamp to affect potentiation would be inconclusive: it could be attributed either to an inability of the recording electrode to control postsynaptic membrane potential or to insensitivity of the potentiation effect to voltage-clamping the postsynaptic membrane. Sensitivity to postsynaptic voltage-clamp, however, can only be interpreted as evidence that the mechanism in question is both postsynaptic and voltage-gated. Thus the observation that synaptic potentials are more strongly potentiated than their underlying currents (Fig. 12) strongly suggests that the potentiation involves a postsynaptic voltage-gated conductance. This conclusion was given further credence by the results of experiments involving pharmacological manipulations discussed in the following text.

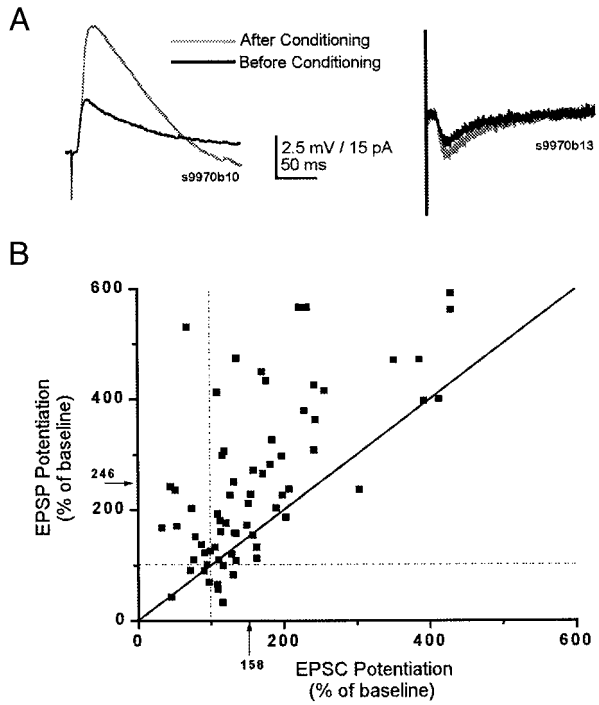

FIG. 12. Potentiation of synaptic potentials is greater than of the underlying currents. *A*, *left*: EPSPs before (black) and after (gray) conditioning; recording was made at the resting membrane potential of −65 mV. *Right*: excitatory postsynaptic currents (EPSCs), recorded from the same cell in the subsequent trial, before (black) and after (gray) conditioning stimulation; membrane potential was clamped at −63 mV. *B*: scatter plot comparing the potentiation of EPSPs and EPSCs on successive trials. The diagonal line (identity line) indicates equal potentiation of EPSPs and EPSCs. Dotted vertical and horizontal lines indicate no effect on EPSCs and EPSPs, respectively. Great majority of the points are located above the identity line, indicating stronger potentiation of EPSPs than EPSCs. Average potentiation of EPSPs and EPSCs shown (arrows) along ordinate and abscissa, respectively.

Pharmacological manipulations

Because the above-described evidence suggested a voltage-dependent postsynaptic locus of the EPSP potentiation that followed layer VI/WM conditioning stimulation, we considered postsynaptic mechanisms that might become operative whenever K^+ accumulates in the extracellular space. Two mechanisms we viewed as most plausible were inactivation of a postsynaptic voltage-gated channel that normally functions to reduce or dampen EPSP amplitude (e.g., the A-type potassium current) and/or recruitment of a postsynaptic voltage-gated channel or receptor that boosts the net inward current caused by a synaptic event [e.g., *N*-methyl-D-aspartate (NMDA) receptors, low-voltage-activated and high-voltage-activated calcium channels, and sodium channels] (see Spruston et al. 1999 and Johnston et al. 1996). The possibility that such mechanisms contributed to the observed EPSP potentiation was independently evaluated in experiments in which the effect of layer VI/WM conditioning stimulation on layer V pyramidal neuron EPSPs was determined both before and after application of a selective antagonist of a specific membrane channel and/or receptor.

