Inhibition Evoked From Primary Afferents in the Electrosensory Lateral Line Lobe of the Weakly Electric Fish

(*Apteronotus leptorhynchus*)

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Berman, Neil J. and Leonard Maler. Inhibition evoked from primary afferents in the electrosensory lateral line lobe of the weakly electric fish (*Apteronotus leptorhynchus*). *J. Neurophysiol.* 80: 3173–3196, 1998. The responses of two types of projection neurons of the electrosensory lateral line lobe, basilar (BP) and nonbasilar (NBP) pyramidal cells, to stimulation of primary electrosensory afferents were determined in the weakly electric fish, *Apteronotus leptorhynchus*. Using dyes to identify cell type, the response of NBP cells to stimulation of primary afferents was inhibitory, whereas the response of BP cells was excitation followed by inhibition. \(\gamma\)-Aminobutyric acid (GABA) applications produced biphasic (depolarization then hyperpolarization) responses in most cells. GABA\(_A\) antagonists blocked the depolarizing effect of GABA and reduced the hyperpolarizing effect. The GABA\(_A\) antagonists weakly antagonized the hyperpolarizing effect. The early depolarization had a larger increase in cell conductance than the late hyperpolarization. The conductance changes were voltage dependent, increasing with depolarization. In both cell types, baclofen produced a slow small hyperpolarization and reduced the inhibitory postsynaptic potentials (IPSPs) evoked by primary afferent stimulation. Tetanic stimulation of primary afferents at physiological rates (100–200 Hz) produced strongly summating compound IPSPs (~500-ms duration) in NBP cells, which were usually sensitive to GABA\(_A\) but not GABA\(_B\) antagonists; in some cells there remained a slow IPSP that was unaffected by GABA\(_A\) antagonists. BP cells responded with excitatory or mixed excitatory + inhibitory responses. The inhibitory response had both a fast (~30 ms, GABA\(_A\)) and long-lasting slow phase (~800 ms, mostly blocked by GABA\(_A\) antagonists). In some cells there was a GABA\(_A\)-antagonist-insensitive slow IPSP (~500 ms) that was sensitive to GABA\(_B\) antagonists. Application of glutamate ionotropic receptor antagonists blocked the inhibitory response of NBP cells to primary afferent stimulation and the excitatory response of BP cells but enhanced the BP cell IPSP; this remaining slow IPSP was reduced by GABA\(_B\) antagonists. Unit recordings in the granule cell layer and computer simulations of pyramidal cell inhibition suggested that the duration of the slow GABA\(_A\) inhibition reflects the prolonged firing of GABAergic granule cell interneurons to primary afferent input. Correlation of the results with known GABAergic circuitry in the electrosensory lobe suggests that the GABAergic type 2 granule cell input to both pyramidal cell types is via GABA\(_A\) receptors. The properties of the GC2 GABA\(_A\) input are well suited to their putative role in gain control, regulation of phasicness, and coincidence detection. The slow GABA\(_A\) IPSP evoked in BP cells is likely due to ovoid cell input to their basal dendrites.

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FIG. 1. Topography and relevant inhibitory circuitry of the electrosensory lateral line lobe (ELL). A: coronal section (cresyl violet stain) from the atlas of Maler et al. (1991; level T-7). ELL layers are approximately indicated for the segment investigated in this study: the centromedial segment. CCb, cerebellum; mol, molecular layer; SIF, stratum fibrosum; PCL, pyramidal cell layer; GCL, granule cell layer; DNL, deep neuropil layer; DFL, deep fiber layer. B: circuit diagram of pyramidal cell types and the relevant inputs that could be driven by stimulation of the DFL. Nonbasilar and basilar cells: both receive GABAergic input from type 2 granule cells (GC2) (Maler and Mugnaini 1994) and inhibitory (based on synapse ultrastructure) (Maler et al. 1981) input of unknown transmitter type from type 1 granule cells (GC1). Basilar pyramidal cells differ only in that their basilar dendrites receive GABAergic input along its length from ovoid cells and direct excitatory (glutamatergic) input from the primary afferents.

(see atlas of Maler et al. 1991 for location). The ELL consists of four independent segments, each containing a topographic electrosensory map (Carr et al. 1982; Heiligenberg and Dye 1982). One segment (medial segment: MS) receives only ampullary input, whereas the other segments (centromedial: CMS, centrolateral: CLS, lateral: LS) receive tuberous input (mostly P units). There are physiological and morphological differences among the segments (Metzner and Juranek 1997; Shumway 1989a,b; Turner et al. 1996) but all three tuberous segments contain similar canonical circuits of excitatory and inhibitory neurons and pathways (Fig. 1) (Fig. 2 in Maler and Mugnaini 1994). This and the companion studies (Berman and Maler 1998a,b) were conducted exclusively in the CMS; hence all results are interpreted for this segment only, as intrinsic cell properties and proportions of cell types vary across the segments. All four segments of the ELL have a similar laminar structure consisting of: a deep fiber layer (DFL) consisting mainly of electrosensory afferents, a granular cell layer (GCL) of interneurons, a pyramidal cell layer (PCL), and dorsal and ventral molecular layers (DML, VML) containing apical dendrites of pyramidal cells, interneurons, and feedback input from higher brain centers. The electroreceptor afferents terminate in a deep neuropil region (ventral to the granular cell layer) where they form chemical synaptic contacts with the basal bush of basilar pyramidal neurons, and mixed (chemical and gap junction) synapses onto dendrites of several types of putative inhibitory interneurons: type 1 granular cells (GC1; transmitter not identified), type 2 granular cells (GC2; GABAergic), ovoid cells (GABAergic) and polymorphic cells (GABAergic) (Maler 1979; Maler and Mugnaini 1994; Maler et al. 1981; Yamamoto et al. 1989).

The principal output neurons of the ELL are the pyramidal cells (Fig. 1). There are two morphologically and physiologically distinct types of ELL pyramidal neurons: basilar (BP) and nonbasilar pyramidal cells (NBP). BP cells (E units) receive direct glutamatergic synaptic input from tuberous afferents onto their basal dendrite and are excited by increases in EOD amplitude (Maler 1979; Maler et al. 1981; Saunders and Bastian 1984; Wang and Maler 1994); this cell type also receives an input from the GABAergic ovoid cell, which climbs on its basal dendrite as well as input from GC1 and GC2 cells onto its soma (Maler and Mugnaini 1994). NBP (I units) somata and somatic dendrites receive input from GABAergic and non-GABAergic granular cells (GC2 and GC1) and are excited by decreases in EOD amplitude (Maler 1979; Maler and Mugnaini 1994; Maler et al. 1981; Saunders and Bastian 1984). The descending excitatory and inhibitory inputs to pyramidal cells, which termi-
nate in the dorsal laminae of the ELL (Carr and Maler 1986; Maler and Mugnaini 1993, 1994) are discussed in the accompanying papers (Berman and Maler 1998a,b).

The responses of pyramidal cells to sensory input bear remarkable resemblance to those of mammalian sensory systems, such as the retina. ELL pyramidal cells have receptive fields that have center-surround antagonism (Shumway 1989a) and flexible gain control/contrast adaptation (Bastian 1986b, 1995, 1996). Maler and Mugnaini (1994) have attempted to relate ELL inhibitory circuitry to its sensory physiology. The inhibitory input to pyramidal cells from GABAergic GC2 was proposed to control the size and strength of receptive field centers (Maler and Mugnaini 1994). They further hypothesized that non-GABAergic input from GC1 contributes to surround regulation. Ovoid cell input to BP cells is thought to mediate common mode rejection (see Bastian et al. 1993; Maler and Mugnaini 1994). However, little is known of the mechanisms and dynamics of inhibition in the ELL. Such data are needed to assess the validity of these hypotheses. In this paper we have investigated the mechanisms of inhibition that may underlie the first stage of electroreceptor processing in the ELL. This study identifies the biophysical and pharmacological nature of inhibitory responses recorded in pyramidal cells in response to stimulation of the DFL. Our results show that BP and NBP cells can be identified by their response to DFL stimulation and that both GABA$_A$, and GABA$_B$ receptors are involved in their inhibitory responses. GC2 are likely to generate mostly GABA$_A$-receptor-mediated inhibition, whereas ovoid cell input is mediated partly by GABA$_B$$_A$$_A$$_B$$_B$$_A$$_B$$_A$_$$_A$$_B$$_B$$_A$_$$_A_$$receptors mediated inhibition.

MATERIALS AND METHODS

Slice preparation

Slices of ELL were prepared according to a modified method of Mathieson and Maler (1988) and Turner et al. (1994). Briefly, fish of either sex were anesthetized by immersion in water containing 0.2% 3-aminobenzoic acid ethyl ester (MS-222; Sigma provided all chemicals unless otherwise indicated). All surgical procedures were approved by the University of Ottawa Animal Care Committee. The fish were then transferred to a foam-lined clamp, and a glass tube was introduced into their mouth that supplied aerated water containing the same anesthetic. The brain was exposed by dissection of the skin and overlying skull while the surgery site was irrigated continuously with chilled artificial cerebrospinal fluid [ACSF, which contained (in mM) 124 NaCl, 3 KCl, 0.75 KH$_2$PO$_4$, 2 CaCl$_2$, 2 MgSO$_4$, 24 NaHCO$_3$, and 10 d-glucose]. Care was taken to avoid touching the ELL while underlying nerves were cut. The brain was blocked to ensure a true transverse section of the ELL by cutting the rostral face of the block at ~4° angle at the level of the optic tectum. The block was removed from the skull after severing the spinal cord, glued to a wide-mouthed plastic pipette and ejected into a holding chamber then stored in PBS overnight. The slices were resectioned on a vibratome at 80 µm, placed on glass slides, dried, and processed according to a modified Horikawa and Armstrong (1988) protocol. Background peroxidase activity was removed by pretreatment of

Histology and reconstruction

After recording, slices were allowed to incubate for ~1 h before fixation. Slices, which were left on individual pieces of lens tissue paper (Kodak, Rochester, NY), were removed from the chamber, placed on a glass slide, and had a droplet of fixative applied [4% paraformaldehyde 0.5% glutaraldehyde in 0.1 M phosphate buffered saline (PBS)]. Slices were postfixed at room temperature for 1–3 h and then stored in PBS overnight. The slices were resectioned on a vibratome at 80 µm, placed on glass slides, dried, and processed according to a modified Horikawa and Armstrong (1988) protocol.
slides with 0.3% H₂O₂ in PBS. Horseradish peroxidase was revealed by using a cobalt-nickel-intensified (Adams 1981) diaminobenzidine (0.2 mg/ml in 0.1 M sodium acetate buffer, pH 6.0) procedure. Treatment with 0.1% (in PBS, 30–90 s) Osmium tetroxide further darkened the reaction product. Sections then were dried, defatted, and cover-slipped. For Lucifer yellow visualization, slices were fixed with paraformaldehyde (4% in PBS). Sections were cleared using dimethyl sulfoxide to minimize shrinkage distortions (Grace and Llinas 1985). Sections were viewed on a fluorescence microscope (BH-2; Olympus America, NY). Pyramidal cells were identified as basilar versus nonbasilar on the basis of the presence of a basal dendrite (Maler 1979). For illustration, sections were scanned on a confocal microscope in 5- or 10-µm optical slices (×16 averaging). Final montages were made by merging all optical slices (Photoshop Version 5.0, Adobe Systems, CA) using the ‘‘lighten’’ algorithm (each pixel has the highest value sampled from each optical slice).

