Distal Versus Proximal Inhibitory Shaping of Feedback Excitation in the Electrosensory Lateral Line Lobe: Implications for Sensory Filtering

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Berman, Neil J. and Leonard Maler. Distal versus proximal and VML cell inhibition allows them to act as low- and high-pass filters respectively on indirect descending feedback to ELL pyramidal cells.

INTRODUCTION

The electrosensory lateral line lobe (ELL) of electric fish is the primary processing structure for electrosensory information. Most of the extrinsic input to the ELL is in the form of descending excitatory feedback pathways (Bastian 1986a,b; Maler 1979; Maler et al. 1981). In the previous study (Berman and Maler 1998b), we examined the characteristics of inhibition that controls the excitatory feedback provided by the Stratum fibrosum (StF) to the ventral molecular layer (VML); a pathway originating from the nucleus praeminentialis (Pd). In this study, we report on inhibition activated by a feedback pathway that terminates in the dorsal molecular layer (DML); the parallel fibers (PFs). PF afferents from the granule cells of the caudal portion of the cerebellum, the eminentia granularis posterior (EGp) (Maler et al. 1981; Sas and Maler 1987), form this pathway, which courses perpendicular to the main orientation of the pyramidal cell dendrites, much like the parallel fibers in the cerebellar molecular layer (Maler et al. 1981). This pathway contains electrosensory and proprioceptive (Bastian 1995; Sas and Maler 1987) information, and there is compelling evidence that it controls the adaptive gain of the ELL pyramidal neurons to electrosensory input (Bastian 1986a).

The PFs form excitatory, probably glutamatergic (Maler and Monaghan 1991; Wang and Maler 1994) synapses on the apical dendrites of pyramidal cells (Maler et al. 1981) and on molecular layer GABAergic interneurons (Maler and Mugnaini 1994), namely the stellate and VML cells. GABAergic polymorphic cells (Maler and Mugnaini 1994) also have dendrites that extend into the DML (Maler 1979) that may be activated by the parallel fibers. However, the major target of polymorphic cells is the type I granule cells (GC1) and therefore they are not considered here. GABAergic type II granule cell (GC2) dendrites extend into the molecular layer but only reach the ventral region of parallel fiber termination (Maler 1979; Maler and Mugnaini 1994; unpublished observations). Thus based on anatomic evidence, the PF pathway will directly (monosynaptically) excite and indirectly (disynaptically) inhibit ELL pyramidal cells; the inhibition will come primarily from VML and stellate cells with a lesser contribution from GC2 cells. Stellate cells contact and VML cell inhibition allows them to act as low- and high-pass filters respectively on indirect descending feedback to ELL pyramidal cells.

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pyramidal cell apical dendrites, whereas VML cells contact their proximal apical dendritic trunk and soma (Fig. 1). ELL stellate cells are morphologically similar to cerebellar stellate cells (Maler 1979), whereas the connectivity of the VML interneurons resembles that of the cerebellar basket cells (Maler 1979; Maler et al. 1981). For the experiments in this study, we restricted our stimulation sites to the dorsal portion of the DML so that we could assess the contribution of VML and stellate cells in isolation. The overall impact of this feedback pathway would be determined by the balance between the excitatory and inhibitory branches (Maler and Mugnaini 1994); Bastian (1986a) unexpectedly found that the net effect of an imposed reduction of activity of the PF pathway was an increased sensitivity or gain of pyramidal cells to electrosensory input. These findings suggest that physiological activation of this pathway recruits potent inhibition of pyramidal cells via the stellate and VML cells and that loss of this inhibition caused the increase in response to electrosensory input in his experiment. Thus characterization of PF pathway inhibitory postsynaptic potentials (IPSPs) would provide some insight into the mechanisms of feedback gain control in the ELL. In the present study, we found that activation of the parallel fibers recruited substantial γ-aminobutyric acid-A (GABA_A)-mediated inhibitory input to the pyramidal cells and that this inhibition controlled both the gain and temporal characteristics of pyramidal cells’ responses to descending input. Comparisons of the effect of GABA antagonists on the proximal versus distal DML-evoked inhibition suggested that distal inhibition (mediated by stellate cells) acts as a low-pass filter, whereas the more proximal site of VML GABA_A inhibition acts as a high-pass filter.

**METHODS**

ELL slices from the brain of *Apteronotus leptorhynchus* were prepared and maintained as described in the companion studies (Berman and Maler 1998a,b). All surgical procedures were approved by the University of Ottawa Animal Care Committee. Briefly, fish were anesthetized [0.2% 3-aminobenzoic acid ethyl ester (MS-222), Sigma provided all chemicals unless otherwise indicated] and then, while artificially respirated with aerated water containing anesthetic, the brain was removed, the ELL blocked, and embedded in agar. True transverse slices (350 μm) were sectioned on a vibratome (Technical Products International, St. Louis,
Stimulation and recording

The parallel fibers of the DML were stimulated (50 to 100 \( \mu \)S, 1 to 50 V cathodal, SIU unit; or Neurolog Stimulus Isolator NL800, 20 \( \mu \)S, 13 to 800 \( \mu \)A, Digitimer, Welwyn Garden City, UK) by placing a monopolar lacquer-coated sharpened tungsten electrode (50- \( \mu \)m exposed tip) into the more dorsal portion of the DML, lateral to the recording site by \( \sim 400 \mu \)m. Note that this very dorsal location ensures that our stimuli will solely activate parallel fibers lying in the dorsal region of this layer. The position and depth of the electrode were varied to obtain the optimal response. In some experiments, a second stimulating electrode was placed at the VML/pyramidal cell layer (PCL) border to activate the StF pathway (see Berman et al. 1997). Intracellular recordings were made in the CMS pyramidal cell layer with 2 M potassium-acetate-filled electrodes bevelled to 55 to 120 M\( \Omega \) DC resistance. All experiments were software controlled (Ad/Advance, McKellar Designs, Vancouver, BC, Canada). Electrical signals were amplified (Axoclamp-2A, Axon Instruments, Foster City, CA), filtered (TM 503, Tektronix, Wilsonville, OR: 10 kHz \( f_c \)), digitized (ITC-16, Intrutech, NY; 1.25 to 50 kHz), and analyzed off-line (Igor Pro, Wavemetrics, Lake Oswego, OR). For spike rate averaging, the spike times for each sweep were converted to estimated instantaneous firing rate using a triangular filter (30 to 60-ms triangle base) (Paulin 1992), and then averaged.

Drug applications. For application to the slice by microdroplet, antagonists were dissolved in ACSF at the following concentrations: 70 \( \mu \)M bicuculline and 100 \( \mu \)M SR-95531 (Tocris Cookson, Ballwin, MO; Research Biochemicals International, Natick, MA); 0.05 to 1 mM 6-cyano-7-nitroquinazoline-2,3-dione (CNQX; with dimethyl sulfoxide, RBI), 2 mM CPP, and 1 to 3 mM phaclofen and saclofen (Tocris Cookson). Microdroplets were applied by pressure (Neurophore, Medical Systems, Great Neck, NY) via a broken-back pipette (10 to 20 \( \mu \)m) to either the DML region or to the pyramidal cell layer/VML region (Turner et al. 1994) (Fig. 1). Control applications of ACSF alone had no significant effects on evoked responses. Large microdroplets were used as global applications to try affect as much of the cell as possible. Small localized microdroplets targeted either the DML or the PCL to selectively affect receptors on the distal dendrites in the DML or on the soma and proximal dendrites respectively. Previous studies (Turner et al. 1994) have shown the myelinated StF to be a highly effective barrier to drug diffusion; this and direct visualization of droplet spread helped ensure the applications were local.

Results

Intracellular recordings were obtained from 12 pyramidal cells in the centromedial segment of ELL slices. No distinction was made between basilar and nonbasilar pyramidal cells as there is no anatomic evidence for differences in their molecular layer synaptology.