The initial experiments of this type yielded uniformly negative results. Specifically, layer VI/WM conditioning continued to elicit robust layer II/III EPSP potentiation in the presence of bath-applied 10 μM nimodipine, which blocks L-type calcium channels ($n = 2$ cells; EPSP amplitude potentiated to 245% of baseline), 100 μM 2-amino-5-phosphonovaleric acid (APV), which blocks NMDA receptors ($n = 3$ cells; EPSP amplitude 200% of baseline), and 100 μM nickel, which blocks low-voltage activated calcium channels ($n = 6$ cells; EPSP amplitude 405% of baseline). Intracellular application of 5 mM 4-aminopyridine to block A-type potassium channels also failed to prevent EPSP potentiation ($n = 3$ cells; EPSP amplitude 171% of baseline).

To evaluate the role of postsynaptic sodium channels in layer II/III potentiation, whole cell recordings were made from layer V neurons ($n = 14$ cells) with pipette solutions containing 10 mM of the lidocaine derivative *N*-(2,6-dimethyl-phenylcarbamoylmethyl) triethylammonium bromide (QX-314). Both fast and persistent sodium currents are blocked by the intracellular application of QX-314 (Connors and Prince 1982). During these recordings, the depolarization caused by QX-314 was offset by hyperpolarizing current injection. After action potentials had been blocked by intracellular QX-314 application, layer II/III EPSPs were still potentiated by a conditioning train delivered at the layer VI/WM border. However, in those cells in which the recording was continued for 30 min to 1 h, the potentiation effect gradually but progressively declined. On average, when QX-314 was included in the re-

cording pipette, EPSP potentiation on trials conducted ≥45 min after rupturing the cell membrane was reduced to 30 ± 8% of the magnitude observed shortly after the establishing the whole-cell recording configuration (Fig. 13*A*). Cells studied over the same extended time period, but in the absence of QX-314, displayed significantly less rundown. EPSP potentiation on trials conducted after 45 min of whole cell recording with the standard pipette solution retained 83 ± 17% of its original magnitude (Fig. 13*A;* a significant difference with the QX-314 data, $P < 0.05$, paired *t*-test).

The ability of QX-314 to block layer II/III EPSP potentiation suggests that postsynaptic sodium channels may, at least in part, underlie the potentiation. Because QX-314 affects a number of voltage-gated ion channels [including potassium (Nathan et al. 1990) and calcium channels (Talbot and Sayer 1996)], the role of postsynaptic sodium channels in potentiation was tested further. Specifically, the effect of raising ECK on the response to glutamate puffs was evaluated in slices in which synaptic transmission had been blocked either by adding 1 μM tetrodotoxin (TTX) or 0.2 mM cadmium. Cadmium disrupts synaptic transmission by blocking presynaptic calcium channels. Glutamate responses recorded in ACSF containing 0.2 mM cadmium were strongly potentiated when the concentration of K^+ in the bath was increased from 3 to 7 mM, with previously subthreshold responses often triggering action potentials (Fig. 13*B; n* = 5 cells). In stark contrast, increasing the potassium concentration in the bath had no effect on the response of the cell to glutamate puffs when synaptic transmission was blocked by TTX (Fig. 13*C;* average response in 7 mM ECK was 109 ± 11% of that in 3 mM ECK; *n* = 5 cells). The ability of elevated ECK to cause EPSP potentiation in the presence of cadmium, but not in the presence of TTX, strongly suggests that the EPSP potentiation does not involve postsynaptic calcium channels, is a direct postsynaptic effect (because synaptic transmission was blocked the response to glutamate puffs was entirely postsynaptic), and involves a recruitment of sodium channels.