RESULTS

Biophysical parameters

Intracellular recordings were obtained from cells impaled in the PCL of ELL slices. This study concentrates on the inhibitory responses that are evoked by DFL stimulation; this inhibition must arise from activation of three inhibitory interneurons: GC1, GC2, and ovoid cells (Maler and Mugnaini 1994) are the only known interneurons that receive direct primary afferent input via glutamatergic and gap junction synapses (Maler 1979; Maler et al. 1981; Mathieson et al. 1987) and project to pyramidal cells.

Of the 101 cells impaled in the PCL, 15 cells were histologically identified as BP (n = 9) or NBP (n = 6). Based on their electrophysiological response to DFL stimulation (see next section), a further 78 cells were classified as either BP (n = 49) or NBP (n = 29). The remaining cells could not be assigned unambiguously to either group or were suspected to be polymorphic type cells (see following text) and were not included in the sample. The summary statistics of the pyramidal cells’ passive and active biophysical characteristics are presented in Table 1. There were no significant differences between the means of the BP and NBP cell groups. The passive parameters are unremarkable and are similar to those reported for ELL pyramidal cells (Berman et al. 1997; Mathieson and Maler 1988; Turner et al. 1994) and for pyramidal cells in the benchmark rat hippocampal slice preparation (Schwartzkroin 1975).

ELL BP and NBP spikes had a half-width (0.4 ms) narrower than typical cortical or hippocampal pyramidal cells (~0.5–0.6 ms) (Mason and Larkman 1990). Two cells, not included in Table 1, had very narrow spikes (half-width <0.18 ms), a large afterhyperpolarization (AHP; ~10 mV), and rapid accommodation of spike rate to injected current. These spike characteristics have been observed in identified polymorphic cells (Maler 1979; R. Turner, personal communication). Twelve cells probably were impaled in their dendrites because their current-evoked spikes decayed slowly [1.00 ± 0.47 (SE) ms], were wide (half-width 0.617 ± 0.147 ms), and lacked a fast AHP (see Turner et al. 1994).

Basilar and nonbasilar pyramidal cells can be identified by their response to DFL stimulation

MORPHOLOGY. Histological recovery of Lucifer yellow (LY)- or biocytin-filled cells were used to correlate their responses to DFL stimulation with their classification as BP or NBP cells. The cellular morphology and intracellular responses of a typical LY-filled BP cell is shown in Figs. 2 and 3. The soma of the BP cell straddled the pyramidal and plexiform layers. In this case the sparse somatic dendrites arose from the apical dendrite trunk within the pyramidal cell layer (the site of termination of granular cell axons) (Maler 1979; Maler and Mugnaini 1994). The axon can be seen leaving the soma and coursing horizontally through the plexiform layer (Fig. 2A). The thick apical dendrite courses through the stratum fibrosum and ramifies in the molecular layer. The thick basal dendrite courses ventrally through the plexiform and granular cell layers to form a compact but dense bush in the deep neuropil layer (Fig. 3C), where it receives excitatory input from primary afferents (Maler et al. 1981). Our fills demonstrated that the basal bush was far denser than seen in typical golgi impregnations (Maler 1979).

The NBP cell soma was situated within the pyramidal cell layer (Fig. 2A) and had a more extensive somatic dendritic arbor than the BP cell (cf. Fig. 3, A with B); this was typical of our sample and corresponds with our previous Golgi description of these cells [although the previous studies (Maler 1979; Saunders and Bastian 1984) did not allow full resolution of these fine processes]. Because these processes receive rich GABAergic input (Maler and Mugnaini 1994; Maler et al. 1981), this implies that NBP cells might have more potent inhibitory responses than BPs. The functional implications of placement of GABAergic inputs on these extensive and fine somatic arbors were further explored by

### Table 1. Summary of biophysical parameters of cells recorded in the ELL slice preparation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BP</th>
<th>NBP</th>
<th>All Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;m&lt;/sub&gt;, mV</td>
<td>−67.9 ± 6.2 (47)</td>
<td>−67.3 ± 4.5 (31)</td>
<td>−67.8 ± 5.7 (80)</td>
</tr>
<tr>
<td>R&lt;sub&gt;m&lt;/sub&gt;, MΩ</td>
<td>17.6 ± 4.4 (45)</td>
<td>16.3 ± 4.7 (33)</td>
<td>17.2 ± 4.8 (78)</td>
</tr>
<tr>
<td>τ&lt;sub&gt;m&lt;/sub&gt;, ms</td>
<td>15.7 ± 6.3 (42)</td>
<td>18.2 ± 6.4 (32)</td>
<td>16.6 ± 6.4 (74)</td>
</tr>
<tr>
<td>Spike parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>80.9 ± 10.3 (41)</td>
<td>77.7 ± 9.7 (31)</td>
<td>79.7 ± 10.0 (74)</td>
</tr>
<tr>
<td>Rise,* ms</td>
<td>0.299 ± 0.278 (40)</td>
<td>0.275 ± 0.107 (31)</td>
<td>0.296 ± 0.231 (73)</td>
</tr>
<tr>
<td>Decay,* ms</td>
<td>0.515 ± 0.3892 (40)</td>
<td>0.435 ± 0.353 (31)</td>
<td>0.453 ± 0.3709 (73)</td>
</tr>
<tr>
<td>Half width,† ms</td>
<td>0.454 ± 0.159 (40)</td>
<td>0.415 ± 0.170 (31)</td>
<td>0.432 ± 0.162 (73)</td>
</tr>
</tbody>
</table>

Number of observations in parentheses. All values are means ± SD. Nonbasilar (NBP) and basilar (BP) pyramidal cell means were not significantly different (Student’s t-test). ELL, electroreceptive lateral line lobe; V<sub>m</sub>, resting membrane potential; R<sub>m</sub>, input resistance; τ, cell time constant. * 10–90% rise or decay time. † Measured at half-height.

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computer simulation (see APPENDIX). The apical dendritic morphology of NBP and BP cells was indistinguishable (Maler 1979; Saunders and Bastian 1984).

PHYSIOLOGY. We recorded the intracellular responses of the same cells to single (Fig. 2B) and tetanic (200 Hz; Fig. 2C) stimulation of the primary afferents via an electrode in the DFL. This BP cell responded to a single stimulus with a short-lasting excitatory postsynaptic potential (EPSP) (<10 ms half-width, Fig. 2B, left) followed by a longer lasting IPSP; when it appeared, the IPSP was always associated with a preceding EPSP. The NBP cell responded with an IPSP alone (2 ms onset, 14 ms to peak, Fig. 2B, right). Postsynaptic potentials were classified as EPSPs if they were depolarizing at resting membrane potential ($V_m$) and, when the cell was depolarized with current injection, increased the probability of spike firing. The polarity of IPSPs depended on resting $V_m$; cells with large (approximately less than $75 \text{ mV}$) resting $V_m$ had depolarizing IPSPs that reversed with depolarizing current injection and reduced the probability of spike firing (see following text).

Tetanic stimulation (Fig. 2C) produced a summating series of EPSPs or compound EPSP in the BP cell and a summating series of IPSPs or compound IPSP in the NBP cell. The cells in Fig. 2 were tested both at rest and during injection of current (+0.2 nA).

The BP cell did not fire at rest, and tetanic stimulation (Fig. 2C, bottom) could evoke spikes only on the last few EPSPs in the tetanus; tetanic stimulation was effective in
producing six spikes in the depolarized condition (Fig. 2C, left, top). Higher stimulation intensities were capable of synapticly evoking spikes at rest both to single stimuli and to every stimulus in the tetanus (not shown). The depolarized condition (Fig. 2C, left, top) revealed a slow IPSP following the compound EPSP. Depolarizing current injection (≥0.6 nA) induced discharge in this cell that was blocked by the slow IPSP. The NBP also fired during current injection, and this discharge was completely suppressed both during the tetanus and during the evoked slow IPSP (>300 ms).

The preceding results were representative for nine BP and six NBP cells filled with either HRP or LY. BP cells always displayed a clear short-latency EPSP (0.96 ± 0.48 ms, n = 8) or compound EPSP to tetanic stimulation (3 of 3 cells tested), whereas filled NBP cells displayed a short-latency IPSP (2.8 ± 0.4 ms to peak, n = 4) or compound IPSP to tetanic stimulation (3 of 3 cells tested). In one filled NBP cell, we observed a short-latency depolarizing potential (0.4 ms to peak) that was presumably due to gap junction input from granular cells (Maler et al. 1981). The shape and amplitude of this potential remained constant at all membrane polarizations, unlike the EPSPs in BP cells the amplitudes of which were voltage sensitive (not shown).

