Patterned Activity in Stratum Lacunsum Moleculare Inhibits CA1 Pyramidal Neuron Firing

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Dvorak-Carbone, Hannah and Erin M. Schuman. Patterned activity in stratum lacunsum moleculare inhibits CA1 pyramidal neuron firing. J. Neurophysiol. 82: 3213–3222, 1999. CA1 pyramidal cells are the primary output neurons of the hippocampus, carrying information about the result of hippocampal network processing to the subiculum and entorhinal cortex (EC) and thence out to the rest of the brain. The primary excitatory drive to the CA1 pyramidal cells comes via the Schaffer collateral (SC) projection from area CA3. There is also a direct projection from EC to stratum lacunsum-moleculare (SLM) of CA1, an input well positioned to modulate information flow through the hippocampus. High-frequency stimulation in SLM evokes an inhibition sufficiently strong to prevent CA1 pyramidal cells from spiking in response to SC input, a phenomenon we refer to as spike-blocking. We characterized the spike-blocking efficacy of burst stimulation (10 stimuli at 100 Hz) in SLM and found that it is greatest at ~300–600 ms after the burst, consistent with the time course of the slow GABA<sub>B</sub> signaling pathway. Spike-blocking efficacy increases in potency with the number of SLM stimuli in a burst, but also decreases with repeated presentations of SLM bursts. Spike-blocking was eliminated in the presence of GABA<sub>B</sub> antagonists. We have identified a candidate population of interneurons in SLM and distal stratum radiatum (SR) that may mediate this spike-blocking effect. We conclude that the output of CA1 pyramidal cells, and hence the hippocampus, is modulated in an input pattern-dependent manner by activation of the direct pathway from EC.

INTRODUCTION

The hippocampus plays a critical role in such high-level brain functions as learning and memory (Eichenbaum et al. 1992; Squire and Zola-Morgan 1991; Wood et al. 1999; Zola-Morgan and Squire 1990) and spatial navigation (Muller 1996; Wilson and McNaughton 1993). In order for the neural computations performed by the hippocampus to be used by the rest of the brain, an output from the hippocampus to neocortex is necessary. The pyramidal cells of area CA1 are the primary population of hippocampal principal cells projecting outside of the hippocampus, with axons projecting to subiculum and entorhinal cortex (EC) as well as subcortical targets (Lopes da Silva et al. 1990; Tamamaki and Nojyo 1995; Van Groen and Wyss 1990; Witter et al. 1989). The primary excitatory input to the CA1 pyramidal cells is the Schaffer collateral (SC) projection from area CA3 (Amaral et al. 1990; Amaral and Witter 1989; Andersen et al. 1966; Lopes da Silva et al. 1990). CA1 pyramidal cells and their SC inputs are therefore crucial sites for the regulation of hippocampal output.

METHODS

Tissue preparation

Slices were prepared by standard procedures from 6- to 8-wk-old male Sprague-Dawley rats. Rats were decapitated following Halothane anesthesia, and the brain rapidly removed to ice-cold, oxygenated artificial cerebrospinal fluid (ACSF; in mM: 119 NaCl, 2.5 KCl, 1.3 MgSO<sub>4</sub>, 2.4 CaCl<sub>2</sub>, 1.0 NaH<sub>2</sub>PO<sub>4</sub>, 26.2 NaHCO<sub>3</sub>, and 11.0 glucose). The posterior half of each hemisphere was glued, ventral side up, onto the stage of a cooled oscillating tissue slicer (Ots-3000–04; FHC, Brunswick, ME) and covered with chilled ACSF immediately after slice preparation. All electrophysiology was done with the slices submerged and constantly perfused with oxygenated ACSF at room temperature.