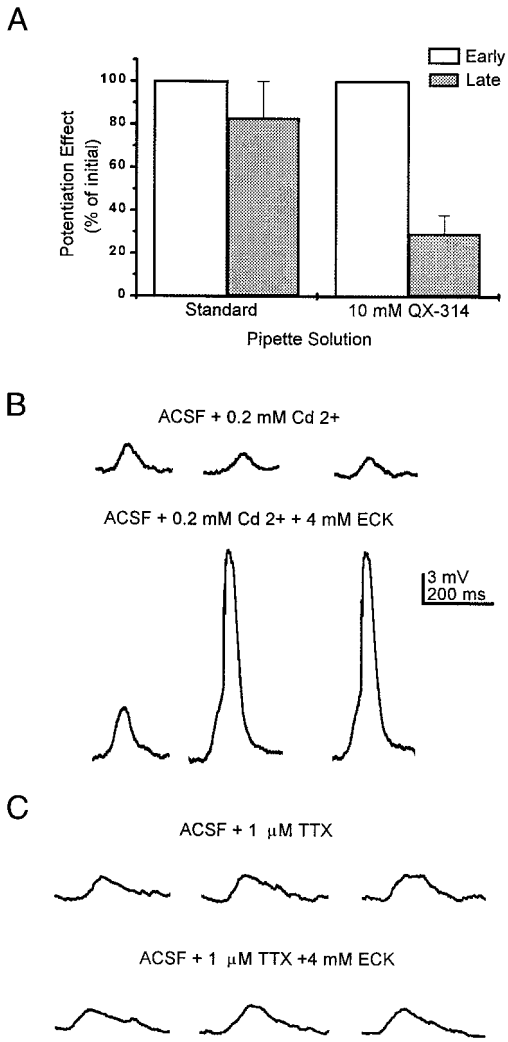

FIG. 13. Layer II/III EPSP potentiation involves the recruitment of postsynaptic sodium channels. *A*: average rundown (compare □ vs. ▨) of the potentiation effect in standard intracellular solution (*left*) and in intracellular solution containing 10 mM *N*-(2,6-dimethyl-phenylcarbamoylmethyl) triethylammonium bromide (QX-314, *right*). "Early" responses were recorded within ten minutes of the commencement of whole cell recording; "Late" responses were recorded ≥45 min later. QX-314 strongly reduced the magnitude of the EPSP potentiation that followed layer VI/WM conditioning stimulation. *B*: responses to glutamate recorded in 0.2 mM cadmium (*top*) are potentiated when potassium in the bath is raised to 7 mM (*bottom*). *C*: responses to glutamate recorded in presence of 1 μM TTX (*top*) are not potentiated by the same increase in ECK (*bottom*).

Finally, we considered the possibility that layer II/III EPSP potentiation may involve stimulus-driven disinhibition. Repetitive stimulation of the type used in this study has been shown to reduce the strength of cortical inhibition, both via the activation of presynaptic $GABA_B$ receptors, which reduces the subsequent release of GABA from inhibitory interneurons for hundreds of milliseconds (Deisz and Prince 1989), and via a breakdown of the transmembrane chloride gradient, which compromises inhibition for tens of seconds (Kalia et al. 1997; Staley et al. 1995). The latter form of disinhibition may be caused, in part, by an increase in ECK that compromises the function of the K-Cl transporter used to maintain the chloride concentration gradient (Jarolimek et al. 1999; Thompson et al. 1988). As a result, this form of disinhibition would be expected to have a time course similar to stimulus-evoked ECK accumulation, raising the possibility that it contributes to the observed potentiation of layer II/III EPSPs. To evaluate this possibility, three layer V neurons were recorded in ACSF containing 0.2 mM cadmium, 5 μM bicuculline methiodide (BMI; a $GABA_A$ antagonist), and 100 μM CGP35348 (a $GABA_B$ antagonist). Increasing the bath concentration of ECK from 3 to 7 mM—in solutions containing cadmium, BMI, and CGP35348—converted subthreshold glutamate responses to suprathreshold events. That the responses to glutamate application recorded in the presence of cadmium, bicuculline, and CGP35348 are potentiated by high ECK, combined with the observation that the EPSP potentiation is reduced by intracellular QX-314, strongly suggests that stimulus-driven cortical disinhibition plays little or no role in the EPSP potentiation evoked by layer VI/WM conditioning stimulation.