**Pyramidal cell response to exogenous GABA and baclofen**

**RESPONSE TO GABA.** GABA was ionophoresed (1–10 s, 10–200 nA) within the PCL close to the intracellular recording site for 13 physiologically identified pyramidal cells while membrane potential and input resistance (R_{m}) changes were monitored. Both BP and NBP cells displayed similar responses to GABA at the resting V_{m} (Fig. 4); a rapid initial...
FIG. 4. Response of BP cell to γ-aminobutyric acid (GABA) application. A1: response to GABA at 3 membrane potentials (resting $V_m = -61$ mV). Input resistance ($R_{in}$; control = 22 MΩ) was monitored by injecting −0.5-nA current pulses (50 ms, 1 Hz) and measuring the resulting voltage deflections. -- -- --, control membrane potential. DC currents were injected into the cell to depolarize (top) and hyperpolarize (bottom) the cell from rest (middle). This and subsequent figures: short depolarizing voltage deflections were caused by 0.5-nA pulses used to monitor cell excitability (firing response). These deflections have been truncated digitally.

A2: normalized $R_{in}$ measurements derived from responses to hyperpolarizing current pulses in A1. B1: same data as in A, but with voltage deflections digitally removed and all 3 traces plotted on same voltage axis. This plot indicates ≥2 components to the GABA response. Middle: hyperpolarizing peak at 1 s after the onset of GABA. Bottom: depolarizing peak at 5 s. Markers indicate latency from onset of GABA application where measurements (plotted in B2) of the GABA response were made.

B2: measurements of the GABA response magnitude vs. the control holding potential indicate the approximate reversal potential (membrane potential where the interpolated GABA response would be 0 mV) of the early (●; −65 mV) and late (○; −62.5 mV) components of the GABA response.

**hyper- or depolarization followed by a lower amplitude sustained hyperpolarization.** The initial response was brief (0.5–4 s), and its polarity dependent on resting $V_m$ (Figs. 4 and 5). It was depolarizing in 8 of the 13 cells.

Polarizing the cell with injected current revealed that this initial response may be composed of more than one phase with reversal potentials near rest (Fig. 4B). The small hyperpolarization seen at resting potential reversed at −65 mV. A second phase appeared as a later depolarizing peak (Fig. 4B; depolarizing peak, ○) with a slightly more depolarized reversal potential (−62.5 mV). The peak of the second component coincided with the greatest decrease in $R_{in}$ (Fig. A2). After the iontophoretic current was turned off, a small hyperpolarization (0.5–1 mV) persisted for ≥15 s. The slow response, which outlasted the GABA application, was of longer duration (>4 s) and did not reverse polarity even in the most hyperpolarized traces (<−86 mV; Fig. 5).

GABA produced a large decrease in $R_{in}$ throughout the applications irrespective of whether the initial response was hyper- or depolarizing (Figs. 4–6); the postapplication hyperpolarization was accompanied by a much smaller decrease in $R_{in}$ (Figs. 4 and 5). The conductance change underlying the GABA response was strongly voltage dependent (e.g., Fig. 4, A2); in this experiment, $R_{in}$ decreased by ≤2.5 MΩ when the cell was hyperpolarized and GABA applied, whereas when the cell was depolarized to −55 mV, $R_{in}$ decreased by as much as 7 MΩ during the GABA application. Although all pyramidal cells showed some voltage dependency (rectification) of their steady-state $R_{in}$, this could not account for the voltage dependence of the GABA response. Indeed, the hyperpolarizing response to GABA (most depolarized case) would offset the intrinsic voltage dependency of $R_{in}$, therefore voltage dependence of the GABA response may be underestimated. The cell in Fig. 4 was typical in that control $R_{in}$ decreased with depolarization and vice versa (compare control period voltage deflections at the 3 membrane potential levels). This rectification was probably due to the voltage dependence of intrinsic currents (e.g., $I_h$ and $I_{Na}$) (see Mathieson and Maler 1988) and is not discussed further. The change in $R_{in}$ produced by GABA followed iontophoretic current, ejection time, and proximity of the drug electrode to the impalement site; $R_{in}$ became unmeasurably low with large GABA ejection parameters.

**EFFECT OF GABA A ANTAGONISTS ON THE GABA RESPONSE.** To determine whether the responses to GABA were due to activation of GABA$_A$ receptors, bicuculline (70 μM) or SR (50 μM) were applied by microdroplet to the pyramidal cell
layer prior to iontophoresis of GABA (Fig. 5). The efficacy of the antagonists was apparent after several seconds by the appearance of membrane potential oscillations (Turner et al. 1991). Both GABA<sub>A</sub> antagonists blocked or substantially decreased the initial (hyper- or depolarizing) part of the GABA response (n = 8). In the example in Fig. 5, the GABA response was initially depolarizing followed by a longer lasting hyperpolarization. Note that the cell’s resting potential was −74 mV and was not polarized (with injected current) beyond the GABA depolarizing response’s reversal potential (−56 mV by extrapolation). SR blocked the depolarizing response but did not affect the longer hyperpolarization (Fig. 5B1) the extrapolated reversal potential of which was −100 mV. This hyperpolarization, which under SR block started at the onset of the GABA response, probably was masked by the depolarization under control conditions. The R<sub>in</sub> changes underlying the GABA responses (Fig. 5, A2 and B2) showed typical strong voltage dependency. The SR block of the depolarizing response was associated with a block of the early peak conductance shift seen in control conditions (1st → in Fig. 5, A2 and B2), while the second peak in the conductance shift was relatively unaffected by SR (2nd →, Fig. 5, A2 and B2).

GABA<sub>B</sub>-MEDIATED RESPONSES TO GABA AND BACLOFEN. Because the slow GABA-evoked hyperpolarization was insensitive to bicuculline or SR, GABA<sub>B</sub> antagonists (saclofen or phaclofen) and an agonist (baclofen) were tested on the pyramidal cells. Figure 6 shows one such experiment; in this cell (which displayed a small oscillation of membrane potential under control conditions) both components of the GABA response were hyperpolarizing at rest. Treatment with saclofen (2 mM) curtailed mainly the duration of the hyperpolarization. Conductance measurements showed a decrease in the magnitude of the GABA-induced conductance increase (Fig. 6B, · · ·). Treatment with both SR and saclofen before the GABA application blocked both the voltage and conductance shifts. Thus at least part of the GABA response was sensitive to the GABA<sub>A</sub> antagonist. GABA<sub>B</sub> antagonists clearly affected part of the GABA response in only 5 of 12 cases.

The GABA<sub>B</sub> agonist baclofen consistently (n = 13) produced a small (1−2 mV) hyperpolarization and increase in spike current threshold (not shown). The conductance change underlying the response was either small (≈1 MΩ) or undetectable. This is expected because the effect of rectification with hyperpolarization (which would tend to increase R<sub>in</sub>) would cancel the small conductance increase associated with the response to baclofen. Treatment of the slice with microdroplets of saclofen (n = 3, 2 mM) was unable to antagonize the response to baclofen. In the example shown in Fig. 6, the cell depolarized slightly (by 4 mV) after saclofen treatment; this actually increased the magnitude of the response to baclofen.

These experiments demonstrated that both types of pyramidal cells respond with both GABA<sub>A</sub> and GABA<sub>B</sub>-like potential and conductance changes and pharmacology, as seen in mammalian pyramidal cells in vitro (hippocampus: Alger and Nicholl 1979, 1982; Scharfman and Sarvey 1988b; neocortex: Scharfman and Sarvey 1988a). Thus although a molecular characterization is presently lacking, we conclude that ELL pyramidal cells (BP and NBP) have both GABA<sub>A</sub> and GABA<sub>B</sub> receptors; traditional GABA<sub>A</sub> antagonists are
FIG. 6. Response of pyramidal cell to GABA and baclofen in the presence of SR314 and saclofen. A: GABA (76 nA, 10 s) applied during control conditions produced a strong hyperpolarization of the cell. After a microdroplet application of saclofen (2 mM), the response to GABA was diminished (middle). When SR314 also was applied to the pyramidal cell layer, the GABA response was blocked almost completely. Note the presence of spontaneous slow membrane potential oscillations during control and saclofen conditions. SR314 enhanced the oscillations causing the cell to fire action potentials on the depolarizing phase. Action potentials are truncated in the figure. B: $R_m$ measurements derived from the data in A. Saclofen reduced the $R_m$ change during GABA applications. Under saclofen and SR314 conditions GABA did not change $R_m$ appreciably. Oscillations caused fluctuations in control $R_m$ that were accentuated during the SR314-induced large amplitude oscillations. Saclofen alone had no effect on the oscillations. C: under control conditions, 20-s iontophoresis (150 nA) of baclofen caused a slow hyperpolarization from rest (---). Prior application of saclofen did not antagonize the baclofen response (bottom). D: change in $R_m$ during the baclofen responses in A. Baclofen caused a small decrease in $R_m$ (~0.5 MΩ) that, if anything, was enhanced by the presence of saclofen.

highly effective in the ELL, whereas the GABA_B antagonists (saclofen and phaclofen) appear to have a relatively low potency.

EFFECT OF BACLOFEN ON INHIBITION. GABA_B receptors have been shown to decrease presynaptic release of GABA in other systems (Thompson and Gähwiler 1992). This was tested in the ELL by observing the effect of baclofen treatment on the inhibition evoked by DFL stimulation (NBP cells, $n = 3$). Under control conditions, tetanic stimulation produced strong hyperpolarizing inhibition (Fig. 7A, →). The cell was current-clamped at various potentials to monitor the efficacy of the inhibition. After baclofen treatment, DFL stimulation did not evoke strong inhibition. To test whether the baclofen-induced postsynaptic decrease in $R_m$ could account for the disappearance of the stimulus-evoked inhibition, the cell was depolarized to levels that previously had produced strong repetitive firing (Fig. 7, B and C). Under control conditions, the stimulus-evoked inhibition potently reduced the firing rate of the cell (Fig. 7C, →), however, after baclofen treatment, it had only a small inhibitory effect on spike rate (Fig. 7, B and C, right). We conclude that GABA_B receptors are likely to be present on GABAergic nerve terminals in the PCL.