To minimize the possibility of disynaptic or trisynaptic activity in CA1, the dentate gyrus and CA3 regions were dissected away from the slice, leaving a CA1 minislice. A cut was made through SR in distal CA1 (near the subiculum) perpendicular to the cell body layer, to prevent antidromic activation of SC axons by the stimulating...
Electrophysiology

Bipolar tungsten electrodes, either concentric or paired needles, were used for stimulation. One electrode was placed in SR to stimulate the SC axons; the other was used to stimulate SLm afferents on the far side of the cut. The level of SR stimulation was set such that the resultant excitatory postsynaptic potential (EPSP) in the pyramidal cell just barely reached spike threshold; this generally required a current of 20–40 μA for 100 μs. Stimulation in SLm was generally stronger, 30–200 μA for 100 μs.

Intracellular recordings from pyramidal cells were made using sharp electrodes whose resistance was 100–200 MΩ when filled with 2 M potassium acetate. Sharp electrode recordings were made blind by lowering the electrode into stratum pyramidale until a penetration was achieved. The voltage reading of the electrode was zeroed with the electrode in the bath, and the bridge was balanced before penetration and rebalanced after penetration. Capacitance compensation was applied after penetration. Neurons included for analysis had an average resting potential of ~62 ± 1.0 (SE) mV, fired overshooting action potentials, and had input resistances of 109 ± 10 ΩM (n = 28). Pyramidal cells were identified by the presence of strong spike frequency accommodation in response to positive current injection. All experiments were performed in current-clamp mode; the cell was generally at its resting potential, although in a few (3/28) cases a small negative current was applied to hyperpolarize the cell and prevent it from spontaneously firing action potentials.

Whole cell electrodes used for interneuron recordings had a resistance of ~5 MΩ when filled with intracellular solution (125 mM KMeSO₄, City Chemical, Jersey City, NJ), 9 mM HEPES, 3.6 mM NaCl, 90 μM EGTA, 4 mM Mg-ATP, 300 μM Li-GTP, 25 mM phosphocreatine, and 0.2–0.4% biocytin). Whole cell recordings were made under visual guidance on an Olympus BX50W1 upright microscope equipped with a MTI VE1000 CCD camera. Positive pressure was applied to the electrode solution while advancing toward the targeted neuron, to keep debris off the electrode as well as to clean the surface of the neuron (Edwards 1995). A gigaseal was obtained under voltage-clamp conditions by applying slight negative pressure; the patch was then clamped down to ~60 mV, and whole cell configuration was achieved by applying further negative pressure. Neurons included for analysis had resting potentials negative to ~50 mV, fired overshooting action potentials, and had input resistances of 562 ± 5 MΩ (n = 41). All experiments were performed in current-clamp mode, with the cell at its resting potential.

Drugs were applied by dilution of concentrated stock solutions into the perfusion medium. Stock solutions were made up in water. CGP 55845A was a kind gift from Novartis (Basel); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2-0H-saclofen were obtained from Tocris Bioscience (Bristol, UK). Intracellular responses displayed are averages of three to five individual traces.

The effectiveness of the SLM burst in blocking SR-evoked spiking was quantified as follows. Trials of SR stimulation following an SLM burst were interleaved with trials where SR stimuli were delivered in isolation. For one test condition, 10 SR + SLM trials were interleaved with ~25 SR-alone trials. SR-induced spike firing probabilities in the presence or absence of the SLM burst were calculated from these trials. Spike-blocking efficacy for each test condition was defined as follows: spike-blocking efficacy = (probability of firing with SR stimulation alone) – (probability of firing with SR + SLM stimulation). Thus spike-blocking efficacy would reach a maximum of 1 if the cell never spiked when the SR stimulus was presented following the SLM burst and always spiked when the SR stimulus was presented in isolation, and would be 0 if the cell was equally likely to fire in the presence or the absence of the SLM burst. A negative value would be possible if the spike firing probability increased in the presence of the SLM burst. In practice, the upper bound of the spike-blocking efficacy was limited by the firing probability in response to SR stimulation alone. Over all tests, the firing probability in response to SR stimulation alone was 0.87 ± 0.01 (n = 176); therefore a spike-blocking efficacy of ~0.9 would indicate maximal spike-blocking. In only 1 test of 176 was the SR-alone spike firing probability <0.5, and in 76% of tests, the SR-alone spike firing probability was >0.8.