Response to activation of parallel fibers

We used both single and tetanic stimulation to assess PF effects on pyramidal cells and the control of these responses by inhibitory circuitry in the ELL. Although there is no physiological data for cerebellar (EGp) granule cells (origin of PF), we estimate that the in vivo frequency of PF activity may be between 30 and 120 Hz, as this is the spontaneous rate of its electroreceptive (Pd multipolar cells) and proprioreceptive input (Bastian and Bratton 1990). We chose tetanic stimulation frequencies of 100 Hz to allow comparisons with the results of StF stimulation (Berman and Maler 1998; Berman et al. 1997).

Graded stimulation of PF with a single pulse evoked an excitatory postsynaptic potential (EPSP) that triggered a spike when threshold was crossed (Fig. 2A). At higher stimulus intensities, the EPSP appeared to consist of two components with a fast and slow decay. The EPSPs peaked at 6.4 ± 1.8 ms (mean ± SE; \( n = 11 \)) but took \( >50 \) ms to decay completely. This was similar to the EPSPs evoked in the same cells by the more proximal StF excitatory synapses (5.6 ± 1.3 ms; \( n = 6 \), not shown) (see also Berman et al. 1997). The response to tetanic stimulation (100 Hz, 100 \( \mu \)S) of PF always consisted of individual EPSPs riding on a depolarizing wave that outlasted the stimulus by 100 to 200 ms. Spikes were triggered off the individual EPSPs peaks where they crossed spike threshold (see Fig. 2B, inset). Some of the depolarizing wave may be due to voltage dependency of the late phase of the EPSPs (Fig. 2C). Depolarizing the cell with current injection increased the amplitude of the late phase (Fig. 2C, arrow 3), but not the early peak of the PF EPSP (arrow 1), to the extent that a second peak appeared (at 8 ms: Fig. 2C, arrow 2). The inward rectification of the voltage-sensitive components of the EPSP (arrows 2 and 3) are illustrated in Fig. 2D. Note the relative constancy of EPSP peak 1 compared with the strongly rectifying EPSP amplitudes 2 and 3. This is similar to the voltage-dependent EPSPs generated by StF stimulation (Berman et al. 1997).

In all but one case (see below), there was no obvious evidence for IPSPs (neither at rest nor when the cell was depolarized) after PF stimulation, therefore the possibility that inhibition was masked by excitation was explored below.

Pharmacology of the PF response

GABA receptor and excitatory amino acid (EAA) receptor antagonists were used to characterize the PF response. The drugs were applied to the slice surface to reach the cell soma and most of the cell’s apical dendritic area; these applications are referred to as global.

GABA\(_{\alpha} \) inhibition. Global blockade of GABA\(_{\alpha} \) inhibition dramatically altered the amplitude of the depolarizing response to PF stimulation. The response to single stimuli (Fig. 3A) was increased by between 5 and 50% after SR (SR-95531: GABA\(_{\alpha} \)-antagonist) treatment, proportional to stimulus intensity. The difference between the control and SR responses revealed GABA\(_{\alpha} \) inhibition to be active throughout the excitatory response, suggesting that excitation normally masks the presence of concurrent inhibition. When tested on the response to tetanic stimulation (Fig. 3B), SR again increased the depolarizing response to the extent that spike threshold was reached (see \( V_{\text{rec} \max} \) case). Note that the increased depolarization was less evident in the hyperpolarized condition (Fig. 3B, bottom, −77 mV), presumably because the depolarizing wave is due mostly to summation of the slow, voltage-dependent phase of the EPSP (see Berman et al. 1997).
FIG. 2. Intracellular electrophysiology of parallel fiber activation in a pyramidal cell. A: graded excitatory postsynaptic potential (EPSP) responses to different intensities of stimulation of the parallel fiber pathway (100–800 μA, 100-μm steps). Highest stimulus intensity (thicker trace) evoked a spike (spikes truncated in this and all subsequent figures). B: response to tetanic stimulation (solid triangles, 100 Hz, 100 ms, 600 μA). Three spikes were evoked. Inset: expanded tetanic response. Note the EPSPs riding on a depolarizing wave. C: response to single stimuli while cell was polarized with injected current. There was evidence of a voltage-dependent EPSP(s) (arrows), but no inhibitory postsynaptic potential (IPSP). pf; parallel fiber pathway stimulus. D: plot of EPSP amplitude (at peaks arrowed in C) vs. prestimulus membrane potential from experiment in C. Note the 2nd and 3rd EPSP peaks are strongly voltage dependent (increasing in amplitude when cell was depolarized above −69 mV).

In one cell, PF stimulation evoked a clear IPSP (Fig. 3C, arrow: latency to peak 27 ms), presumably derived from both stellate and VML cell input. The IPSP was visible when the cell was depolarized as it had a reversal potential (−68 mV, plotted in Fig. 3D) just above resting membrane potential. Application of SR blocked the IPSP (Fig. 3D) but did not affect the preceding short latency EPSP (2.7 ms to peak, not shown).

**Blockade of EAA Transmission.** To test whether our stimulus protocols directly activated inhibitory fibers, we examined the effects of EAA antagonists (CPP and CNQX) on the PF-evoked response. When applied to drug-naïve or cells exposed to SR, EAA antagonism completely blocked the response to PF stimulation (n = 3). An example is shown in Fig. 3E; SR, applied first, enhanced the response to tetanic PF stimulation. Subsequent application of CNQX + CPP completely blocked the response to PF stimulation. This effect contrasts the effect of EAA blockade of the StF pathway (Fig. 3F) (Berman and Maler 1998b; Berman et al. 1997): SR enhanced the amplitude of a slow GABA_A IPSP (Fig. 3F, asterisk), which was unaffected by EAA blockade (was evoked by direct activation of GABAergic bipolar cell fibers) (see Berman and Maler 1998b).

**GABA_B Inhibition.** The PF pathway does not appear to evoke any significant GABA_B inhibition. As seen above, there is little evidence for a slow hyperpolarizing IPSP typical of GABA_B inhibition. Furthermore, application of a GABA_B antagonist (saclofen, n = 3) had little or no effect on the response to PF stimulation, even though the drug antagonized the StF-evoked slow IPSP in the same experiments. Saclofen had little effect on the depolarizing response to tetanic stimulation either after SR treatment (Fig. 3G) or on a drug-naïve cell (Fig. 3H). Note that the cell in Fig. 3H responded to StF stimulation with a slow IPSP (shown in Fig. 3F) that was antagonized by the same saclofen application that had little effect on the response to PF stimulation. An example is shown in Fig. 3F, asterisk).

**Effect of PF-evoked GABA_A inhibition on cell firing**

To assess the effect of GABA_A inhibition on the response of pyramidal cells to PF stimulation in the cells’ dynamic range (i.e., suprathreshold), we paired injected current with tetanic
stimulation of the PF. When hyperpolarized, the response to PF stimulation did not cross threshold (Fig. 4A, −0.5-nA trace and inset), unless GABA<sub>A</sub> inhibition was blocked. At rest, SR increased the spike rate during the tetanus. At depolarized levels, SR caused an increased spike rate both during and for ~200 ms after the tetanus (Fig. 4A, 0.2-nA thin trace, double asterisk). The estimated instantaneous firing rates (Fig. 4B, various currents) were enhanced by SR during the tetanus (shaded region) and remained elevated for a further ~200 ms; under control conditions the spike rate was depressed during this latter period. These experiments suggest that GABA<sub>A</sub> inhibition is controlling firing both during PF activation and for ~200 ms after the stimulus.