Electrophysiological and pharmacological characteristics of synaptically evoked inhibition in NBP cells

ELECTROPHYSIOLOGY OF NBP CELL INHIBITION. As shown in Fig. 2, the response of NBP cells to DFL stimulation was inhibitory. IPSPs could be classified into at least two categories, fast and slow. Fast IPSPs peaked at between 1.8 and 25 ms (mean $8.4 \pm 8.7$ to peak, $n = 28$), whereas slow IPSPs peaked at between 150 and 600 ms (mean $501.3 \pm 371.7$ ms to peak, $n = 20$) and endured for $\approx 2$ s. The slow IPSP was absent in 26% of NBP cells. The fast IPSP reversed at $-71.1 \pm 7.1$ mV ($n = 8$); this is more negative than the reversal potential of the early phase of the response to GABA application (~65 mV; recall that the early phase of the response to exogenous GABA lasts $>500$ ms and may be a combination of $\approx 2$ components). The slow IPSP reversed
at $-83.9 \pm 6.5$ mV, which is very negative to the GABA$_A$ (antagonist sensitive) hyperpolarization caused by exogenous GABA. The probably mixed origin of the slow IPSP is discussed later (see GABA$_A$ ANTAGONISM).

In vivo, the probability coding receptors (P units) of high-frequency wave species have high spontaneous firing rates ($\sim 200-400$ spikes/s) and stimulus-evoked firing rate increases of several hundred spikes per second (Bastian 1981a; Xu et al. 1996). Although single stimulus pulses could evoke IPSPs in the slice preparation, more physiological tetanic stimulation ($100-200$ Hz) was far more reliable in evoking both fast and slow IPSPs. An example of a NBP cell that responded well to both single and tetanic stimulation is shown in Fig. 8. Single-pulse stimulation at rest evoked a hyperpolarization that appeared to consist of at least two components, a fast and a slow IPSP (Fig. 8, A and C). The fast IPSP was strongly inhibitory; it prevented spike firing during depolarization, albeit for only a few milliseconds. The slow IPSP was only weakly inhibitory. Tetanic stimulation, however, produced a summating IPSP that was strongly inhibitory; it prevented firing in the face of strong depolarizing currents ($\pm 0.6$ nA).

The slow IPSPs were variable in duration and effect. Figure 9 shows a NBP that was spontaneously active. Single-pulse stimulation (Fig. 9A) evoked a slow IPSP that was moderately inhibitory (some spikes “broke” through after a few hundred milliseconds). Tetanic stimulation (Fig. 9B) evoked a large, strong summating IPSP that prevented spiking for $\approx 1.5$ s. Note the tight clamping of the membrane potential during the early part of the summating IPSP.

**Pharmacology of NBP Cell Inhibition.** NBP cells receive inhibitory input from at least two cell populations, GC1 and GC2, of which the latter are GABAergic (Maler and Mugnaini 1994). GABA$_A$ (bicuculline, picrotoxin, or
After bicuculline was applied, the EAA transmission was tested on NBP cell tetanus-evoked IPSPs were blocked almost completely and the tetanic responses to single stimulus (5 trials superimposed). Cell exhibited spontaneous and somatic dendrites (Maler et al. 1981) and the voltage was +70 mV for the fast IPSP and +85 mV after bicuculline, suggesting it was K⁺-mediated. This explains the generally very negative reversal potentials of the drug-naive slow IPSP: it is a mix of Cl⁻-mediated responses (GABA_A; reverses at -62 with exogenous GABA and -70 mV for the fast IPSP) and K⁺-mediated (unknown transmitter, likely GC1 in origin) responses.

In some NBP cells (n = 3), not only were the IPSPs antagonized, but a depolarizing or excitatory component was evoked by DFL stimulation after bicuculline application. This took the form of a slow depolarization (Fig. 11; n = 2) or spikes activated at very short latency (formed part of the stimulus artifact, n = 1). The cell shown in Fig. 11 responded to DFL stimulation with IPSPs alone. The resting membrane potential was very negative (-74 mV), therefore the short-latency IPSP evoked by a single DFL stimulus was depolarizing (Fig. 11, inset, control trace). This depolarizing IPSP and the compound IPSP evoked by tetanic stimulation (Fig. 11A, control) were abolished completely by bicuculline (Fig. 11B, bicuculline).

Under these conditions, the tetanic stimulus evoked a slowly depolarizing potential; this potential was clearly voltage sensitive. We attribute these responses to the electrotonic synapses made by dendrites of GC1 and GC2 cells onto NBP somata and somatic dendrites (Maler et al. 1981) and the voltage-sensitive persistent Na⁺ currents present in these cells (Matheson and Maler 1988; Turner et al. 1994). The rarity of this response is presumably also due to the low density of these connections (Maler 1979) and the difficulty of activating them in the slice preparation. This interpretation must however still be experimentally confirmed.

It has been suggested that gap junction input from granule cells and disinhibition may be responsible for NBP cell responses (Maler et al. 1981; Shumway and Maler 1989); however, the response of NBP cells to DFL stimulation when inhibition was blocked was at best only weakly excitatory in our experiments. Our data predict that other excitatory inputs, perhaps descending feedback, must be contributing to the excitatory responses of NBP cells seen in vivo.

GABA_A ANTAGONISM. The effects of GABA_A antagonists (saclofen and phaclofen) were tested on IPSPs evoked in NBP cells (n = 7). Only one cell showed any sensitivity to the antagonists, and even then the effect was marginal (Fig. 11, C and D). The tetanic stimulus produced a large compound IPSP. Several trials are shown to indicate the variability in the response. After 2 mM saclofen application, the IPSP showed a small reduction in duration. In all other NBP cells tested, the antagonists failed to significantly alter the time course or amplitude of IPSPs evoked by tetanic or single stimuli. We conclude that there is no significant GABA_A component to the NBP cell IPSPs evoked via primary afferent stimulation.

EFFECT OF EXCITATORY ANTAGONISTS ON NBP CELL INHIBITION. The effect of blockade of excitatory-amino-acid (EAA) transmission was tested on NBP cell tetanus-evoked responses (GABA_A, left, →) that inhibited cell firing (Fig. 10, A, top 2 traces, and B, ——). After bicuculline was applied, the IPSPs were blocked almost completely and the tetanic stimulus caused a much smaller reduction of the firing rate evoked by current injection. In another NBP cell (Fig. 10C), bicuculline substantially reduced the size of the IPSPs, but there remained a significant slow IPSP that resisted further attempts at antagonism, either with additional applications of bicuculline or with saclofen (data not shown). The reversal potential of this slow IPSP shifted from -78 to less than -85 mV after bicuculline, suggesting it was K⁺-mediated. This explains the generally very negative reversal potentials of the drug-naive slow IPSP: it is a mix of Cl⁻-mediated responses (GABA_A; reverses at -62 with exogenous GABA and -70 mV for the fast IPSP) and K⁺-mediated (unknown transmitter, likely GC1 in origin) responses.

1 Note that in his in vivo intracellular study of NBP cells, Bastian (1993) showed that the cells can be excited moderately by stimulation of commissural avoid cell fibers, presumably via disinhibition of granule cells. This is consistent with morphological evidence for a projection from ovoid cells to granule cell interneurons (Maler and Mugnaini 1994). Under conditions shown to isolate ovoid cell activity, we failed to observe such an excitatory effect (e.g., Fig. 12). Presumably granule cells are not (or less) spontaneously active in vitro and hence would not be susceptible to disinhibition.
FIG. 10. Effect of GABA<sub>A</sub> antagonist bicuculline on NBP cell IPSPs. A: control responses (left) of a NBP cell to tetanic stimulation of DFL (200 Hz) were obtained during current injection (0.45-, 0.15-, 0-, 0.15-, 0.3-, 0.45-, and 0.6-nA step pulses). --- , resting membrane potential (~69 mV). →, strong compound IPSP. After bicuculline was applied to the slice surface and the responses retested (right), the compound IPSP was abolished except for a small component (0.3 nA trial, →). Effect of bicuculline on the capacity of the compound IPSPs to inhibit cell firing is measured from the traces obtained during depolarizing current injection (0.45 and 0.6 nA). Instantaneous firing rates during control and bicuculline conditions are plotted in B. Under control conditions (→), the cell showed some adaptation to current injection (slow decline in firing rate over time). At the onset of the tetanic stimulus (t = 0), the evoked IPSP reduces the firing rate, strongly during 0.45-nA injection and weakly during 0.6-nA injection. After bicuculline application (→), the tetanic stimulus caused a much smaller reduction in firing rate (0.45 nA; compared with current injection alone, not shown). C: response of NBP to tetanic stimulation before and after bicuculline application; in this cell there was a substantial (~25%) component of the slow IPSP that was insensitive to bicuculline. Bicuculline caused the reversal potential of the slow IPSP to shift from ~78 to less than ~85 mV.

FIG. 11. Effect of GABA antagonists on NBP cell IPSPs. A and B: GABA<sub>A</sub> antagonist bicuculline. A: cell responded to single-pulse stimulation with a reversed (depolarizing) IPSP (see inset, control trace) at rest (~74 mV). Current injection during control conditions shows the compound IPSP to reverse a few millivolts above resting potential (main plot, →, control traces on left). B: application of bicuculline not only completely abolished the compound IPSP but revealed a slow depolarizing potential. Note that bicuculline blocks the depolarizing IPSP in the single-stimulus pulse experiment (A, inset). C and D: GABA<sub>B</sub> antagonist saclofen. Cell displayed spontaneous oscillations. C: tetanic stimulation (200 Hz, ◊) produced a robust long-duration IPSP (control trials, left). D: after saclofen application, the IPSP showed a small reduction in peak amplitude and duration. Approximate resting potential was ~70 mV (~--~).
IPSps. Non-NMDA (CNQX) and NMDA receptor antagonists (CPP or APV) were applied to the slice surface. The NBP cell in Fig. 12 responded to tetanic stimulation of the DFL with a strong compound IPSP. After CNQX and APV (1 mM) application, the IPSP mostly was abolished; during the stimulus period (Fig. 12, right, □), there remained a small inhibitory effect on current-evoked spiking. CNQX, with or without CPP/APV, reduced (n = 3) or eliminated (n = 2) the IPSPs produced by tetanic stimulation. Hence the inhibition evoked via primary afferent stimulation is mostly due to EAA-mediated synaptic activation of granule cell interneurons.

Electrophysiological and pharmacological characteristics of synaptically evoked inhibition in BP cells

BP CELL INHIBITION. Electrophysiology. Cells were classified as BP cells if their response to DFL stimulation included a depolarizing and excitatory potential (EPSP, single stimulus) or a compound EPSP in their response to tetanic stimuli. As in NBP cells, tetanic stimulation was more effective than single stimuli at producing the strongest response. A representative range of responses to DFL stimulation is shown in Fig. 13.