All numerical values are listed as means ± SE; error bars in bar graphs are SE. Data were analyzed and plotted using Microcal Origin. Some statistical analyses were performed in Microsoft Excel or STATISTICA for Windows (StatSoft, Tulsa, OK). A paired Student’s t-test was used to test statistical significance of the spike-blocking effect, using spiking probabilities in the presence or absence of the SLM burst as the dependent variables. For multiple comparisons, e.g., comparing the effectiveness of spike-blocking at different interstimulus intervals (ISIs), a repeated-measures, one-way ANOVA was performed across that subset of the data for which all levels (e.g., ISI) were tested on each neuron included in the analysis; the Neuman–Keuls test was performed to assess the statistical significance of all pair-wise post hoc comparisons. Results were considered significant when P ≤ 0.05; P values >0.05 are reported as NS.
RESULTS

Burst stimulation in SLM results in a large IPSP and blocks SC-induced spiking in a GABA_{B}-dependent manner

A single stimulus in SLM usually resulted in a biphasic response in the postsynaptic pyramidal cell, with a small EPSP (0.9 ± 0.1 mV, peaking 31.3 ± 1.7 ms after the stimulus artifact, n = 23), presumably mediated by excitatory axons from layer III of EC (Empson and Heinemann 1995a,b), nucleus reuniens thalami (Dolleman-Van Der Weel and Witter 1996; Wouterlood et al. 1990), or intertemporal cortex (Yukie and Iwai 1988), followed by a slow, small inhibitory postsynaptic potential (IPSP) (−1.1 ± 0.1 mV, peaking 281 ± 10 ms after the stimulus artifact, n = 28; Fig. 1A). With the addition of the GABA_{A} antagonist bicuculline (20 μM), the time-to-peak and amplitude of the EPSP and late IPSP were increased, suggesting the presence of a GABA_{A} receptor–mediated IPSP (Empson and Heinemann 1995a,b). Burst stimulation in SLM, i.e., 10 stimuli at 100 Hz, resulted in a significantly larger IPSP (−4.4 ± 0.4 mV, peaking 391 ± 13 ms after the stimulus artifact, P < 0.0001, n = 27; Fig. 1B). This burst-elicited IPSP was mediated by GABA_{B} receptors, because it was significantly reduced in the presence of the GABA_{B} antagonist 2-0H-saclofen (100 μM; peak IPSP amplitude, −2.0 ± 0.3 mV, significantly different from the IPSP in the same cell under control conditions, P < 0.05, n = 4) and virtually eliminated in the presence of the more potent GABA_{B} antagonist CGP 55845A (2 μM; peak IPSP amplitude −0.5 ± 0.3 mV, significantly different from the IPSP in the same cell under control conditions, P < 0.05, n = 3; Fig. 1C).

We wished to examine whether the inhibition evoked by high-frequency stimulation in SLM could have a functional effect on the output of the hippocampus, namely action potentials in CA1 pyramidal cells. SC stimulation strength in SR was set to such a level that the response was just suprathreshold for action potential generation (Fig. 1D). Bursts of stimuli in SLM were tested for their ability to block spiking evoked by SR stimulation. Spike-blocking efficacy can range from 0 (no block; spiking equally likely in presence or absence of SLM stimulation) to 1 (maximal block: spikes never evoked following SLM stimulation), or be negative if spiking probability increased following SLM stimulation (see METHODS). When the SR stimulus was delivered in the middle of the SLM burst-evoked IPSP, spike generation was blocked (Fig. 1E); spike-blocking efficacy 400 ms after an SLM burst of 10 stimuli at 100 Hz was 0.73 ± 0.05 (n = 19). This spike-blocking effect was mediated by GABA_{B} receptors, because spike-blocking efficacy was reduced from 0.69 ± 0.01 to 0.15 ± 0.003 in the presence of 100 μM 2-0H-saclofen (P < 0.01, n = 3) and was reduced from 0.82 ± 0.02 to 0.17 ± 0.03 in the presence of 2 μM CGP 55845A (P < 0.001, n = 3; Fig. 1F).