DISCUSSION

The principal finding of this study is that repetitive stimulation at the layer VI/WM border differentially alters the efficacy of layer I and layer II/III inputs to layer V pyramidal neurons. Specifically, repetitive stimulation at this cortical location potentiates layer II/III EPSPs and simultaneously at-

tenuates layer I EPSPs. Together, these results suggest that pyramidal cell dendrites compartmentalize their inputs, dynamically altering the ability of inputs deriving from different sources to influence cell spiking. The results suggest a novel type of cortical circuit dynamics that may contribute to the sensory cortical neuron RF dynamics that others have observed in vivo.

ECK as a mediator of synaptic dynamics

While our primary goal was to characterize the effects of repetitive stimulation on the efficacy of different inputs to layer V pyramidal cells, we conducted a number of experiments aimed at elucidating the potential mechanisms contributing to the observed phenomena. These experiments suggest that the potentiation of layer II/III EPSPs involves a stimulus-driven increase in ECK because *1*) the potentiation dissipates at a rate similar to the repolarization of glia after conditioning, *2*) increasing the bath concentration of ECK potentiates layer II/III EPSPs and glutamate response, and *3*) postsynaptic firing evoked by depolarizing current injection—a manipulation unlikely to alter ECK significantly—had no effect on EPSP amplitude.

The proposal that ECK accumulation can affect synaptic transmission has been considered in a number of previous studies. For example, Weight and Erulkar (1976) reported that ECK accumulation, due to repetitive postsynaptic activity, caused a decrease in EPSP amplitude at the squid giant synapse. Malenka et al. (1981) reported that stimulus-driven increases in ECK, due to repetitive activity in a population of Purkinje cells, can modulate the excitability of parallel fibers in rat cerebellar cortex. Small increases in ECK led to a slight potentiation of field potential amplitude, whereas larger increases caused a reduction in amplitude. Eng and Kocsis (1987) found similar results in the turtle olfactory nerve and stated that the effect of ECK switches from excitatory to depressive when its concentration increases by $>3-4$ mM. Poolos and Kocsis (1990) reported that elevating ECK caused an enhancement of postsynaptic responses by a selective enhancement of NMDA receptors in hippocampal neurons. Finally, a form of transient synaptic potentiation following a stimulus paradigm similar to ours has been described in the hippocampus (MacVicar and Dudek 1979) in which mossy fiber stimulation at 10 Hz caused a transient EPSP potentiation that outlasts the effect of the stimulation on the somatic membrane potential. The authors suggested stimulus-evoked changes in ECK may underlie the potentiation.

While the preceding studies are consistent with our proposal that stimulus-evoked changes in ECK are capable of transiently modifying synaptic efficacy, our results extend those findings by showing that the effects of ECK accumulation on sensorimotor layer V pyramidal cells involve interactions between different sources of input to the cell and that the effect of ECK appears to involve a novel effect on a postsynaptic voltage-gated conductance (see following text).