The cell in Fig. 13A (1–3) responded mostly with excitation to both single (with a bimodal EPSP) and tetanic stimulation. The tetanic stimulation was most effective, evoking several spikes (Fig. 13A3). However, in most cells, IPSps also were evoked by single and tetanic stimulation (e.g., Fig. 13, B and C). The IPSpS appeared to curtail the summating EPSPs (e.g., Fig. 13B), resulting in a net hyperpolarization. The interaction of EPSPs and IPSps (clearly separable in the response to single pulses, Fig. 13C1) could produce quite complex responses. For instance, in the cell in Fig. 13C, a short tetanus of five pulses had a net inhibitory effect (Fig. 13C, 2 and 3) that prevented cell spiking, whereas tetani with more than five pulses (Fig. 13C4) were strongly excitatory.

In cells in which a single EPSP could be discerned after a single DFL stimulus, the first EPSP peaked at 1.2 ± 0.8 ms (n = 50). This was followed by a decaying depolarization (27 cells), which in some cells had a clear later EPSP peak at 18.8 ± 22.4 ms (n = 14) or by a clear early IPSP after the EPSP (23 cells), similar to the cell in Fig. 13C1. These early IPSps peaked at 8.7 ± 7.6 ms (n = 6) and reversed at 71.7 ± 8.4 mV (n = 8). Slow IPSps, such as those in the cells in Fig. 13, B and C, peaked at 197 ± 102 ms (n = 17) and reversed at −88.5 ± 0.52, with a mean duration of 813 ± 396 ms (n = 15). Compared with NBP cells (see Electrophysiological and pharmacological characteristics of synaptically evoked inhibition in NBP cells), BP-cell IPSp characteristics (early and late IPSps) were not significantly different except for the slow IPS duration, which was significantly shorter in NBP cells (NBP cell slow IPS duration was 501 ± 371 ms, n = 17, P < 0.05). To determine whether the BP-cell IPSps evoked by DFL stimulation showed pharmacological sensitivities similar to NBP cell IPSps, the effects of GABA antagonists and EAA antagonists were tested on the BP-cell responses.

Pharmacology. 1) GABA A antagonism. The effects of GABA A antagonists were tested on the response of 10 BP cells to DFL stimulation. All of these cells showed varying degrees of mixed excitatory and inhibitory responses to DFL stimulation (see above). The example in Fig. 14 shows a typical effect of bicuculline. This BP cell responded to single-pulse stimulation of DFL with a large EPSP, followed by a fast IPS (Fig. 14A), whereas tetanic stimulation (Fig. 14B) evoked a mixed excitatory and inhibitory response. Bicuculline completely blocked the fast IPS (Fig. 14A) and the inhibitory component of the mixed response (Fig. 14B). It is noteworthy that the peak of the EPSP is unaffected by bicuculline, suggesting that the IPSP is delayed so as to overlap only the slower phase of the EPSP (see Berman and Maler 1998b for discussion of EPSP/IPSP timing). The small slow depolarizing response that followed the tetanus at rest (Fig. 14B, left) increased in magnitude after the bicuculline treatment.

In all 10 cells GABA A antagonists increased the cells’ net excitatory response to DFL stimulation. The nature of the effect depended on whether there was clear evidence of fast and slow IPSps. Where fast IPSps followed a DFL-evoked EPSP (n = 4), they were blocked completely by GABA A.
FIG. 13. Responses to single and tetanic stimulation of the DFL in 3 BP cells. Responses ranged from excitatory to mixed excitatory and inhibitory. A: BP cell with mostly excitatory response. A1: DFL stimulation (single stimulus) evokes EPSP with 2 peaks; at 1 and 4.8 ms. A2: current-clamp experiment (0.25-nA increments and as indicated) shows the voltage sensitivity of the EPSPs (−0.5-nA trace has the largest amplitude EPSP). A slow slow IPSP appears to be present on the depolarized trace (sag toward end of response to current step), but it cannot prevent spiking in the more depolarized trials (0.5 and 0.75 nA). A3: response of same cell to a 200-Hz tetanic stimulus (m). Several spikes are evoked during the stimulus, whereas a single stimulus could not generate a spike (A1). There is no evidence of IPSPs. B: BP cell responded with similar EPSPs (see inset, 5-trial average) as the cell in A to a single stimulus; however, this cell also showed a slow (400-ms duration) IPSP after the EPSPs (single, 5-trial average). Tetanic stimulation (3-trial average) produced a mixed response with the IPSPs summating at first but then were overwhelmed by a strong IPSP(s) that also persisted for 400 ms. C1: BP cell responding to single stimulus mostly with an EPSP followed by a fast (peak at 2.5 ms) and slow IPSP (peak at ~260 ms). In some trials a spike was evoked by the stimulus (1 trial shown). C2: tetanic stimulation at 200 Hz but with only 5 stimulus pulses produced a mixed EPSP/IPSP response, with the inhibitory effect dominating; current clamp (rest, −0.25 and −0.5 nA) showed the reversal of the IPSPs just below resting potential (~71 mV), which prevents the EPSPs from reaching threshold. C3: net effect of the EPSP/IPSP mix is inhibitory as the tetanic stimulus (m, 200 Hz, 5 pulses) interrupts a current-evoked spike train (0.5 nA). C4: tetanic stimulation of 30 pulses at 200 Hz produces a summating EPSP that overcomes the inhibition to generate 4 spikes followed by a slow IPSP.

antagonists (e.g., Fig. 14A). Long-lasting IPSPs evoked by single or tetanic stimulation were blocked partially by GABA_A antagonists (4 of 4 cells). Slow EPSPs (e.g., Fig. 14B), present in three BP cells, were augmented by GABA_A antagonism. Two of these cells responded with a fast EPSP without evidence of a fast IPSP; bicuculline increased the amplitude of the fast EPSP, suggesting the presence of a masked IPSP. Hence in BP cells, primary afferent-evoked EPSPs always will be followed immediately by a fast GABA_A IPSP.

2) GABA_B Antagonism. Unlike NBP cells, BP cells did have a saclofen/phaclofen-sensitive component of the slow IPSP. Figure 15 shows one such cell in which single-pulse stimulation evoked a EPSP followed by IPSPs (Fig. 15A). Tetanic stimulation evoked a mixed EPSP/IPSP where the EPSP had a short-lived effect on cell excitability; the sum- mating IPSP quickly gained control over the cell after only two pulses of the stimulus (Fig. 15B, note the spike on the 1st pulse in the tetanus). Application of saclofen partially antagonized the slow component of the IPSP but had no effect on the early component. Of five BP cells tested, saclo- fen or phaclofen completely blocked (n = 3) or reduced the amplitude (n = 2) of the slow IPSP. Two additional BP cells were exposed to both bicuculline and saclofen; the effects of the two drugs were additive but failed to fully block the slow IPSP.

Effect of EAA Antagonists on BP-cell IPSPs

Non-NMDA (CNQX) and NMDA (APV or CPP) receptor antagonists were tested on the responses of 16 BP cells to DFL stimulation. In all 16 cases, the antagonists reduced or blocked the EPSPs. Where present under control conditions, evoked IPSPs were augmented by the drugs (n = 6) or appeared in the response where previously masked or absent (n = 4). The two cells featured in Fig. 16 (A and B) show such an effect. One cell (Fig. 16A) responded with vigorous excitation during the tetanus that gave way to a slow IPSP. After CNQX + APV treatment, the excitatory component was completely abolished, whereas the IPSP was augmented. There may be two components to the IPSP as the membrane potential was clamped at around rest during
the tetanic stimulus, then hyperpolarized after the stimulus. A more depolarized trace revealed the effect of this early part of the IPSP (Fig. 16A, inset). In the second cell (Fig. 16B), a large component of the mixed response to tetanic stimulation was inhibitory (→). The CNQX + APV application blocked the EPSP (see inset) evoked by single stimulus pulses. After drug treatment, tetanic stimulation evoked a large IPSP.

In two of the preceding experiments, the GABA<sub>B</sub> antagonist saclofen reduced the amplitude of the slow IPSP that had been augmented/unmasked by CNQX + CPP. An example is shown in Fig. 16C where the peak amplitude of the slow compound IPSP (during exposure to CNQX + CPP) was reduced by >30% after application of saclofen.

In summary, EAA antagonism blocks primary afferent EPSPs but enhances the compound IPSP in BP cells. Given that EAA antagonism blocked inhibition in NBP cells, it is likely that the EAA antagonism removed GC1 and GC2 inhibition of BP cells and the enhanced IPSP originates from ovoid cells, because they will be activated directly by the DFL stimulating electrode. As the enhanced IPSP is sensitive to GABA<sub>B</sub> antagonism, this is further evidence that it originates from a source other than GC1 and GC2 (their inhibition was insensitive to GABA<sub>B</sub> antagonism).

**GC2 firing patterns may determine slow IPSP time course in NBP cells**

The duration of the GABA<sub>A</sub> IPSPs evoked by tetanic stimulation was unexpectedly long. Classical GABA<sub>A</sub> IPSPs are usually 20–50 ms in duration, whereas the bicuculline-sensitive IPSP after tetanic stimulation could last for hundreds of milliseconds in NBP (e.g., Fig. 10) and BP cells (not shown). To determine whether the duration of the IPSP is due to the receptor-transmitter kinetics, passive postsynaptic
FIG. 16. Effect of EAA antagonists on BP cells’ responses to DFL stimulation. A: under control conditions, this BP cell responded to 200-Hz (20 pulse) stimulation with a summating EPSP that crossed threshold for spiking only after 17 pulses (4-trial average), this was followed by a slow IPSP. After CNQX + APV treatment, the depolarizing response was abolished completely, and the amplitude of the slow IPSP was increased (6-trial average, spikes truncated by averaging). Response to tetanic stimulation actually contained a ‘silent’ IPSP that was obvious in some trials in which the cell was somewhat depolarized (1 trial shown in inset); stimulus brings the potential down to rest (∓⋯∓). B: under control conditions, this BP cell responded to a single stimulus pulse with an EPSP followed by an IPSP (inset, 8-trial average). Tetanic stimulation (ø) produced a predominantly inhibitory response that also consisted of a slow IPSP (6-trial average). Treatment with CNQX + APV antagonized the early EPSP (see inset) and augmented the inhibitory response to the stimulus (6-trial average). C: BP cell responded to single pulse DFL stimulus with an EPSP and IPSP (inset). EPSP was blocked completely by CNQX + CPP. With EAA transmission blocked (CNQX + CPP, main plot) tetanic stimulation evoked a typical slow IPSP. This slow IPSP was partially blocked by the GABAB antagonist saclofen.