Spike-blocking efficacy is dependent on relative timing of the SLM and SR stimuli

The dependence of spike-blocking efficacy on the relative timing of SR and SLM stimulation may suggest under what circumstances spike-blocking may occur in vivo. Dependence on ISI (measured from the 1st stimulus in the SLM burst to the SR stimulus) was tested in 23 neurons using an SLM burst pattern of 10 stimuli at 100 Hz; ISIs measured ranged from 25 ms (with the SR stimulus thus arriving during the SLM burst) to 1,500 ms. Spike-blocking efficacy reached a maximum of 0.77 ± 0.04 (n = 19; significantly different from 0, P < 0.00001) at an ISI of 400 ms, a time interval consistent with the slow time course of the G protein–mediated GABAB signaling pathway (Misgeld et al. 1995; Mott and Lewis 1994), and dropped off at shorter or longer ISIs (Fig. 2A). At 1,500 ms, the spike-blocking effect was not significant (spike-blocking efficacy, 0.15 ± 0.15, NS, n = 4).

To compare spike-blocking efficacy at different ISIs, an ANOVA was performed on data from nine neurons on which ISIs of 100, 200, 400, 600, and 800 ms had been tested. There was significant variation in spike-blocking efficacy between ISIs (F = 6.78, DF = 4, P < 0.001). Spike-blocking efficacy at 100, 200, and 800 ms was significantly lower than that at 400 ms (Newman-Keuls test, P < 0.01, n = 9), and spike-blocking efficacy was also significantly lower at 800 than at 600 ms ISI (Newman-Keuls test, P < 0.01, n = 9). This confirms the observation that spike-blocking efficacy was greatest at intermediate ISIs.

Spike-blocking efficacy is dependent on the number of stimuli in the SLM burst

We sought to determine how spike-blocking efficacy varied with the number of stimuli in the SLM burst. IPSP amplitude and duration increased when the number of stimuli in the burst was increased (e.g., see Fig. 2B, inset). We tested spike-blocking with single SLM stimuli as well as bursts consisting of 2–15 stimuli delivered at 100 Hz in 9 neurons. With only a single SLM stimulus, there was no significant spike-blocking effect (spike-blocking efficacy, 0.12 ± 0.07, NS different from 0, n = 9); spike-blocking efficacy was greatly increased by repetitive stimulation (Fig. 2B). There was a significant effect...
of number of stimuli/burst on spike-blocking efficacy \( (F = 6.04, \text{DF} = 8, P < 0.01) \). One stimulus was significantly less effective in spike-blocking than three or more stimuli (Newman-Keuls test, \( P < 0.05 \) for all comparisons). Two stimuli were significantly less effective than 8 or 10 stimuli (Newman-Keuls test, \( P < 0.05 \)). No other significant differences were observed between different numbers of stimuli.

Repeated presentation of the SLM burst results in a reduction of the IPSP and of spike-blocking efficacy

Having characterized the effect of SLM-evoked inhibition on excitatory SC transmission, we wished to examine whether this inhibitory effect could itself be modulated. GABA-mediated responses are known to undergo frequency-dependent depression by means of presynaptic \( \text{GABA}_B \) autoreceptors (e.g., Davies et al. 1990). We found that repeated presentation of the SLM burst (10 stimuli at 100 Hz) at 1 Hz resulted in an exponential decay of IPSP amplitude, with a time constant of 3.7 ± 0.2 s \( (n = 8; \text{Fig. 3}) \).