Proposed role for postsynaptic sodium channels in EPSP potentiation

Several observations suggest that the layer II/III EPSP potentiation evoked by layer VI/WM conditioning stimulation may involve ECK-mediated recruitment of postsynaptic sodium channels. First, the potentiation is strongly reduced by intracellular application of QX-314. Second, responses to glutamate puffs are potentiated by elevated ECK when synaptic transmission is blocked by cadmium but not when it is blocked by TTX. Third, the extended duration of EPSP potentiation suggests that the mechanism involved must be capable of resisting rapid inactivation, a hallmark property of the persistent sodium current (Stafstrom et al. 1985). Although the cellular location of the sodium channels that are involved is uncertain, the finding that potentiation persists for $30-45$ min after action potentials are blocked by QX-314 suggests they *may* be dendritic. The long delay required for intracellularly applied QX-314 to block EPSP potentiation presumably reflects the time required for the diffusion of the compound from the soma to the dendrite. While the effects on dendritic sodium channels of QX-314 applied via a dendritic recording pipette occur within several minutes (Stuart and Sakmann 1994), dendritic effects of QX-314 applied via a somatic patch pipette can be substantially delayed (Lee and Heckman 1999). The delayed effect of QX-314 may further be attributable to the high access resistance of the recordings and the location of the channels in the dendrite (e.g., spines or narrow dendritic branches). Fourth, EPSP potentiation occurred even though the soma was slightly hyperpolarized by the conditioning train (Fig. 5*A*). If ECK causes EPSP potentiation by depolarizing the cell membrane and allowing synaptic potentials to activate sodium channels more effectively, it seems unlikely that ECK could potentiate EPSPs by recruiting somatic channels. The somatic hyperpolarization we observed after conditioning is likely due to the measured increase in conductance, which would likely counter the depolarizing effect of ECK on the soma. When ECK was increased directly in the bath, there was no change in membrane conductance and the soma depolarized by ~ 5 mV.

Published reports have shown that dendritic sodium channels and the dendritic persistent sodium current are capable of boosting synaptic events in layer V pyramidal cells (Lipowsky et al. 1996; Magee and Johnston 1995; Mittman et al. 1997; Schwindt and Crill 1995). On the other hand, some researchers have reported that only somatic sodium channels contribute to pyramidal neuron EPSP amplification (Andreasen and Lambert 1999; Stuart and Sakmann 1995). The relative contribution of dendritic versus somatic sodium channels to EPSP potentiation may depend on the duration of the synaptic events with more prolonged events better capable of activating dendritic channels (Spruston et al. 1999). Finally, it has recently been reported that inhibitory interneurons (Martina et al., 2000) and layer II/III pyramidal cells (Waters et al. 2001) express dendritic sodium channels, consistent with our observation of EPSP potentiation in these cell types.

Our suggestion that increases in ECK depolarize the dendrite, thus changing the functional coupling between the dendrite and the somatic compartment, is consistent with the recent findings of Larkum et al. (2001). These authors reported that increasing ECK from 2.5 to 7.5 mM depolarizes the dendrite substantially, causing dendritic bursting and enhanced activation of dendritic sodium and calcium channels. Our results differ from their findings in that the change in ECK in our study is stimulus-evoked, caused by short periods of repetitive activity in a population of cells activated by the input drive.

Layer I EPSP attenuation

The mechanism responsible for the strong attenuation of layer I EPSPs after layer VI/WM conditioning is unclear and will require further study. Given that layer I EPSPs are relayed via the apical dendrite, where EPSPs are strongly potentiated, it seems likely that the attenuation involves a strong shunting of the distal apical tuft. This suggestion is consistent with our finding that layer I EPSP attenuation is paralleled by a reduction in EPSP rise time, time to peak, and full width at half-maximum. A potential basis for this shunting was reported by Yuste et al. (1994), who found that repetitive stimulation is capable of eliciting a local calcium accumulation ("apical band") in the distal dendrite of layer V pyramidal cells. The location of the apical band corresponds to the laminar location at which the effect of conditioning switched from potentiation to attenuation of synaptic inputs (layer II). If, for instance, local intracellular calcium accumulation in the apical band activates Ca^{2+}-activated potassium channels, the resulting increase in distal dendritic membrane conductance could underlie the attenuation of layer I EPSPs. Alternatively, layer I EPSP attenuation could be due to GABAergic modulation of the distal apical tuft or to the inactivation of conductances in the apical tuft that normally function to boost layer I synaptic input (Spruston et al. 1999). It is unlikely that layer I EPSP attenuation is due to a decrease in extracellular potassium (i.e., the inverse of the proposed mechanism of layer II/III EPSP potentiation). Previous studies have consistently found that repetitive stimulus drive causes an increase in ECK in layer I, although the increase in that layer is smaller than that seen in layers II–VI (Cordingley and Somjen 1978; Dietzel et al. 1980; Greenwood et al. 1981).