**Distal versus proximal GABA<sub>A</sub> inhibition**

The GABA<sub>A</sub> inhibition that was manipulated in the preceding experiments likely originated from stellate and VML cells (see Fig. 1) because they are the only known ELL interneurons that project to pyramidal cells and are contacted by PF in the upper portion of the DML. To parse the relative contributions of these two populations of interneurons to inhibitory control of the PF response, we selectively blocked distal, followed by proximal, GABA<sub>A</sub> receptors using focal applications of SR to the DML or the PCL (see METHODS). A range of stimulus intensities (50–900 µA) was used to activate the PF (range was adjusted to prevent overdriving the cell).

The cell in Fig. 5 responded with a typical EPSP to single shock PF stimulation (Fig. 5A). After SR was applied focally to the distal dendritic area (in the DML), the EPSP peak amplitude increased with a smaller increase in the decaying phase of the EPSP. SR then was applied (no recovery allowed) to the region of the cell soma in the PCL; the EPSP peak amplitude was unaffected by the drug, but the amplitude of the slow phase of the EPSP was increased greatly (effectively lengthening the duration of the EPSP). These effects were observed over a wide range of control EPSP amplitudes (Fig. 5B), including minimal EPSPs (0.1–0.3 mV). Thus distal blockade of GABA<sub>A</sub> inhibition affected mostly the early part of the EPSP, whereas proximal blockade affected the decay phase (7 ms onwards) of the EPSP.
To understand how these differential effects would affect the output of the cell, the response to PF stimulation during active spike firing was investigated in the same experiment. Figure 5C shows the response of the same cell to 0.3-nA current pulses that triggered spike trains. When PF was stimulated (100 Hz, 100 ms) during the train, the firing rate transiently increased; mostly during the early part of the tetanus (see Fig. 5D for plots of firing rate from C), and then was depressed for ~100 ms after the tetanus. After SR was applied to the DML, the firing rate was further increased during PF stimulation, mostly toward the end of the tetanus (Fig. 5, C and D). Blockade of distal GABA_A inhibition did not, however, block the spike rate depression that occurred just after the stimulus. Further application of SR to the PCL caused PF stimulation to greatly increase the firing rate both during the tetanus and for the following 100–200 ms. These effects were independent of the prestimulus firing rate (compare 0.3-, 0.4-, and 0.5-nA conditions), though the effect of SR applied to the DML was most obvious when the prestimulus firing rate was low (<20 spikes/s, 0.3-nA condition). Thus blockade of GABA_A receptors had a short-term affect during the tetanus when applied distally and a sustained effect when applied proximally.

The above effects of distal versus proximal GABA_A blockade were consistent for all cells tested (n = 7). Figure 6 shows the summary data from seven cells where GABA_A receptors were blocked distally (DML) and then proximally (+PCL). The data were normalized across the EPSP amplitude and stimulus intensity range. SR to the DML significantly (P < 0.05) increased the EPSP peak amplitude (Fig. 6, top) but had an insignificant effect on the decay phase of the EPSP (Fig. 6, bottom). SR to the PCL had the reverse effect, significantly (P < 0.05) increasing the decay phase of the EPSP with little additional increment to the EPSP peak.

The degree of inhibition evoked by PF stimulation varied from cell to cell; some cells (n = 3) experienced a net inhibitory response when PF was stimulated. The cell shown in Fig. 7 responded to single-pulse stimulation with a depolarizing EPSP that was affected by proximal and distal inhibition as described above (Fig. 7A). When tetanic stimuli were applied (Fig. 7B), the evoked depolarizing train of EPSPs did not evoke spikes at rest or when hyperpolarized. Note how the evoked response plateaus after five stimuli during the tetanus (~0.2- and ~0.4-nA control traces). SR applied to the DML caused a dramatic change in the tetanic response; the response no longer plateaued but ramped past spike threshold, triggering spikes (even with ~0.4 nA injected). SR applied to the PCL further increased the number of triggered spikes, and when the cell was at rest, caused an additional prolonged depolarization after the train (see +PCL trace at rest). Thus it seems that there was a strong and long-lasting (continuing for >200 ms after stimulation) underlying excitatory drive that was being controlled by
GABAₐ inhibition. This was particularly evident when depolarizing current was applied (Fig. 7, C and D); PF tetanic stimulation interrupted a current-evoked (0.1–0.4 nA) spike train. The spike rate took ≈200 ms to recover. The reduction of current-evoked spike rate was reversed to an increase in spike rate after distal GABAₐ blockade (Fig. 7, C and D). Further application of SR to the proximal region (PCL) mainly increased the spike rate in the 200 ms after the stimulus. Similar potent inhibitory effects of PF stimulation have been observed in vivo; PF stimulation reduces primary afferent-driven increases in pyramidal cell spike rates (Bastian 1997).

The above experiments demonstrated that PF-evoked inhibition could be very potent: sufficient to suppress spiking in the face of substantial currents (0.4 nA). The distal inhibition in the DML appeared to control the amplitude of the early phase of the EPSP and spike response during a tetanus, whereas proximal inhibition appeared to control the amplitude of the slow phase of the EPSP, its summation, and hence the sustained firing rate evoked by the pathway.

To control for possible saturation effects in the above experiments, the order of SR application was reversed (Fig. 8). In response to various stimulus intensities, SR applied first to the PCL (Fig. 8A) caused an increase in the EPSP decay phase duration and amplitude. There was some increase in peak EPSP amplitude in response to strong stimuli (≥350 μA). When SR subsequently was applied to the DML, there was an additional increase in peak EPSP amplitude (which then triggered spikes) but no effect on EPSP decay phase duration or amplitude. The same DML and PCL SR applications both increased the overall depolarization during and after tetanic stimulation (Fig. 8B), though their effects on individual EPSP size was different (this is explored below). When the effect of SR was tested on PF modulation of spike trains (Fig. 8C), SR applied first to the PCL enhanced the overall PF-induced increase in spike rate. There was an additional increment in this effect when SR then was added to the DML (the effect saturated at the tetanus frequency: 100 Hz). Hence the order of application did not alter the qualitative differences in proximal versus distal GABAₐ antagonism.

As a further control, the response to StF stimulation was monitored in the same experiment (Fig. 8C). SR applied to the PCL had a large augmenting effect on the StF-induced increase in spike rate (again this effect saturated at the tetanus frequency: 100 Hz) similar to the effect observed with DML stimulation. Subsequent application of SR to the DML + VML had, however, no further effect on spike rate.

**GABAₐ inhibition and PF synaptic depression**

In the previous experiment, there appeared to be a depression of the individual EPSPs evoked during a PF tetanus. This was examined more directly in experiments where the cells first were hyperpolarized to minimize spike contamination of the EPSP measurements (Fig. 9). During the tetanus, individual EPSPs rode on a depolarizing wave; presumably due to summation of the slow decay phase of the EPSP. Note that the overall depolarization plateaued after about four stimuli. The amplitude of the individual EPSPs in the response (measured from the prestimulus region preceding each EPSP) showed an increase in amplitude for the second
Further application of SR to the PCL did not further increase the EPSP amplitudes (though spike threshold was crossed earlier in the tetanus).

**DISCUSSION**

As predicated by anatomic studies (Maler and Mugnaini 1994), the descending feedback pathway (PF) terminating in the dorsal molecular layer evokes GABAergic IPSPs in pyramidial cells via molecular layer interneurons. These IPSPs control the gain and duration of the EPSPs and spike rate modulations caused by stimulation of this pathway. This study explored the relative effects of PF-evoked distal and proximal IPSPs and found significant differences in their control of EPSP gain and duration. The implications of this for gain control and filtering properties of these pathways are discussed with regard to their putative role in theories of electrosensory processing.

**Physiology of PF-evoked responses**

**EXCITATION.** The physiology of the PF-evoked response was very similar to that evoked by the more proximally terminating descending pathway, the StF (Berman and Maler 1998b; Berman et al. 1997; this study). The EPSPs evoked by graded stimuli peaked at similar latencies to StF EPSPs and appeared to be composed of more than one component: a voltage-insensitive component and one or more voltage-sensitive components (including a late depolarized tail). The latter increased in amplitude with depolarization; this is typical of an NMDA-receptor-like EPSP (Berman et al. 1997). Tetanic stimulation generally could evoke several spikes from rest, though some cells were inhibited overall by such stimulation. Each tetanic stimulus evoked a single EPSP pulse, but these were superimposed on a depolarizing wave that summated to a plateau early on during the train.