DISCUSSION

The aim of this study was to characterize the inhibition evoked in ELL pyramidal cells by stimulation of primary afferents. The source of the various IPSPs then could be assigned to the inhibitory circuitry previously identified by our laboratory (Maler and Mugnaini 1994). An identification strategy was developed by correlating the responses of recorded cells to DFL stimulation with their morphological type (NBP or BP) as determined by intracellular staining. NBP cells responded with IPSPs only, whereas BP cells responded with EPSPs and IPSPs. This agrees with the presence or absence (NBP cells) of direct primary afferent glutamatergic excitatory input (Maler 1979; Wang and Maler...
FIG. 17. Time course of NBP IPSP and unit activity in the granule cell layer evoked by tetanic stimulation (100 Hz, 100 ms, ø) of DFL. A: response of NBP cell to stimulation of the DFL produces a compound, long-lasting (400 ms) IPSP. B: input resistance (R_in) of the same cell monitored (30 ms, −0.2 nA current pulses) under the same conditions in A (4 trials). C: single trial showing a granule layer unit response to stimulation of DFL. Positive-going stimulus artifacts removed in all traces. D: histograms showing the average firing rate for 3 such units. Stimulation artifacts prevented reliable monitoring of spikes during stimulation. E: single trial records from 2 of the cells in D showing the trial-to-trial variability in the responses.

1994) via the basal dendritic bush (BP cells) and the in vivo classification of BP and NBP cells as E and I cells (excited vs. inhibited by increased electrosensory input) (Bastian 1981b; Saunders and Bastian 1984).

The discussion focuses on 1) the similarity in ELL pyramidal cell responses to GABA with mammalian pyramidal cells, 2) the strong voltage dependence of GABA responses and its implications, 3) evidence for GABA_A and GABA_B receptors on BP and NBP cells and the interneurons associated with these receptor subtypes, and 4) functional implications of the parsed inhibitory circuitry for first stage electrosensory processing in the ELL.

Effect of GABA on pyramidal cells

Consistent with mammalian pyramidal cells, the response of ELL pyramidal cells to exogenous GABA consisted of more than one phase. The early component was usually depolarizing and reversed at about −60 mV. The later component, which outlasted the GABA iontophoresis pulse, was hyperpolarizing and reversed at very negative potentials (>100 mV). The early component was blocked by GABA_A antagonists, and the late hyperpolarizing component was sensitive to GABA_B antagonists. The latter effect varied from cell to cell and between applications. This is perhaps not surprising given the low potency and variable efficacy of GABA_A antagonists in the mammalian CNS (Baumfalk and Albus 1987) and the possibility of pharmacologically distinct subsets of GABA_B receptors (Matthews et al. 1994; Scherer et al. 1988). Thus the reversal potential, time course, and pharmacology of the response of pyramidal cells to GABA strongly suggest that both BP and NBP cells express classic fast GABA_A-receptor-gated (Cl^-) channels and slow GABA_B-receptor-gated (K^+) channels.

The hyper- versus depolarizing nature of the early GABA response and the presence of two phases with slightly different reversal potentials may be due to action of GABA on somatic versus dendritic GABA_A receptors. In our experiments, the iontophoretic pipette was placed in the PCL, hence GABA applications would reach the soma, the somatic dendrites, and the proximal apical and basal (in BP cells) dendritic trunks. There is much evidence that dendritic applications of GABA result in depolarizing potentials while applications that recruit somatic GABA_A receptors result in mixed or purely hyperpolarizing potentials due to conductance increases to Cl^- (Alger and Nicholl 1982; Andersen et al. 1980; Connors et al. 1988). There is no consensus on the ionic mechanisms mediating the dendritic response (see Alger and Nicholl 1982; Djoorup et al. 1981; Smith et al. 1995; Thompson et al. 1988; Wong and Watkins 1982) although there are suggestions it involves some ion in addition to Cl^- (Thompson et al. 1988) and perhaps extrasynaptic
receptors that are not usually activated by physiological levels of synaptic GABA release (Wong and Watkins 1982).

The late component of the GABA response and the hyperpolarization induced by baclofen likely both were mediated by K⁺-linked GABA_B receptors (see Bormann 1988); they were both hyperpolarizing at rest and were associated with more modest conductance changes than the early GABA response (but see Ogata et al. 1987). It should be emphasized that exogenous GABA will activate a mix of somatic and dendritic GABA_A and GABA_B receptors and that the final response will be a complex mix determined by the receptors’ activation and desensitization time constants (Otis and Mody 1992; Tia et al. 1996; Wong and Watkins 1982) and affinity for GABA (Sodickson and Bean 1996).

**Voltage dependence of the GABA_A response**

The conductance changes due to GABA applications were larger when the neuron was depolarized and smaller when the neuron was hyperpolarized. This is consistent with the voltage sensitivity of ligand-gated chloride channels (GABA: Segal and Barker 1984; glycine: Faber and Korn 1987; Legendre and Korn 1995). GABA-activated chloride channels increase their conductance (Segal and Barker 1984) and decrease their rate of desensitization (Yoon 1994) with depolarization. This net increase in GABA_A-receptor-mediated inhibition with depolarization may have important consequences for sensory processing in the ELL.

GABA_A-mediated IPSPs usually are described as ‘shunting’ inhibition (Andersen et al. 1980) because the opening of these Cl⁻-permeable channels results in large decreases in input resistance with relatively small voltage changes (due to the close proximity of Cl⁻ equilibrium potential and resting membrane potential). However, the voltage sensitivity of these channels implies that the shunting effect is nonlinear. For example, the voltage sensitivity of GABA_A channels might provide for a simple form of gain control or normalization of primary afferent input: stronger input will produce greater depolarization of BP cells and thus automatically make GABA_A (GC2 cell) inhibition more effective by augmenting the associated conductance increase. This simple view does not take into account the possible interactions of GABA_A input with that of the more hyperpolarizing K⁺-mediated IPSPs (GC1 and ovoid cells) that may attenuate the conductance changes due to GC2 IPSPs, the autoinhibition of GABA synapses via GABA_B receptors and the complex kinetics of granule cell firing. Although detailed modeling studies are needed, it is clear that GABA_A-receptor-mediated inhibition by GC2 cells might mediate more complex computations than previously anticipated.

**GABA_A and GABA_B inhibition of BP and NBP cells**

Electrical stimulation of the primary afferents in the DFL produced fast GABA_A-type IPSPs (bicuculline/SR-sensitive and reversed close to rest) in both BP and NBP cells; as discussed later, we attribute these IPSPs to activation of the GC2 interneuron.

Both BP and NBP cells also responded to DFL stimulation with a slow IPSP with a very negative reversal potential. There are, however, striking differences between the slow IPSP in these two cell types: slow IPSPs in BP cells were significantly longer than those in NBP cells (mean of 813 vs. 501 ms), only the BP slow IPSP was sensitive to GABA_B antagonists, and the slow IPSP of BP cells was enhanced by blockade of excitatory transmission, whereas NBP IPSPs were blocked under the same conditions. As discussed later, we attribute the slow IPSP of NBPs to activation of GC1 interneurons; the transmitter/receptor involved is unknown, but the channel is likely to be K⁺ selective (due to its relatively hyperpolarized reversal potential). We propose that the slow GABA_A IPSP in BP cells is mainly due to the GABAergic (Maler and Mugnaini 1994) ovoid cell fibers that densely invest the basal dendrite of this cell type and can be stimulated directly from the DFL.

**NBP cells.** Nonbasilary pyramids receive input from GC1 and GC2 cells, but not from ovoid cells (Bastian et al. 1993; Maler 1979; Maler and Mugnaini 1994). As the type 2 granular cell is GABAergic, primary afferent input therefore can cause GABAergic inhibition only via the type 2 interneuron. Although the transmitter used by GC1 cells is unknown, morphological studies suggest that it is an inhibitory interneuron (Maler et al. 1981). Single-pulse electrical stimulation of primary afferents always evoked fast GABA_A IPSPs in NBPs and often evoked slow long-lasting IPSPs as well, consistent with this interpretation. Tetanic stimulation with frequencies similar to those of probability coders in vivo (Bastian 1981a; Hopkins 1976) generated prominent long-lasting IPSPs that were very effective in reducing current-evoked spiking. The glutamate antagonist block of DFL-evoked IPSPs confirmed that primary afferents can only affect NBPs via GC1 and GC2 cells and that the gap junction input to these interneurons (from primary afferents) (Maler et al. 1981) does not strongly activate them.

Based on the GABA_A antagonist block of the fast IPSPs and its reversal potential of −71 mV, we conclude that it originates from GC2 cells activating GABA_A receptors on NBP somata and somatic dendrites. The slow IPSP, which was reduced by GABA_A but not GABA_B antagonists and had a more negative reversal potential (−84 mV), is likely to be due to prolonged activation (see Slow IPSP kinetics) of the same GC2 input and input from non-GABAergic GC1 cells. The intermediate reversal potential of the slow IPSP is simply due to combined activation of GABA_A channels (−62 to −70 mV: exogenous GABA and fast IPSP) and the K⁺ channels putatively activated by GC1 cell input; the true reversal potential of the GC1 cell contribution to the IPSP is thus presumably even more negative than −84 mV. GC1 cells are less numerous than GC2 cells (Maler and Mugnaini 1994) and have sparse long-range projections to pyramidal cells and may not always be activated by our focal stimulation in DFL. This explains why the non-GABAergic component of NBP cell IPSPs was small and variable.

The question then remains: what drives the NBP GABA_B receptors responsible for the GABA_A-like responses evoked by exogenous GABA? Our companion study (Berman and Maler 1998a) suggests that it is the descending bipolar cell inhibitory pathway (Maler and Mugnaini 1994) as it produces GABA_B-receptor-like inhibition in NBP and BP cells.