Limitations of the current study

A limitation of the current study is that none of the evidence we obtained concerning the mechanisms involved in layer II/III potentiation is direct. Specifically, we suggest, based on recordings in the somatic compartment, that the EPSP potentiation that follows layer VI/WM conditioning stimulation may involve dendritic depolarization due to a stimulus-evoke ECK accumulation and a consequent improved recruitment of postsynaptic (dendritic) sodium channels. While the data are consistent with this hypothesis, additional experiments involving dendritic recordings would be necessary to test whether a conditioning train at the layer VI/WM border depolarizes the dendrite, causing EPSP potentiation. Finally, QX-314- and TTX-sensitive postsynaptic currents appear to underlie the potentiation we observe, but a number of additional mechanisms capable of modifying EPSP amplitude might be recruited by ECK accumulation. For instance, astrocytic depolarization by ECK accumulation would be expected to interfere with glutamate clearance from the synaptic cleft (Szatkowski et al. 1990), potentially altering synaptic efficacy. Our data suggest that such the effect of such mechanisms on synaptic efficacy is substantially weaker than that of QX-314- or TTX-sensitive channels, at least following the type of conditioning stimulation we employed.

A second potential complication is the possibility that some of the EPSPs evoked by electrical stimulation in the present study were not monosynaptic. While the onset latency and reliability of EPSPs was consistent with monosynaptically relayed inputs, it is conceivable that the EPSP potentiation involved polysynaptically mediated input in some cells. Several findings suggest that this is not the case. First, the potentiation is sensitive to postsynaptic pharmacological and membrane potential manipulations. If the potentiation was due to a polysynaptic effect (e.g., a change in the strength of inhibition in a local cortical circuit), it should be relatively insensitive to manipulations of the recorded cell. Second, the magnitude of EPSP potentiation was not correlated with layer II/III EPSP onset latency ($r = 0.017$, $P = 0.78$), suggesting that monosynaptic responses are potentiated as strongly as potential polysynaptic responses. Finally, the results obtained with glutamate puffs recorded in the presence of extracellular cadmium show that the postsynaptic responses to directly applied glutamate (the response to glutamate in the presence of cadmium must be direct since synaptic transmission is blocked by cadmium) are potentiated by changes in ECK. Our conclusion is therefore that EPSP potentiation does not rely on effects mediated by intermediary neurons.

Because the mechanism responsible for the attenuation of layer I EPSPs was not identified, the question of whether this modulation requires polysynaptically relayed inputs remains unresolved. However, as with the layer II/III EPSP potentiation, the possibility that layer I EPSP attenuation requires polysynaptic inputs is viewed as unlikely because the magnitude of layer I EPSP attenuation was not correlated with EPSP onset latency ($r = 0.17$, $P = 0.23$).

Functional implications

The functional implications of the results depend critically on whether observations obtained from the sensorimotor cortical slice accurately reflect events that occur in vivo. A number of published observations suggest that the ECK accumulation that underlies the EPSP potentiation observed in our experiments does occur in vivo. For example, Singer and Lux (1975) reported that appropriately oriented bars of light elicit substantial increases in ECK in cat visual cortex. Kelly and Van Essen (1974) found that glial cells in cat primary visual cortex could depolarize by >5 mV in response to appropriately oriented bars of light, a depolarization similar to that observed in the current study. Other studies have provided evidence for significant ECK accumulation in vivo after electrical stimulation in the thalamus (e.g., Dietzel et al. 1980; Karahashi and Goldring 1966). In addition, because neurons in the slice are tonically hyperpolarized compared with their in vivo counterparts and because the persistent sodium current is only activated at a relatively depolarized membrane potential (approximately -60 mV) (Stafstrom et al. 1985), the ECK accumulation needed to produce EPSP potentiation in the slice appears to be larger than that required in vivo. Much less is known concerning the physiological plausibility of layer I EPSP attenuation, but the finding that V5/MT feedback to primary visual cortex is strongest for stimuli of low contrast (Hupe et al. 1998) seems consistent with our finding that strong stimulation reduces the efficacy of layer I inputs, the primary target of MT feedback to V1. (Salin and Bullier 1995).