**INHIBITION.** The presence of inhibition in the PF response affected. When SR was applied to the DML, however, the amplitude of the overall depolarization and the individual EPSPs increased, and some of the synaptic depression was relieved to the extent that spikes were triggered.

For comparison, the behavior of the StF-evoked EPSPs were monitored during the same experiment in the same cell. StF EPSPs also showed facilitation for the first pair of EPSPs, but thereafter their amplitudes remained stable. SR applied to the PCL region increased the late phase of the EPSP and the overall depolarization evoked by the tetanus but had little effect on the peak EPSP amplitude (Fig. 9A, inset). SR applied subsequently to the molecular layer (VML region) caused a small increase in EPSP amplitude for only the first two EPSPs. This contrast between PF (synaptic depression) responses and StF (no synaptic depression) also occurs during stimulation of these pathways in vivo (Bastian 1997).

In a second example of PF synaptic depression (Fig. 9C), the decrease in EPSP amplitude during the train was overcome completely by removal of GABA_A inhibition. After SR was applied to the DML, the synaptic depression of individual EPSPs was blocked completely and replaced by synaptic facilitation: EPSP amplitudes increased during the tetanus. Further application of SR to the PCL did not further increase the EPSP amplitudes (though spike threshold was crossed earlier in the tetanus).

**NO GABA_B.** In contrast to their effects on StF responses (Berman and Maler 1998b), GABA_A antagonists did not reveal a slow IPSP in response to PF stimulation. This, together with the lack of effect of a GABA_B antagonist, suggests that GABA_A receptors are not involved to any significant degree in the inhibition evoked by PF. As stellate and VML cells are the only candidates for inhibition of pyramidal cells via activation of the PF in the DML, they therefore do so only via GABA_A receptors. This agrees with the in vivo findings that saclofen had no effect on the PF descend-
FIG. 7. Effects of distal and proximal GABA\(_A\) antagonism on a net inhibitory parallel fiber response. A: single stimulus-evoked EPSP had its peak increased by DML SR application and then its decay phase increased/prolonged by PCL SR application. B: response to tetanic stimulation at rest and at hyperpolarized potentials showed that after application of SR to the DML, the enhanced EPSP summated significantly, evoking spikes at all polarization levels. Subsequent PCL SR dramatically increased the spike rate during the tetanus but only enhanced the depolarizing wave evoked after the tetanus at resting membrane potential. C: stimulating PF (100 Hz, 100 ms, shaded region) during depolarization of the cell with injected current (0.3 nA shown) revealed a net inhibitory effect of PF activation (control) that was blocked by SR application to the DML to reveal the excitatory component of the response to PF stimulation. D: mean firing rates from experiment in C and the effects of SR on PF tetanus-evoked spike rate modulation (control). PF stimulation (100 Hz, 100 ms, □) slowed spike firing during and for 200 ms after the train. DML SR blocked this effect; PF stimulation then increased spike rate, mainly during the tetanus. Subsequent addition of SR to the PCL facilitated a further tetanus-induced increased spike rate, mostly in the 200 ms after the tetanus.

Distal versus proximal inhibition

DISTAL INHIBITION. Focal DML GABA\(_A\) antagonist application will affect the DML stellate cell input to pyramidal cell distal dendrites, leaving the vml cell inhibitory input to pyramidal cell proximal dendrites and somata intact. DML GABA\(_A\) blockade mainly increased the peak EPSP amplitude but had little effect on the slow decay phase. Bearing the circuitry constraints in mind, this suggests that stellate cell IPSPs are fast and short lasting and are coincident with PF-evoked EPSPs in pyramidal cells and that they exert potent control of EPSP gain (peak EPSP amplitude increased up to threefold after GABA\(_A\) receptor blockade).

A further conclusion that may be drawn is that under our stimulation protocols, stellate cell were activated at lower stimulus intensities than pyramidal cells. GABA\(_A\) antagonism increased the EPSP amplitude throughout the range tested even when there was no measurable EPSP amplitude in response to low stimulus intensities. This could be simply due to the fact that stellate cells have compact local dendritic arbor (Maler 1979) that are likely to be enveloped completely by the beam of parallel fibers activated by the stimulus electrode; the same beam will contact only a subset of
the total pyramidal cell dendritic spines because they span the full dorsoventral extent of the molecular layer (Maler 1979). The situation in vivo may be quite different as the spatial nature of the PF fiber activity might not be as concentrated as in our experiments. Although stellate cells therefore may not always be activated for a given descending input (subset of PF activated), when activated, their IPSPs always will overlap with PF-evoked EPSPs on pyramidal cell dendrites.

Bastian (1986a) clearly showed in vivo that the net effect of removal of EGp descending input (i.e., PF) was always an increase in pyramidal cell response to electrosensory input even at the lowest stimulus intensities that produced a significant change in response after the EGp lesion. This suggests that inhibitory cells are active even at these low stimulus intensities; this is consistent with our in vitro findings. In addition, PF stimulation reduces primary afferent-driven increases in spike rates in vivo, suggesting that the net effect of PF activity is often inhibitory (Bastian 1997). This is consistent with our results for tetanic stimulation where PF activation sometimes caused a net inhibition of current-evoked spike rate.

Note that Bastian (1986a) found that the spontaneous activity of pyramidal cells was decreased significantly by EGp lesion. Hence at low levels of PF activity with presumably spatially diffuse activity patterns, the net effect may be excitatory in vivo. The large apical dendritic tree will average all of the infrequent activity, whereas the small local stellate cell dendritic tree will sample a smaller fraction of the activity). Hence spatial aspects of PF activity may form a significant fraction of the information content of the descending feedback.

PROXIMAL INHIBITION. PF activity will recruit proximal inhibition via vml cells. When GABA_A receptors in the PCL
region were blocked, the PF-evoked EPSP peak was relatively unchanged, whereas its slower decaying phase increased in amplitude and duration. We conclude that proximal inhibition had a longer latency of onset and acts over a slower time course than distal inhibition.

If vml cells are responsible for control of the late phase of the EPSP, then the response to StF stimulation should show similar effects of GABA$_A$ receptor antagonism as vml cells lie in the StF termination field. This was indeed what we found when SR was applied first to the PCL region; only the late phase of the StF-evoked EPSP was increased. Subsequent application of SR to the molecular layer increased the amplitude of the EPSP peak, presumably where it could affect stellate cell synapses. These experiments are more difficult to interpret because StF stimulation probably recruits GC2 interneurons and the bipolar cell pathway (see Berman and Maler 1998b).

**Tetanus-evoked inhibition**

As was seen in the studies on primary afferent and StF-evoked inhibition (Berman and Maler 1998a,b), tetanic stimulation was far more effective at evoking inhibition that single stimuli. Although tetanic stimulation sometimes had a net depressive effect on excitability, more commonly PF stimulation had a net excitatory effect on spike rate, and GABA$_A$ antagonism was required to reveal the presence of inhibition. Inconsistencies in orientation of the cut slice and position of stimulating electrode may account for the response variation.

The effect of DML and PCL GABA$_A$-receptor antagonism on the response to PF tetanic stimulation was also variable. In general the effects were simply additive, though some temporal distinctions could be seen between DML and PCL applications. First, DML GABA$_A$ antagonism increased the spike rate in the early part of the tetanus, whereas PCL GABA$_A$ antagonism affected mostly the latter part of the tetanus. Second, both sites of application increased the slow depolarizing wave amplitude and duration, but only DML applications increased the amplitude of individual EPSPs. This mirrored the effects on single stimulus-evoked EPSPs. Thus removing DML inhibition increased firing probability by increasing the peak amplitude of individual EPSPs, whereas removal of PCL inhibition increased firing probability by increasing temporal summation of the EPSPs (by increasing their slow phase amplitude and duration). As these effects depend on EPSP and IPSP time course and DML inhibition appears to act over a shorter time span than PCL inhibition, changes in stimulation rate will vary the influence of PCL versus DML inhibition. At present the physiological firing pattern of EgP granule cells is unknown.