Is spike-blocking efficacy modulated along with IPSP amplitude? To determine how spike-blocking efficacy varied with position in the train of SLM bursts, we used a modified stimulation paradigm. We presented 10 SC stimuli at 1 Hz alone, followed by 10 SC stimuli at 1 Hz offset by 400 ms from 10 SLM bursts at 1 Hz (Fig. 4A), followed by 10 SC stimuli again. This set of stimuli was repeated 5–10 times at ~5-min intervals to allow for recovery of the \( \text{GABA}_B \) response. Spike-firing probability for SC stimulation alone and in the presence of the SLM bursts was determined for each position in the train of bursts (Fig. 4B; \( n = 8 \)). Spike-blocking efficacy decayed in an exponential manner with repeated SLM burst stimulation, from 0.73 ± 0.07 during the first burst, to 0.08 ± 0.04 during the last burst (Fig. 4C; \( n = 8 \)). The time constant of this decay was 2.8 ± 0.3 s, similar to the time course of IPSP depression. Spike-blocking efficacy varied significantly with position in the train (repeated-measures ANOVA, \( F = 11.10, \text{DF} = 9, P < 0.0001 \)); post hoc analysis showed that spike-blocking efficacy was significantly greater during the first burst in the train than at any other position (Newman-Keuls test, \( P < 0.05 \)) and significantly greater during the second burst than in any of the 4th through 10th bursts (Newman-Keuls test, \( P < 0.05 \) for all comparisons).

Because of our observation that SC spike firing probability was not constant during a train of 10 stimuli at 1 Hz (Fig. 4B), we wished to verify that the apparent decrease in spike-blocking efficacy was not due, rather, to a facilitation in the SC response owing to repeated stimulation. To test this, we compared spike-blocking efficacy during the first and last SLM bursts by presenting only two SC stimuli nine seconds apart, thus occurring during the first and last IPSPs of a train of bursts. Spike-blocking efficacy was 0.87 ± 0.13 during the first burst and 0.17 ± 0.12 during the last burst, a significant difference \( (P < 0.05, n = 3) \), indicating that spike-blocking efficacy did in fact decrease over the course of the burst train.

**FIG. 3.** Repeated presentation of the SLM burst results in a decrease in the IPSP amplitude. A: SLM bursts (10 at 100 Hz) repeated 10 times at 1 Hz result in IPSPs of decreasing amplitude. Scale bar: 2 mV/2 s. B: the decrease in IPSP amplitude (normalized to the amplitude of the 1st IPSP in the train) is very well fit by a single exponential decay curve. IPSP amplitudes for 8 different cells are plotted along with exponential curve fits; \( r^2 \) values range from 0.901 to 0.994.
Recovery from the decay of spike-blocking was measured by performing single probe tests 2 min following a train of SLM bursts. After 2 min, spike-blocking efficacy had returned to \(0.46 \pm 0.12\) (\(n = 5\); Fig. 5); spike-blocking efficacy at 5–10 min following the previous burst train was \(0.72 \pm 0.08\) (\(n = 5\)), very similar to the \(0.74 \pm 0.05\) spike-blocking efficacy observed when single bursts were tested at 400 ms ISI (compare with Fig. 2A).

**SLM interneurons may contribute to spike-blocking**

We made recordings from 41 SLM interneurons, potential mediators of this spike-blocking effect, to determine their responses to high-frequency SLM stimulation. Some (14/41) interneurons were not very responsive at all to stimulation in SLM, despite having dendrites in that layer. Of this subset (14/41), some interneurons could be driven directly (i.e., spiking due to direct depolarization or antidromic activation of the axon) by SLM stimulation. Another set of interneurons (3/41) responded solely with an IPSP to temporoammonic (TA) stimulation. The last set (24/41) of SLM interneurons responded with a large EPSP to stimulation in SLM and could be made to spike by repeated SLM stimulation at high frequencies; an example of such an interneuron is shown in Fig. 6. The spike-blocking effect we characterized is likely to be mediated by interneurons that were driven synaptically, as well as those that were driven directly by the stimulating electrode.