The ability of repetitive stimulus drive to modify upper layer EPSPs has significant functional implications. First, it suggests that the astrocytes may play a role in cortical information

processing. By controlling the magnitude and duration of stimulus-driven changes in ECK (Newman 1995), glial cells may modulate both the strength of synaptic input and the manner in which the postsynaptic neuron integrates its inputs. Second, the large-amplitude layer II/III EPSPs observed after conditioning may be important for facilitating permanent changes in the synaptic efficacy of corticocortical connections. In this way, EPSP potentiation could complement Hebbian-type mechanisms for permanently altering synaptic strength; the recent history of activity would function as a gate controlling the likelihood of LTP and prevent spurious coincidences from changing synaptic strength. Consistent with this proposal, Hess et al. (1996) found that repetitive feedforward drive, of the type used to elicit EPSP potentiation in this study, was necessary for allowing permanent modification of horizontal inputs in rat motor cortex. Finally, the differential modulation of layer I and II/III EPSPs suggests that repetitive stimulation may enable dynamic compartmentalization of the inputs to different regions of the dendrite. This proposal is consistent with a number of recent studies that have shown that the relative efficacy of inputs to different dendritic compartments can change substantially due to changes in the relative timing of the input and a somatic spike (Hausser et al. 2001; Larkum et al. 1999) or the overall level of depolarization in the relevant dendritic segment (Larkum et al. 2001).

In a more general context, our results are consistent with a growing body of both systems- and cellular-level literature that suggests that the response properties of neurons in sensory cortex and the circuits in which they are embedded are capable of substantial transient stimulus-driven modification. At a systems level, it is well known that the RF properties of cortical neurons are affected by the recent history of stimulation, over time scales lasting hundreds of milliseconds to many minutes (see Gilbert 1998 for review). The effects of stimulus history include changes in a number of RF characteristics (Bonds 1991; Cavanaugh et al. 1999; Das and Gilbert 1995; Dinse et al. 1990; Dragoi et al. 2000; Movshon and Lennie 1979; Ohzawa et al. 1982; Saul and Cynader 1989a,b). It has been proposed that these forms of cortical dynamics may be manifestations of a stimulus-driven optimization of the network (Barlow 1990).

While the physiological substrate for such effects is far from clear, there is a rich literature describing cellular mechanisms that could contribute to the changes of RF properties observed in vivo. The possible mechanisms include pre- and postsynaptic metabotropic receptors that operate on time scales extending from hundreds of milliseconds to many minutes, changes in both intracellular and extracellular ion concentrations (e.g., intracellular calcium accumulation, either in the presynaptic terminal or in local dendritic compartments), depletion of synaptic vesicles, and changes in the activation state of various voltage-gated conductances present in the dendrite. Many of these mechanisms have been studied extensively, but their overall effect on the functioning of cortical circuits remains unclear.

Our results suggest a specific and novel way in which repetitive stimulus drive can alter the functioning of cortical circuits. They reveal that the efficacy of both feedback and horizontal inputs to layer V pyramidal cells depends on the recent history of activity relayed via feedforward/columnar pathways. Although a mapping of specific examples of in vivo response dynamics onto the types of transient synaptic modification observed in this study is currently not possible—due largely to a lack of understanding concerning the role of each type of input in determining the RF properties of cortical neurons—it seems highly plausible that repetitive feedforward drive leads to substantial but fully reversible modifications of the efficacy of horizontal and feedback inputs and, in turn, to the cortical neuron RF dynamics extensively documented in recently published studies of both anesthetized and conscious behaving animals.

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