**Slow IPSP kinetics.** The duration of the GABA_A compo-
ment of the NBP slow IPSP was too long (>1 s) to be accounted for by typical GABA_A receptor kinetics (Berman et al. 1992; Mody et al. 1994; Pearce 1993) or by the electrotonic properties of the thin somatic dendrites of NBPs: conductance changes tracked the IPSP time course, suggesting that the GABA_A receptors remained active throughout the IPSP, and modeling the effect of IPSPs on pyramidal cells demonstrated that IPSPs generated on distal thin somatic dendrites were only slightly prolonged compared with those on the soma (see Appendix). Unit recording of granule cells suggested that sustained firing of throughout the IPSP period accounts for the long time course of this slow IPSP in NBP cells. The response of granular interneurons was highly variable; we do not know whether this is due to slice conditions or to intrinsic properties of these interneurons (see Phasic properties).

BP CELLS. Unlike NBP cells, BP cells, in addition to the input from GC1 and GC2, also receive an input from GABAergic ovoid cells that invests their basal dendrite (Maler and Mugnaini 1994). BP cells responded to DFL stimulation with a complex mixture of EPSPs and IPSPs that complicates the interpretation of the response during tetanic stimulation. The response to single stimuli and tetani demonstrates that primary afferent stimulation evokes both fast and slow IPSPs. The fast IPSP has the same reversal potential and time to peak as the fast IPSP seen in NBP cells and was blocked completely by GABA_A antagonists. We therefore propose that the fast IPSP of BP cells is also due to GC2 cell activation of postsynaptic GABA_A receptors. The late phase of the IPSP has a reversal potential of about –88 mV and, unlike the case for NBP cells, was antagonized partially by saclofen. Further, applications of EAA antagonists enhanced the slow IPSP, which then could be reduced by saclofen. In the presence of EAA antagonists, granular interneurons are not activated (see preceding section for NBP cells) but ovoid cell axons, which run in the DFL (Maler and Mugnaini 1994), still can be stimulated. Therefore this GABA_b component is likely to be due to activation of ovoid cells.

Thus we propose that the IPSP that can be evoked in the absence of EAA transmission probably is entirely due to ovoid cell input to BP cells; the increase in the evoked IPSP (during EAA antagonism) can be attributed to the fact that, in the absence of GABA_A (Cl^-mediated) inhibition and EAA excitation, the reversal potential of the IPSP moves in the negative direction toward that of the GABA_b (K^+ -mediated) channel. Tetanic stimulation of commissural fibers (ovid cell axons) in vivo evokes a similar slow hyperpolarizing IPSP in BP cells (Bastian et al. 1993). It is not clear whether the IPSP due to ovoid cell input to BP cells has a GABA_A as well as a GABA_B component. The fact that saclofen could never completely antagonize the DFL-evoked response in the presence of EAA antagonists suggests this to be the case. Our current view of the GABAergic circuitry associated with electroreceptor input is summarized in Fig. 18 the major granule cell inhibitory input to BP and NBP cells is from the GABAergic GC2 and is mediated by GABA_A receptors, there is a lesser input to pyramidal cells from the non-GABAergic GC1 that probably involves a K^+ -mediated hyperpolarization, and BP cells receive a

strong GABA_B-mediated input, and this is due to fibers from GABAergic ovoid cells, which climb up the basal dendrite of these cells. This input also may involve a GABA_A component.

Functional interpretations

Our current understanding of the role of inhibition in generating ELL pyramidal-cell receptive fields and temporal response characteristics (Maler and Mugnaini 1994; Shumway and Maler 1989) has been based on ELL circuitry (Maler 1979; Maler and Mugnaini 1994; Shumway and Maler 1989), GABA immunohistochemistry (Maler and Mugnaini 1994), in vivo physiology (Bastian 1981b; Bastian and Courtright 1991), and pharmacology (Bastian 1993; Shumway and Maler 1989).

GC2 CELLS CONTROL GAIN AND TEMPORAL RESPONSES TO ELECTROSENSORY INPUT. Blockade of feedback input to the ELL (Bastian 1986b,c) or application of bicuculline to the PCL (Bastian 1993; Shumway and Maler 1989), increased the responsiveness of pyramidal cells and made them more tonic in their response to electroreceptive input. Because the GC2 is the only GABAergic interneuron type that receives both primary afferent and feedback input, it was hypothesized that this cell was primarily responsible for gain and
temporal properties of pyramidal cell responses; bicuculline would block directly GC2 synaptic input to pyramidal cells, while blocking feedback input would eliminate a major excitatory input to these interneurons and thus reduce their activity. The results of this study are entirely consistent with this interpretation.

**Gain control.** We have demonstrated that the synapses made by GC2 use only GABA\textsubscript{A} receptors; thus the in vivo application of bicuculline to the PCL will block the effects of GC2 cell activity. The fast IPSPs generated by GC2 overlap primary afferent input to basilar pyramidal cells and determine whether it will drive them to spike threshold; this presumably underlies the putative ability of the GC2 to control the gain of the pyramidal cell response.

**Temporal control: phasic properties.** The inhibition due to the GC2 cell is also very potent in terminating pyramidal cell depolarization; this is consistent with its presumed role in regulating pyramidal cell temporal response properties; GC2 GABA\textsubscript{A}–mediated inhibition is likely to be a major contributor to the phasicness of the pyramidal cell response to sustained electrosensory input. In the ELL slice, the GABA\textsubscript{A}–summed IPSP evoked by primary afferent tetanic stimulation often outlasts the stimulus tetanus for >500 ms due to the burst firing of granular interneurons. Although it is possible that this burst firing is an artifact of our in vitro preparation, it is noteworthy that Tharani et al. (1996) have suggested that ELL granular neurons express N-type Ca\textsuperscript{2+} channels and that these might regulate their oscillatory and burst activity (Turner et al. 1991, 1996; R. W. Turner, personal communication). It is therefore possible that feedback (Bastian 1986b,c) and intrinsic ELL circuitry (Maler and Mugnaini 1994) regulate temporal properties of pyramidal cells in part via the voltage-dependent Ca\textsuperscript{2+} channels of GC2 cells. This may be especially important with regard to the different temporal properties of pyramidal cells in the three ELL tuberous segments because Shumway (1989a) has shown that, although cells in the centromedial segment are low-pass filters (consistent with the long summed GABA\textsubscript{A} IPSPs we see in this segment), those in the lateral segment respond optimally to high-frequency input (see also Metzner and Heiligenberg 1992). It may be instructive to investigate the time course of DFL-evoked pyramidal cell inhibition in the lateral segment and the effect of local (GCL) application of N-channel antagonists on this inhibition.

**Temporal control: coincidence detection.** The fast time course of the primary–afferent-evoked IPSP may be important for coincidence detection. The primary afferent EPSP is rapid and appears to be shortened considerably by the GABA\textsubscript{A} IPSP (e.g., Fig. 15). As the EOD discharge frequency is extremely high in these fish (≈1,000 Hz), the rapid repolarization of the EPSP by GABA\textsubscript{A} IPSP may be essential to allow the cell to sample the environment at EOD rates. The fast kinetics of the primary afferent EPSP/IPSP favor the decoding of input spike patterns via coincidence detection (Gabbiani et al. 1994; Softky and Koch 1993; Traub et al. 1993) rather than via long-term temporal integration. Bastian (1981b) has already suggested, based on behavioral and electrophysiological data, that the detection of weak electrosensory input requires coincidence detection, and more precise recent studies have supported this idea (Nelson et al. 1997).

**Surround inhibition.** The same manipulations (blockade of feedback input or bicuculline in the pyramidal cell layer) that caused pyramidal cells to become more tonic also caused their inhibitory surrounds to increase in strength and size (Bastian 1986b, 1993; Shumway and Maler 1989). This implies that surround inhibition in the ELL is mediated by either GABA\textsubscript{B} receptors or by a non-GABAergic mechanism. Further, because bicuculline actually increased surround inhibition, it was likely that the interneuron responsible received GABAergic input via GABA\textsubscript{A} receptors. The GC1 cell is not GABAergic (Maler and Mugnaini 1994), although the morphology of its contacts onto pyramidal cells have the characteristics of inhibitory synapses (Maler et al. 1981). Because GC1 itself receives GABAergic input (from polymorphic cells) we hypothesized (Maler et al. 1981; Shumway and Maler 1989) that it was primarily responsible for surround inhibition in the ELL: bicuculline would block inhibition of GC1 cells, thus increasing their activity, while blocking feedback input to polymorphic cells (these interneurons have extensive apical dendrites in receipt of feedback input) (Maler 1979; Maler et al. 1981) also would reduce inhibition of GC1 interneurons; in either case, increased activity of GC1 would cause increased surround inhibition.

Our results are also consistent with this hypothesis. NBP cells receive input from primary afferents only indirectly via GC1 and GC2 cells (Maler 1979). After application of GABA\textsubscript{A} antagonists, stimulation of primary afferents still evoked a saclofen-insensitive hyperpolarizing potential in five of nine NBP cells. We hypothesize that this potential was an IPSP generated by activation of GC1 cells. This response is not always seen, perhaps because these cells are relatively rare (1 GC1:3.7 GC2 cells) (Maler and Mugnaini 1994) and therefore may be difficult to stimulate.

**Ovoid cells: common mode and noise rejection?** BP cells can be inhibited disynaptically by primary afferent input via ovoid cells as well as by GC1 and GC2 interneurons. For these cells, GABA\textsubscript{A} antagonists block part of the evoked IPSP; we hypothesize this is, as in NBP cells, due to GC2. GABA\textsubscript{B} antagonists block a slow component; we attribute this to ovoid cell activation. The remaining rather variable component of the IPSP is presumably due to activation of GC1. The ovoid cell is believed to mediate common mode rejection in the electrosensory system (Bastian et al. 1993; Maler and Mugnaini 1994). Both the rapid synaptic transmission and fast conduction velocity of the ovoid cell (Bastian et al. 1993) were proposed to adapt it to this role. It was therefore surprising to find that ovoid cell synaptic input to basilar pyramidal cells is likely to be mediated by both GABA\textsubscript{A} and GABA\textsubscript{B} receptors because the GABA\textsubscript{B} component has a slow time course. Consistent with our in vitro data, commissural stimulation of ovoid cell fibers in vivo produces a slow IPSP in pyramidal cells (Bastian 1993). Further, this slow IPSP is effective in quenching weak electrosensory stimuli, but strong stimuli are unaffected (Bastian 1993), suggesting that the input may act via conditional inhibition (see Berman and Maler 1998b). Bastian et al. (1993) also reported that ovoid cells had a high level of
resting activity (73 spikes/s); hence there is tonic GABAAergic inhibition of the BP basal dendrite. This points to a role for ovoid cell input in reducing noise in addition to its role in common mode rejection.