**DISCUSSION**

We have shown that high-frequency stimulation in SLM can regulate the output activity of CA1 pyramidal cells in response to excitatory SR inputs. This result suggests a role for the direct projection from EC to SLM of CA1, the TA pathway (Fredens et al. 1984; Maccaferri and McBain 1995; Reeves et al. 1997), in regulation of the output of the hippocampus. Individual EC layer III neurons, whose axons are activated by SLM stimulation, can naturally fire at the high frequencies used in this study (Finch et al. 1986; but see Mizumori et al. 1992; but see Stewart et al. 1992; but see Dickson et al. 1997; Gloveli et al. 1997). EC layer II/III neurons also fire in high-frequency population bursts (Chrobak and Buzsáki 1998). A similar inhibitory phenomenon has been observed in area CA3 of the hippocampus, where a single stimulus to the perforant pathway in SLM can block spontaneous firing of CA3 pyramidal cells (Kehl and McLennan 1985a,b) and reduce the amplitude of a population spike evoked by subsequent stimulation of the fimbria (Kehl and McLennan 1985a,b).

Previous reports have suggested that the TA pathway may inhibit SC responses in area CA1 (Empson and Heinemann 1995a,b; Levy et al. 1998). However, the relative timing of SR and SLM stimulation in these studies was based on the difference in synaptic delays between the mono- and trisynaptic inputs from EC to CA1. This approach assumes that the cells of origin of the two pathways, which consist of discrete populations within EC (Steward and Scoville 1976), are active simultaneously. Because of the difficulty of identifying the layer of origin of single units recorded in vivo (e.g., see Chrobak and Buzsáki 1998; Quirk et al. 1992; Stewart et al. 1992), it is not known whether this is the case. Because afferent inputs are segregated to different layers of the EC (e.g., see Witter 1993), it is likely that the cells of layers II and III of EC have different activity patterns. The slow and long time course of the SLM-mediated inhibitory effect reported in our study suggests that spike-blocking may act as an overall damping of the output of the hippocampus, rather than a more synapse-specific or temporally restricted effect.
The dependence of the spike-blocking effect on GABA_B receptors was confirmed by its elimination in the presence of the GABA_B receptor antagonist CGP 55845A (Fig. 1). The spike-blocking effect may be mediated by presynaptic GABA_B receptors located on the SC axon terminals, as seen elsewhere (e.g., Isaacson et al. 1993), by the hyperpolarization evoked by activation of postsynaptic GABA_B receptors on the pyramidal cells (e.g., Connors et al. 1988), or a combination of both factors; we did not address this issue in our study.

The variation of spike-blocking efficacy with ISI is consistent with the time course of GABA_B-mediated phenomena (reviewed in Mott and Lewis 1994). The IPSP evoked by SLM burst stimulation peaked at 397 ± 14 ms, and spike-blocking efficacy was greatest at an ISI of 400 ms. Some spike-blocking was observed as early as 100 ms, at which point the somatic membrane potential was near its resting potential, suggesting that postsynaptic hyperpolarization may not be essential to the spike-blocking effect. At short ISIs, shunting of the SC input by the GABA_A or EPSP components of the SLM response may have contributed to the spike-blocking effect. We found that the spike-blocking effect also had a longer time course than the IPSP, because there was no significant difference between spike-blocking efficacy at 400 and 600 ms ISI, and spike-blocking could still be observed even 800–1,000 ms after the SLM burst. Spike-blocking at long ISIs may have been mediated by the activation of presynaptic GABA_B receptors on SC axon terminals (Isaacson et al. 1993).