**FIG. 9.** Inhibitory control of parallel fiber synaptic depression. A: tetanic (100 Hz, 100 ms, triangles) stimulation of pf pathway evokes a train of EPSPs riding on a summating depolarizing wave (top). SR added to the PCL increases the overall depolarization of the response but had little effect on individual EPSP peak amplitudes (see bottom). Subsequent application of SR to the DML increased the individual EPSP amplitudes and the overall depolarization achieved. Bottom: individual EPSP amplitudes (measured against each prestimulus baseline) during the response to PF tetanus, before (control) and after SR application to PCL and DML regions. Note the overall depression of EPSP amplitude during the train. B: response to StF stimulation recorded in same cell and at the same time (3 s between PF and StF tests) as the experiment in A. EPSP amplitude increased from the 1st and 3rd stimuli, then stabilized for the remainder of the trains. SR to the molecular layer (DML + VML) increased the EPSP peak of the 1st and 2nd EPSP (inset and bottom) but had negligible effect on subsequent EPSPs; SR to PCL increased the slow phase of the EPSP (inset) and enhanced the depolarizing wave. C: different cell in which the synaptic depression during the response to PF tetanus reduced the individual EPSP amplitude to near 0. SR applied first to the DML dramatically increased the individual EPSP response revealing frequency potentiation. Additional application of SR to the PCL region (+PCL) had little further impact on the response (there appeared to have been some recovery of inhibition between the 2 drug applications).
FIG. 10. Functional summary of inhibition in the centromedial segment of ELL. Diagram is based on conclusions arising from this and the companion papers on ELL inhibition (Berman and Maler 1998a,b). The spatial extent of each inhibitory pathway (local or diffuse) is indicated. Present study suggests that DML stellate cells control the gain of PF EPSPs with a rapid short-lasting GABA<sub>A</sub> IPSP. This inhibition will quench transient excitation but will have no effect on sustained inputs, thereby acting as a high-frequency gain controller or low-pass filter. Similarly, the longer lasting GABA<sub>A</sub> IPSPs attributed to vml cells in this study suggest they control low-frequency gain or act as a high-pass filter. Note that the VML cells receive a GABAergic innervation (unknown source) (Maler and Mugnaini 1994); this implies that gain at low frequencies is itself controlled by local circuitry or feedback to ELL. For comparison, the temporal characteristics of the other inhibitory inputs investigated in the previous studies also are shown: bipolar-cell inhibition (mostly GABAB, some GABA<sub>A</sub>) will provide conditional inhibition of pyramidal cell electroresponsiveness; this is well suited to attentional mechanisms (acts as a novelty detector) (see Berman and Maler 1998b). Descending (StF) and ascending (primary) afferents will activate type 2 granule cells, which provides GABA<sub>A</sub> input to pyramidal cells. Temporal nature of this input depends on GC2 firing properties (see Berman and Maler 1998a) and is suitable for temporal filtering of electroreceptive inputs (e.g., controlling adaptation). Ovoid-cell input appears to act almost exclusively via GABA<sub>B</sub> receptors, which are associated with slow K<sup>+</sup>-mediated IPSPs; the suitability of this mechanism to its proposed role in common mode rejection (Berman and Maler 1998a) is unclear. Type 1 granule cells appear to provide a slow K<sup>+</sup>-mediated input to pyramidal cells, possibly related to surround inhibition.

COMPARISON WITH IN VIVO STUDIES. Bastian (1993) found that injection of bicuculline to the DML produced a modest increase in the response to step increase in electric organ discharge (EOD), probably via local blockade of stellate cell inhibition. In the present study, GABA<sub>A</sub> blockade in the DML greatly increased the response to PF tetani. Taken together, these findings suggest that there may be a nonlinear summation of PF and electroreceptor afferent input to pyramidal cells. A similar conclusion was reached by Bastian (1997) with reference to the non(sub)-linear interaction of primary afferent and PF input. At a longer latency, in vivo application of bicuculline caused a large increase in spike rate for several hundred milliseconds after the electroreceptive stimulus. This is similar to the long-lasting excitation after tetanic stimulation with global GABA<sub>A</sub> antagonism or after blockade of vml cell inhibition. When Bastian blocked GABA<sub>A</sub> transmission in the VML/PCL region, there was a large increase in the response to step EOD changes. This may be similar to the large increases in response to PF or StF tetani (Berman and Maler 1998b; this study) or even primary afferent tetani (Berman and Maler 1998a), as there was no way of determining which of the three pathways underwent a change in gain using natural stimuli in the in vivo preparation.

A further effect of GABA<sub>A</sub> antagonists in vivo was the loss of adaptive gain control; ELL basilar pyramidal cells lost their ability to adjust to overall changes in electric field when bicuculline was applied near the PCL (Shumway and Maler 1989). The PF pathway has been implicated in adaptive gain control (Bastian 1986b), hence GABA<sub>A</sub> blockade of stellate cell and/or vml input may underlie the loss of gain control in their experiments. The slower dynamics of the effect of vml inhibition favor this cell for modulation of slow time-course gain control. Modeling studies have shown how the PF may dynamically regulate pyramidal cell gain via inhibitory interneurons (Nelson 1994a; Payne et al. 1994).

Time-dependent plasticity of PF responses

A further striking feature of PF-evoked EPSPs was that after an initial facilitation (1st to the 2nd stimulus), there was depression of EPSP amplitude during the tetanus that
was somewhat relieved by DML GABA<sub>A</sub> receptor antagonism. By contrast, StF-evoked EPSPs did not show EPSP depression during tetanic stimulation. There are several possible explanations for these results.

**SUMMATING IPSPS DEPRESS LATER EPSPS IN THE TETANUS.** This is supported by the evidence that removal of inhibition (GABA<sub>A</sub> to DML region) reduces or blocks EPSP depression. This hypothesis would suggest that, contrary to our results, StF-evoked EPSPs also should depress because VML stellate cells should be activated by this input. We cannot explain this discrepancy, although it is possible that StF-evoked excitatory synaptic currents are much stronger than those evoked from PF and/or stellate cells in VML are subject to inhibitory input emanating from StF (this may be the case for vml cells) (Maler and Mugnaini 1994).

**CONDUCTION FAILURE.** The thin unmyelinated PFs may not be able to follow high-frequency tetani. Further analysis requires information on the physiological rates of cerebellar (EGp) granule cell activity.

**PRESYNAPTIC DEPRESSION.** Classical presynaptic depression also could be occurring because there was still significant depression even after GABA<sub>A</sub> antagonists were applied to both DML and PCL regions. There is anatomic evidence that depressing PF terminals differ from the nondepressing StF terminals in one important respect: PF terminals have low numbers of vesicles, whereas StF terminals are packed densely (Maler et al. 1981). Thus in PF there may be depletion of the immediately releasable vesicles, whereas in StF there may be rapid replenishment of immediately releasable vesicles from a larger reserve pool. This is consistent with Bastian’s (1997) findings that continued stimulation at frequencies >16 Hz resulted in depression of PF but not StF field EPSPs. Hence our results are not an artifact of the slice preparation. However, as PF firing rates in vivo are unknown, we cannot state whether this depression/depletion is physiological. If depression is presynaptic, then recent studies (Debanne et al. 1996; Dobrunz and Stevens 1997) imply that the probability of release will be high at PF boutons and low at StF terminals in VML.