The complex mode of transmission at ovoid-basal dendrite synapses (see discussion on voltage dependence of GABAergic transmission) (voltage dependence of GABAergic transmission: Berman and Maler 1998a; conditional inhibition: Berman and Maler 1998b) suggests that our understanding of ovoid cell function is incomplete. The presence of a large saclafen-sensitive component in ovoid-basal pyramidal cell IPSPs may provide a pharmacological tool for delineating the function of the ovoid cell in vivo.

In summary, our in vitro results are, on the whole, remarkably consistent with the interpretation of ELL pyramidal-cell-receptive fields based on in vivo physiology and circuit analysis; fast GC2 inhibition is consistent with its role in gain control and shaping of temporal responses. However, two of our results were unexpected: granular interneurons respond to electroreceptor input with very variable spike bursts and ovoid cell-BP-cell IPSPs probably mediated by both GABAergic and GABAergic receptors. These findings suggest that GABAergic inhibition at this level of sensory processing may be regulated more dynamically than previously thought. Furthermore, the strong voltage dependency of GABAergic receptor-mediated inhibition, which is a common feature of mammalian GABAergic somatic dendrites as well, must be included in models if we are to fully understand the complex nature of GABAergic inhibition.

**APPENDIX**

*Computer simulations of the effect of synaptic input location on IPSP duration*

In the experiments above, activation of primary afferents produced long ( >300 ms) IPSPs in pyramidal cells; these IPSPs were blocked by bicuculline/SR and therefore attributed to GABAergic receptors. Based on the firing patterns of GABAergic interneurons, we hypothesized that the duration of the IPSP most likely was caused by sustained presynaptic firing and hence summation of many successive IPSPs rather than by some postsynaptic process that produced a long IPSP from a single volley of presynaptic firing. One alternative hypothesis was that GABAergic inputs on the thin ( <1-μm diameter) dendritic processes around the soma (Maler and Mugnaini 1994) would produce IPSPs that would be smeared temporally by the cable properties of these dendrites (Jack and Redman 1971; Jack et al. 1975) and therefore produce a prolonged IPSP when measured at the soma. To test the feasibility of this explanation, we used a passive compartmental model of a basilar pyramidal cell to simulate the effect these fine dendritic diameters would have on IPSP time course.

**Compartmental model**

A simple passive 35 compartmental model neuron (Fig. A1) was constructed based on measurements of a biocytin-filled ELL basilar pyramidal cell (R. Turner, personal communication) and representative measurements of the fine somatic dendrite processes of the basilar pyramidal cell shown in Fig. A1. Membrane capacitance was 0.75 μF/cm² and resistance 150 kΩ cm² throughout, cytoplasmic resistance 250 Ω cm. A passive leak current conductance (reversal potential ∼70 mV) was set to 0.05 mS/cm² so as to produce an input resistance of ∼20 MΩ when measured from the soma with hyperpolarizing current. Inhibitory synaptic conductances (7.6 mS/cm², reversal potential ∼70 mV) were modeled as an alpha function (Jack et al. 1975) and were placed on the soma, somatic dendrites, and proximal apical and basal dendrites. These regions receive substantial GABAergic input (Maler and Mugnaini 1994). GABAergic inhibitory conductances were not specifically included in the model. The estimate of the conductance load was derived from counts of GABAergic boutons on pyramidal cells that were cut en face (Maler and Mugnaini 1994) and estimates of GABA receptor synapse conductance (Douglas and Martin 1990). The accuracy of the conductance parameter is not critical because the aim of the simulations was to assess the factors affecting the duration of the IPSP. The somatic dendritic trunks were difficult to measure accurately (LY-filled cells, see Fig. 1) because of their thin diameters and varicosities, therefore two diameter measures (1 and 2.5 μm) were used to span the average range of thicknesses observed. A terminating compartment 15 μm long and 1 μm thick (referred to as somatic dendrite tip) was used to simulate inputs terminating on the ends of the somatic dendrites.

The model was implemented on Nodus (version 3.2.2), a Macintosh-based compartmental model simulator (De Schutter 1989; http://biff-www.uia.ac.be/).

**RESULTS.** Simulations were performed to explore the effects that the somatic dendrites’ fine diameters would have on IPSP duration. To test this, two parameters were varied, the location of the input and the thickness of the somatic dendrites. The performance of the compartmental model (Fig. A1A) to a simple alpha function IPSP is shown in Fig. A1B. The model cell was depolarized (0.5 nA to the soma) to move the membrane potential away from IPSP reversal (∼70 mV). With synaptic inputs on the somatic dendrites activated, the IPSP amplitude scaled with dendrite diameter in part because the GABA conductance was based on membrane area. The duration of the IPSP was little changed. The different dendrite diameters had little effect on IPSP amplitude when the soma and proximal regions of the apical and basal dendrites were activated. Combined activation of all these compartments, which may occur in our slice experiments, caused the longest IPSP (up to ~30 ms).

Varying the site of inhibition had little effect on IPSP time course measured at the soma (Fig. A2A). Comparisons of the IPSP profile at the soma with the conductance changes applied to the dendritic compartments (Fig. A2A, compare top with bottom row) showed that the soma voltage change closely tracked the synaptic conductance change. There was little evidence that the IPSP voltage change outlasted the conductance change. Tetanic activation of synapses (Fig. A2B) produced a summating IPSP that lasted up to ~60 ms after the onset of the last pulse in the stimulus; however, this was due to the summating conductance increases that also took that long to decay (not shown). IPSP time to peaks of 2−5 ms were used as these approximate the range measured electrophysiologically (single-pulse fast IPSPs, this study).

**DISCUSSION.** The thin diameter and profusion of pyramidal cell somatic dendrites seen in the filled cells raises the question as to their function. The inputs to the soma and the somatic dendrites arise from the same interneurons (Maler 1979; Maler et al. 1981); morphological (Maler et al. 1981) and electrophysiological evidence suggests that this input is strictly inhibitory. The model simulations show that inhibitory inputs on the fine somatic dendrites are effective in generating IPSPs at the soma, the thinness of the dendrites do not greatly alter the time course of IPSPs measured at the soma, and the IPSPs generated at the soma reach the Cl⁻ reversal potential and the addition of somatic dendritic IPSPs produces little additional hyperpolarization or conductance increase at the soma.

The degree of time course slowing due to membrane properties (Jack et al. 1975) is insufficient to account for the electrophysiological data on GABAergic IPSP durations. Thus we hypothe-
FIG. A1. Compartmental model of ELL basilar pyramidal cell. 

A: diagram of model compartmental dimensions. Leak and GABA conductances were applied as mS.cm⁻² of membrane surface area. Calibration bars: vertical, compartment lengths; horizontal, compartment diameters. 

B: model response to simulated inhibitory synaptic inputs. Single IPSPs (Alpha function time to peak: 2 ms) at t = 0. Simulated current injection (begins at t = −29 ms) of 0.5 nA to depolarize cell to −60 mV to enable the IPSP (reversal potential = resting potential = −70 mV) to produce a voltage change. Simulations were run with 2 models having different somatic dendrite trunk diameters (1 and 2.5 μm). Left: graphics indicate compartments with synaptic inputs that were activated (blank: not activated; shaded: activated). Varying somatic dendrite trunk diameter affected mainly the amplitude of the IPSP but not the duration (top). Alone, somatic dendrite inhibition produces a sizable IPSP. When added to somatic and proximal dendrite inhibition, the somatic dendrite inhibition causes a small increment in the IPSP amplitude.

FIG. A2. Temporal characteristics of somatic dendrite inhibition. 

A: single IPSP activations (t = 0 ms, Alpha function time to peak = 2 ms). Active compartments indicated (see Fig. 1). Top: soma voltage plots; bottom: soma and somatic dendrite (trunk only) synaptic conductances. Note that the IPSP voltage change tracks closely the conductance change. There is little evidence of the IPSP voltage change outlasting the conductance change (somatic dendrite trunks = 2.5 μm diam). Steady depolarizing current, 0.5 nA. B: tetanic IPSP activation (10 pulses, 200 Hz). Tetanus activation produced a summating compound IPSP. Smoothness depended on the time to peak of the IPSP alpha function (2 and 5 ms tested). Various traces are from simulation runs in which the somatic dendrite tips alone or with somatic dendrite trunks or all synaptic conductances were activated (see Fig. A1, A).
size that the long GABA_A IPSP durations recorded in pyramidal cells are caused by sustained presynaptic GABA release due to burst firing of GC2 interneurons.

As to the function of the somatic dendrites, there are three options where placing inhibitory inputs on the somatic dendrites may confer a computational advantage: prolong the inhibitory input, provide increased membrane real estate for inhibition, and perform local AND-NOT computations (see Koch 1997) on gap junction input. Our simulations suggested that the first option was not likely; somatic dendrite IPSPs were not significantly prolonged due to the electrotonic characteristics of the fine dendrites. As for the second option, the extra inhibitory synaptic drive onto somatic dendrites significantly affects the overall inhibition only to the extent that the somatic dendrite inhibitory inputs form the dominant fraction of active inhibitory inputs. When there is a large fraction of somatic inhibition, the soma saturates near the reversal potential of the inhibitory conductance and further somatic dendrite inhibition does not significantly affect soma $V_m$ or $R_m$. The available evidence indicates that GC2 innervate both soma and somatic dendrites (Maler et al. 1981) and hence selective activation of somatic dendrite GABAergic inputs is unlikely. However, the somatic dendrites would permit more GC1 and GC2 cells to contact pyramidal cells (especially the NBP). With regard to the third option, the colocalization of inhibitory and dendro-dendritic gap-junction inputs on NBP somatic dendrites (Maler et al. 1981) would make local computations feasible on this extensive somatic dendritic arbor.

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