Although spike-blocking efficacy was not directly dependent on the IPSP amplitude, the size of the IPSP still appears to be a good indicator of the amount of GABA_B activation. Consistent with this, an increase in the number of stimuli in the SLM burst resulted in increases both in IPSP amplitude (and duration) and in spike-blocking efficacy. Increasing the number of SLM stimuli/burst could result both in the recruitment of more interneurons, because of EPSP summation, and in more action potentials per interneuron. Previous studies have shown that activation of individual SLM interneurons does not result in a GABA_B response visible at the soma of CA1 pyramidal cells, even when a train of action potentials is elicited in the interneuron (Ouardouz and Lacaille 1997; Vida et al. 1998). In general, it is believed that several interneurons must be activated to evoke a GABA_B-mediated response (Fortunato et al. 1996).
The stimulation used may have resulted in depletion of the available pool of synaptic vesicles (Liu and Tsien 1995; Stevens and Tsujimoto 1995). The slow time course of recovery from activity-dependent depression is consistent with synaptic vesicle depletion (Lass et al. 1973; Liu and Tsien 1995; Wiley et al. 1987).

Of the many different kinds of GABAergic interneurons in the hippocampus (see Freund and Buzsáki 1996 for review), the interneurons of SLM are likely candidates for mediators of the spike-blocking effect. SLM interneurons can be driven either synchronically (Fig. 6) (Lacaille and Schwartzkroin 1988a,b; Williams et al. 1994) or by direct depolarization (unpublished observations) in response to stimulation in SLM. Focal stimulation in SLM has been used to evoke GABA_B-mediated synaptic responses in pyramidal cells, presumably by the activation of SLM interneurons (Benardo 1995; Miles et al. 1996; Williams and Lacaille 1992). Trains of action potentials in SLM interneurons can block action potentials from being evoked by depolarizing current injection in pyramidal cells (Lacaille and Schwartzkroin 1988a,b). Other types of interneurons may also contribute to SLM-activated spike-blocking, including vertically oriented orien/veimus interneurons (McBain et al. 1994), stratum pyramidale basket cells (Han 1996; Sik et al. 1995), and chandelier cells (Buhl et al. 1994; Li et al. 1992), all of which have dendritic arborizations in SLM. Basket and chandelier cells have been identified as postsynaptic targets of TA axons (Kiss et al. 1996).

Under what physiological circumstances is the spike-blocking effect likely to be evoked? If SLM interneurons are indeed responsible for spike-blocking, then it needs to be determined under what circumstances they are active. SLM receives projections from layer III of EC (Steward and Scoville 1976), nucleus reuniens thalami (Dollemann-Van Der Weel and Witter 1996), inferotemporal cortex (Yukie and Iwai 1988), and amygdala (Petrovich et al. 1997; Pikkarainen et al. 1999), all of which might activate SLM interneurons. Disynaptic inhibition of CA1 pyramidal cells via the TA input may contribute to facilitation of the trisynaptic pathway (Her
eras et al. 1987). In the presence of natural patterns of activity, such as theta rhythms, the efficacy of spike-blocking could be continuously up- and downregulated. The long-term depression of excitatory TA responses following low-frequency (1 Hz) stimulation (Dvorak-Carbone and Schuman 1999) may also contribute to a rebalancing of inhibitory and excitatory transmission in this pathway.

Activity-dependent depression of inhibitory responses has been well characterized (Ben-Ari et al. 1979; Davies et al. 1990; Deisz and Prince 1989; McCarren and Alger 1985; Thompson and Gähwiler 1989) and is mediated by GABA_B autoreceptors (Davies et al. 1990; Roestorff and Lambert 1994) as well as by a GABA_A-independent process, possibly synaptic vesicle depletion (Fortunato et al. 1996; Lambert and Wilson 1994). Although most studies of modulation of inhibition have focused on GABA_A responses, GABA_B responses are also reduced with repeated stimulation (Ling and Benardo 1994; Williams and Lacaille 1992). We found that repeated presentation of the SLM burst stimulus at 1 Hz resulted in a decrease in the IPSP amplitude along with a decrease in spike-blocking efficacy. In addition to processes intrinsic to inter-neuron axon terminals, a decrease in recruitment of SLM interneurons may also have contributed to the decreased IPSP size (Congar et al. 1995). Also, the repeated high-frequency stimulation used may have resulted in depletion of the available pool of synaptic vesicles (Liu and Tsien 1995; Stevens and Tsujimoto 1995). The slow time course of recovery from activity-dependent depression is consistent with synaptic vesicle depletion (Lass et al. 1973; Liu and Tsien 1995; Wiley et al. 1987).