**DESENSITIZATION OF AMPA AND/OR NMDA RECEPTORS.** During the tetanus, the decrease in EPSP amplitude may be due to desensitization/inactivation of the postsynaptic receptor. This has been demonstrated for AMPA receptors (Partin et al. 1996) and NMDA receptors (Legendre et al. 1993; Sather et al. 1992). If desensitization does account for PF EPSP depression, this implies that there is a dramatic difference between desensitization of ionotropic glutamate receptors in DML (desensitizing) and VML (nondesensitizing).

**PRESYNAPTIC INHIBITION.** The PF terminals may have presynaptic metabotropic glutamate or GABA receptors that reduce synaptic release; tetanic stimulation then may have a negative feedback effect on EAA synaptic release in DML. This has been demonstrated recently in mammalian cerebellar slices (Dittman and Regehr 1997); high levels of inhibitory interneuron (stellate cell) activity induced spillover inhibition of PF terminals via presynaptic GABA<sub>A</sub> receptors. This hypothesis may be tested in our system by using GABA<sub>A</sub> to block the putative presynaptic site and relieve synaptic depression. Presynaptic inhibition would be expected to increase paired pulse facilitation (Dunwiddie and Haas 1985). However, Bastian (1997) has shown that manipulations that depress PF transmission result in reduce paired pulse facilitation. This suggests that presynaptic inhibition did not underlie our results.

We propose that EPSP depression in the DML is a combination of postsynaptic summating inhibition, presynaptic depression, and perhaps postsynaptic receptor desensitization. Whatever the relative contribution of these factors, EPSP depression in DML may be a physiologically relevant normalization mechanism as recently proposed for both cerebellar (De Schutter 1995) and cerebral cortex (Abbott et al. 1997). The response to weaker inputs (low-frequency presynaptic release) would remain consistent over the input signal, whereas strong inputs (high-frequency presynaptic release) would decrement over the signal duration. This process normalizes the input, perhaps to keep the amplitude of the DML input within the dynamic range of the neuron (Abbott et al. 1997). The lack of depression of StF synapses in VML perhaps reflects the searchlight role of this pathway (Berman et al. 1997; Bratton and Bastian 1990; Maler and Mugnaini 1993, 1994) because depressing EPSPs during a train would not be effective in enhancing the response to moving electrolocation targets.

**Mechanisms and role of time course differences between VML and stellate cell inhibition.**

The underlying mechanism behind the different time courses of vml and stellate cell inhibition could not be directly determined in our experiments, but several factors may be relevant.

First, vml cells may fire more prolonged burst than stellate cells in response to PF stimulation. Bottai et al. (1997) have shown by in situ hybridization that vml cells have higher levels of NMDA NR1 subunit mRNA than stellate cells. The slower EPSP time course of NMDA-receptor currents may cause vml cells to fire for longer periods than stellate cells in response to the same input.

Second, postsynaptic GABA<sub>A</sub> receptor dynamics may be different. Differences in the subunit composition of proximal versus distal GABA<sub>A</sub> receptors may underlie differences in IPSP dynamics (Perez-Velazquez and Angelides 1993; Verdorn et al. 1990).

Third, electrotonic differences. There is evidence for differences between distal and proximal GABA<sub>A</sub> IPSP time courses in pyramidal cells in other preparations (hippocampus: Miles et al. 1996; Pearce 1993; pyriform cortex: Kapur et al. 1993). However, these studies cannot rule out the possibility that the differences are solely due to electrotonic distance. In the ELL this is unlikely as the more proximal IPSPs were the slowest; electrotonic filtering would predict the opposite (Rall 1967). In addition, GC2 (Berman and Maler 1998a) and bipolar-cell (Berman and Maler 1998b) GABA<sub>A</sub> IPSPs also are generated proximally (at the soma), yet have a rapid time course suggesting the second factor (preceding paragraph).

Fourth, different I<sub>NaP</sub> currents in soma versus dendrites: computer modeling (see APPENDIX) of the distal and proximal IPSPs showed that NMDA receptor and I<sub>NaP</sub> currents amplify differences in IPSP timings. However, asymmetries...
in dendritic versus somatic $I_{\text{st}}$ itself may underpin apparent differences in IPSP time course. For hippocampal pyramidal cells, Lipowsky et al. (1996) suggested that differences in dendritic versus somatic $I_{\text{st}}$ effect on EPSP time course and amplitude (dendritic $I_{\text{st}}$ mostly enhanced peak amplitude and not time course, whereas somatic $I_{\text{st}}$ enhanced the slow decay phase) may be due to different densities of $I_{\text{st}}$ in dendritic versus somatic compartments. In ELL pyramidal cells, sodium-channel density is greater on somatic versus dendritic membranes (Turner et al. 1994), hence some of the time-course differences between proximal and distal inhibition are likely due to the ability of proximal, but not distal, inhibition to block the activation of somatic $I_{\text{st}}$. It therefore appears that proximal GABA$_A$ receptor inhibition may act by regulating a voltage-dependent (inward) current rather than as a simple shunt (“divisive” element) (Vu and Krasne 1992). We have demonstrated previously that GABA$_A$-mediated inhibition is not merely subtractive but requires a complex interaction with voltage-gated (outward) currents and have proposed the term “conditional inhibition” to designate this phenomenon (Berman and Maler 1998b). We now propose that some GABA$_A$-mediated inhibition also may act in a “conditional” manner with $I_{\text{st}}$. Although it is well known that excitatory currents interact with $I_{\text{st}}$ (e.g., Schwindt and Crill 1995), this has been suggested only recently for GABA$_A$ IPSPs (Stuart 1997).

The reverse IPSP dynamics in our system (longer duration IPSPs are proximal rather than distal as in hippocampus and cortex) may reflect the matching of IPSP dynamics with the required function of the pathway. In the ELL, the vml cell receives input in both DML and vml, hence its slow time course of its IPSPs (on pyramidal cells) will be able to control simultaneously the amplitude of the slow phase of both StF and DML-evoked EPSPs and/or slow somatic inward currents ($I_{\text{st}}$). Stellate cells receive restricted local input in the DML, and their IPSP dynamics may be tuned to match and control the DML input on an impulse basis. The functional reason for the time course of dendritic versus somatic inhibition in cortex remains to be elucidated.

**IPSP dynamics and electrosensory filtering**

Irrespective of the mechanism underlying the differences in IPSP dynamics, we predict that vml and stellate cell inhibition will have distinct filtering roles in controlling descending input. Their filtering properties are discussed in relation to current models of feedback input in the ELL.

The diagram (Fig. 10) summarizes the relative dynamics of IPSPs originating from the principle inhibitory neurons in the ELL, as determined by the present and companion studies (Berman and Maler 1998a,b). In addition, the spatial characteristics of the neuron projection field within the ELL are indicated (local vs. diffuse; based on morphological data) (Maler 1979; Maler and Mugnaini 1994). For simplicity, the case for basilar pyramidal (BP) cells is considered.

**STELLATE CELLS.** The rapid onset, low threshold, and short duration of their IPSPs make stellate cells well suited to act as an adjustable gain, low-pass filter. When activated, these IPSPs will overlap short-lasting inputs (EPSPs, high-frequency inputs) and depress them. However, sustained inputs will outlast the inhibition and excite the cell. Control of stellate cell excitability (e.g., via the GABAergic boutons on their somata) will be able to change the gain of the low-pass filter. These features are well suited to the putative adaptive gain control role assigned to the EGP to ELL pathway; high-frequency signals will be damped by the stellate cell inhibition.

The confined nature of stellate cell termination field (Maler 1979) suggest that they may modulate specific sets of pyramidal cells or even subsets of pyramidal cell apical dendrites, thereby regulating the gain of particular beams of parallel fiber excitation (Maler 1979).

**vml CELLS.** These cells may have a dual function as they are activated by both PF and StF pathways. Their slower temporal characteristics make them suitable as high-pass filters; high-frequency inputs (EPSP peaks) will not be affected by vml inhibition, whereas slower or sustained inputs would be suppressed. Again, modulation of the vml cells themselves by inhibitory contacts on their somata will change the gain of this filter. Vml cells inhibitory dynamics would be suitable for a level-setting role in adaptive gain control (Maler and Mugnaini 1994) but still would allow the pyramidal cell to remain sensitive to strong rapid inputs, such as would be expected from the StF pathway in its putative role as an attentional searchlight (Bratton and Bastian 1990; Crick 1984; Maler and Mugnaini 1993,1994).

The division of the ELL into four topographic segments/maps (MS, medial; CMS, centromedial; CLS, centrolateral; LS, lateral) with specific signal-filtering properties enables us to correlate anatomic segmental differences with sensory-filtering functions. Of the maps receiving high-frequency input (tuberois electroreceptors), the LS has high-pass, and CMS low-pass filtering characteristics (Shumway 1989). The ratio of vml to stellate cells in the molecular layers of the segments correlates with the filtering characteristics of the maps. The LS has the highest vml to stellate cell ratio, whereas the MS has the lowest (Maler and Mugnaini 1994). This agrees with the filtering characteristics of vml and stellate cell inhibition suggested by the present study; i.e., vml: high-pass, stellate: low-pass. Further studies are required to determine whether the vml-cell- and stellate-cell-generated IPSPs have common dynamic properties in all segments, and whether the IPSP dynamic differences generate high-pass- and/or low-pass-filtering properties.

**General implications for the role of GABA$_A$ inhibition**

Computational analyses of inhibition often have described GABA$_A$ receptors as mediating shunting or “divisive” inhibition, whereas GABA$_A$ receptors supposedly underlie “subtractive” inhibition (Amthor and Gryzwacz 1991; Holt and Koch 1997; Koch and Poggio 1987; Nelson 1994b; Rose 1977). We already have discussed why the voltage dependence of both types of GABA receptors suggests that these ideas (subtractive and divisive inhibition) are simplistic (Berman and Maler 1998a,b).

These models generally discuss unsynchronized trains of EPSPs and IPSPs (typically trains with different parameterized Poisson statistics) (Tuckwell 1988), whereas our data in ELL suggest that there is a tight temporal linkage between EPSPs and IPSPs evoked by the same input: e.g.,
stimulation of primary afferents or feedback input always will evoke coupled EPSPs-IPSPs. A comparison of the EPSP-IPSP temporal linkage of primary afferent versus feedback (StF or PF) input also suggests that a richer computational model may be necessary. Primary afferent EPSPs have a very fast component that is completely unaffected by the delayed IPSP (Berman and Maler 1998a); only subsequent EPSPs will overlap with and be affected by the delayed IPSP. Presumably this allows GC2 IPSPs to make the pyramidal cell response phasic and to produce coincidence detection (Berman and Maler 1998a; Maler and Mugnaini 1994; Shumway and Maler 1989 for discussion) while still permitting strong inputs to activate pyramidal cells. Feedback EPSPs have a slower time to peak (compared with Berman and Maler 1998a; Berman et al. 1997) so that they coincide with the stellate and vml cell IPSPs. Therefore, as we have discussed above, stellate-cell-evoked IPSPs will act as high-gain feedback controllers and low-pass filters for PF EPSPs (but not PA EPSPs: see below).

These ideas are summarized in Fig. 11. In the DML, both the PF (due to NMDA component) and the stellate cell inputs (due to 
\[ \text{GABA}_A \text{-receptor chloride channels} \] ) are voltage sensitive (Berman and Maler 1998a; Segal and Barker 1984). In the DML, this will cause them to act in concert; e.g., when the cell is depolarized, both NMDA-receptor currents and 
\[ \text{GABA}_A \text{-chloride currents} \]  will be strongest. This scaling of synaptic strength may serve an important linearizing function. PCL GABA inhibition controls a slow voltage-sensitive conductance (persistent sodium); however, the voltage shift generated by this conductance is likely to be modest (a few millivolts) (Berman et al. 1997) and hence the slow time course of PCL (from vml cells) inhibition, rather than its voltage sensitivity, is likely more important in regulating excitability. The highly filtered input to DML (Sas and Maler 1987) is no longer synchronized to primary afferent EPSPs (Bastian and Bratton 1990). Therefore the shunting produced by both excitatory and inhibitory input in DML still may serve to globally regulate the response of a pyramidal cell to primary afferent input, as proposed by Nelson (1994b). Furthermore, if ELL stellate cell activity is spontaneous, or uncorrelated with PF input, then it would have a global effect on excitatory inputs via an increase in pyramidal cell membrane conductance and decrease in time constant, as recently suggested for cerebellar stellate and basket cells (Hütten and Clark 1997). Thus in ELL, the function of an inhibitory synapse may differ depending on its relation to excitatory input (synchronized or uncorrelated) and cannot be described readily with a single term. Presumably the far more complex circuitry of mammalian cortex implies an even greater complexity of inhibition.

**APPENDIX**

**Computer simulations of IPSP and EPSP interactions**

In experiments on DML PF stimulation, we found that DML EPSP peak amplitude was controlled mainly by DML inhibition, whereas soma/vML region inhibition controlled mainly DML EPSP duration. Our interpretation of this phenomenon included the hypothesis that soma/vML inhibition was slower than inhibition in the DML dendrites. This appendix describes a simple model in which this hypothesis could account qualitatively for the PF results.

In the model, the somatic inhibition was made slower and weaker than more distal inhibition in the apical dendritic compartment that received PF EPSPs. Simulations showed that this asymmetry was sufficient to generate the differential effects of simulated GABA antagonism at the soma versus the apical dendrites that were seen in the real cell.

A simple compartmental model neuron (Fig. 11) was constructed based on measurements of a bicucullin-filled ELL basilar pyramidal cell (R. W. Turner, personal communication), intracellular data on NMDA and AMPA receptor EPSPs evoked by parallel and StF fibers (Berman et al. 1997; this study), GABA, AMPA, and somatic persistent sodium current (I\( \text{NaP} \)) (Berman et al. 1997; Mathieson and Maler 1988; Turner et al. 1994).

**COMPARTMENTAL MODEL.** The 35 compartment model was mostly passive. Spike currents were not included. Membrane capacitance was 0.750 \( \mu \text{F} \cdot \text{cm}^{-2} \) and resistance 150 k\( \Omega \) \cdot \text{cm}^{-1}. Table A1 shows a list of currents and synaptic inputs. A passive leak current was applied to produce a input resistance of 20 M\( \Omega \) when measured from the soma with hyperpolarizing current. A persistent sodium current (De Schutter and Bower 1994) was added to the soma. AMPA and GABA synaptic inputs were modeled as simple alpha functions (Brown and Johnston 1983). The dendritic IPSP was given a similar time course to the AMPA receptor EPSP, whereas the soma IPSP was given a slower time course and smaller maximum conductance (\( G_{\text{max}} \)). The smaller somatic \( G_{\text{max}} \) was required to prevent total quenching of the input from the single dendritic compartment. See Table A1).
The NMDA-receptor conductance was made voltage dependent to simulate the voltage-dependent Mg$^{2+}$ blockade of NMDA EPSPs (Berman et al. 1997; Mayer et al. 1984). The NMDA voltage dependency was adjusted so that NMDA would be ~10% activated near in vitro resting potential (~70 mV) (see Berman et al. 1997). The NMDA-receptor activation equation of Zador, Koch, and Brown (1990) was used to simulate the voltage-dependent Mg$^{2+}$ block of NMDA conductance by using the Hodgkin-Huxley voltage-dependent gate formulation as follows

$$G(V, t) = G_{\text{max}} * M^4$$

$$\frac{dM}{dt} = (M_e - M)$$

$$M_e = \frac{1}{1 + \theta[Mg^{2+}]/(H)} \exp(-\gamma V)$$

with $[\text{Mg}^{2+}] = 1.2$ mM. To match the steep voltage dependence and low threshold of NMDA EPSPs recorded in ELL pyramidal cells (Berman et al. 1997), we set $h = 2.75 \times 10^{-7}$ M$^{-1}$ and $g = 0.06$ mV$^{-1}$ (cf. Zador et al. 1990; $h = 0.33$ mM$^{-1}$ and $g = 0.2$ mV$^{-1}$). The time course of NMDA synaptic activation was set as a double exponential (see Table A1).