Patterns of activity in the nervous system do not occur in a vacuum; responses to synaptic activity are conditioned by the prior history of the synapse. The balance of excitation and inhibition in the hippocampus varies constantly due to activity-dependent regulation of synaptic transmission. Here, we have shown that short-term depression of an SLM-activated spike-blocking effect can shift the balance between excitation and inhibition in the inputs onto CA1 pyramidal cells. Spike-blocking evoked by burst SLM stimulation was depressed when bursts were repeated at 1 Hz (Figs. 4 and 5). Down-regulation of the inhibitory TA input may contribute to frequency-dependent facilitation of the trisynaptic pathway (Herreras et al. 1987). In the presence of natural patterns of activity, such as theta rhythms, the efficacy of spike-blocking could be continuously up- and downregulated. The long-term depression of excitatory TA responses following low-frequency (1 Hz) stimulation (Dvorak-Carbone and Schuman 1999) may also contribute to a rebalancing of inhibitory and excitatory transmission in this pathway.
Burst firing of SLM interneurons may be required for spike-blocking; SLM interneurons may fire in bursts when recovering from hyperpolarization (Lacaille and Schwartzkroin 1988a,b), possibly due to low-threshold, transient Ca$^{2+}$ currents (Fraser and MacVicar 1991; but see Williams et al. 1994). SLM interneurons are also depolarized and fire action potentials in the presence of the muscarinic acetylcholine receptor agonist carbachol (Chapman and Lacaille 1998); the SR/SLM border receives substantial cholinergic innervation (Matthews et al. 1987), suggesting that SLM interneurons in vivo may be activated by cholinergic inputs.

SLM-evoked blockade of SC excitation of pyramidal cells may be important for selective regulation of excitatory inputs to CA1. Although SC inputs are a primary source of excitatory input to area CA1 (Amaral et al. 1990; Amaral and Witter 1989; Andersen et al. 1986; Lopes da Silva et al. 1990), under some circumstances, the TA pathway can also have a strong excitatory effect (Buzsáki et al. 1995; Yeckel and Berger 1990). Responses in CA1 to SC or TA inputs are differentially sensitive to the GABAB agonist baclofen, with SC field responses greatly reduced while TA responses are unaffected (Colbert and Levy 1992). A similar differential suppression of SC versus TA inputs to CA1 in the presence of carbachol has been demonstrated (Hasselmo and Schnell 1994); such regulation is proposed to be important in switching between encoding and retrieval modes of associative memory systems (Hasselmo and Schnell 1994). GABA_B-mediated selective suppression of inputs to CA1, such as that shown here during SLM activity, could also mediate such a switch (Hasselmo et al. 1996). Other models of memory decoding in area CA1 require a strong excitatory input from the EC to CA1 (McClelland and LaMesse 1996); other models of memory decoding in area CA1 require a strong excitatory input from the EC to CA1 (McClelland and LaMesse 1996).

In conclusion, we have shown that afferent inputs to SLM, including the direct projection from EC, are ideally positioned to gate information flow through the trisynaptic pathway by means of appropriately timed inputs. In vivo studies, where fiber tracts are intact and can be stimulated independently, would be helpful to determine which of the many afferents to SLM mediate the inhibitory spike-blocking effect, and under what circumstances the gating is physiologically effective.

The authors thank Dr. A. M. Suter at Novartis for a kind gift of CGP 55845A, and the Howard Hughes Medical Institute for funding, including a predoctoral fellowship to H. Dvorak-Carbone.

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Received 3 June 1999; accepted in final form 27 August 1999.

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