The NMDA-receptor conductance was made voltage dependent and low threshold of NMDA EPSPs recorded in ELL pyramidal cells (Berman et al. 1997), we set $h = 2.75 \times 10^{-7}$ M$^{-1}$ and $g = 0.06$ mV$^{-1}$ (cf. Zador et al. 1990; $h = 0.33$ mM$^{-1}$ and $g = 0.2$ mV$^{-1}$). The time course of NMDA synaptic activation was set as a double exponential (see Table A1).

### TABLE A1. Synaptic and ionic currents incorporated in the compartmental model

<table>
<thead>
<tr>
<th>Current</th>
<th>$G_{\text{max}}$*</th>
<th>Time to Peak, ms</th>
<th>Reversal Potential, mV</th>
<th>Compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leak</td>
<td>0.05</td>
<td>—</td>
<td>−70</td>
<td>Soma, dendrite</td>
</tr>
<tr>
<td>NaP</td>
<td>5</td>
<td>—</td>
<td>45</td>
<td>Soma</td>
</tr>
<tr>
<td>AMPA</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>Dendrite‡</td>
</tr>
<tr>
<td>NMDA</td>
<td>10</td>
<td>3 (open)†</td>
<td>0</td>
<td>Dendrite‡</td>
</tr>
<tr>
<td>Fast IPSP</td>
<td>30</td>
<td>3</td>
<td>−70</td>
<td>Dendrite‡</td>
</tr>
<tr>
<td>Slow IPSP</td>
<td>5</td>
<td>15</td>
<td>−70</td>
<td>Soma</td>
</tr>
</tbody>
</table>

AMPA, $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, $N$-methyl-$\alpha$-aspartate; IPSP, inhibitory postsynaptic potential. * Units are in nS except for leak value, which is in mS·cm$^{-2}$; † Dual exponential with open and closed time constraints; ‡ See Fig A1 for location of synaptically active dendrite compartment.
Simulations of the effect of somatic and dendritic inhibition on the EPSPs. A: membrane voltage measured in the soma compartment at rest (bottom) and during current step injection (top). Legend indicates synaptic conductances included in each run. Note that the somatic IPSP was given a slower time to peak and smaller maximum conductance than the dendritic IPSP. Combined somatic and dendritic inhibition reduced the amplitude and duration of the EPSP. EPSP duration was reduced in simulation runs with only somatic IPSP included, whereas with only dendritic IPSP included, the main effect was to reduce the amplitude. This observation was clearer in the 0.2-nA cases. B: graphs of NMDA and NaP conductance profiles during the simulation runs in A. NaP conductance was the most sensitive to the different effects of dendritic vs. somatic IPSPs. In the 0.2-nA case, control of the EPSP duration (in A) by somatic inhibition was mainly due to the IPSP effect on the NaP.

Single EPSP/IPSP simulations

To model the experiments in which SR was applied to the soma and DML, the simulation was run with both somatic and dendritic IPSPs, with no IPSPs, or with either somatic or dendritic IPSPs. Of main interest was whether the experimental data showing the somatic high-pass versus dendritic low-pass gain control could be qualitatively replicated. The simulation results are shown in Fig. A2. When the combined NMDA/AMPA-receptor EPSP was exposed to dendritic inhibition, the EPSP was reduced in amplitude but less so in duration, especially in the depolarized case. Somatic inhibition reduced mainly the duration of the EPSP. In plots of conductance (Fig. A2B), the EPSP conductance durations are not much affected by inhibition, but rather it was the $\tau_{\text{soma}}$ prolongation of overall EPSP duration that was susceptible to weak somatic inhibition. Thus the slower, smaller somatic IPSP could exert substantial control over the duration of the EPSP via its action on the $I_{\text{NaP}}$ activated by the EPSP depolarization. This observation parallels the experimental evidence in vitro where blockade of somatic inhibition prolonged the duration of an EPSP generated in the apical dendrites (see DML stimulation experiments, Fig. 5). Note that with GABA$_A$ receptor not being made voltage sensitive, addition of distal inhibition produced similar degrees of enhancement at resting and depolarized membrane potentials (Compare to Fig. 3B to Fig. A2A).

Train simulations

The in vitro experimental data on train stimulation of PF showed that blockade of somatic region inhibition (with SR) increased the amplitude and duration of the compound EPSP (or depolarizing wave) but did not alter the amplitude of the individual EPSPs riding on the depolarizing wave. The model reproduced this phenomenon (Fig. A3). Simulations of train stimulation of dendritic input (100 Hz, no IPSPs) produced a large summating wave with EPSP peaks riding on the wave. Addition of the weak, slow somatic inhibition reduced the duration and amplitude of the slow depolarizing wave (or compound EPSP), while the individual EPSP peaks were not much affected. Addition of the strong, fast dendritic inhibition greatly reduced the amplitude of the individual EPSP peaks (and the overall depolarized level of the compound EPSP) but did not change the compound EPSP duration.

Model sensitivity to parameters

The above effects of the somatic IPSP scaled according to the size of $G_{\text{max}}$; increasing $G_{\text{max}}$ reduced EPSP amplitude, but the effect on the EPSP duration was then even more pronounced (under our experimental conditions, VML cells are likely to be less activated than stellate cells for a given PF input due to the diffuse versus compact spread of their respective dendritic fields. Hence the smaller somatic $G_{\text{max}}$ is probably more realistic). Decreasing the time to peak of the somatic IPSP made its effects more like the rapid dendritic IPSP but maintained its preferential effect on $I_{\text{NaP}}$ as long as time to peak of the IPSP was >8 ms. Changing $G_{\text{max}}$ for the inward currents had quantitative effects on the model performance, but the basic observations remained the same. Similarly, altering the steepness or threshold of the voltage dependence of the ionic currents reduced the magnitude but not qualitative characteristics of the overall effects.

Conclusions

The model demonstrates that given few assumptions about the excitatory and inhibitory inputs, the differential effects on duration and amplitude of EPSPs as seen in the electrophysiological experiments could be accounted for by simple control over the intensity and time course of somatic versus dendritic inhibition. In the ELL there are morphological characteristics that may underlie the assumption about inhibitory intensity; somatic or proximal dendritic inhibition recruited by parallel fibers are relayed most likely via the VML interneurons. These cells make GABAergic inhibitory contacts on the proximal dendrites and somata of pyramidal cells. Although the somatic region is invested heavily with GABAergic
boutons, a majority of these are from GC2 cells, which would not be activated by parallel fiber stimulation (their apical dendrites do not extend that far dorsal). This, combined with the fact that the CMS has the fewest number of vml cells of the segments (Maler and Mugnaini 1994), supports the assumption in the model that the amount of vml cell inhibition is relatively small.

Conversely, the number of stellate cells, the probable source of GABAergic input to pyramidal cell dendrites in the DML (Maler 1979; Maler and Mugnaini 1994) is high in the CMS. Thus the relative strength of stellate DML inhibition versus vml cell somatic/proximal dendrite inhibition is compatible with the assumption in our model. The lateral segment could serve as a test of these assumptions, as the ratio of stellate to vml cells is reversed. Here we would predict that vml cell somatic inhibition would have a marked effect on EPSP amplitude, whereas stellate cell IPSPs would have a smaller effect.

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