Dendritic Modulation of Burst-Like Firing in Sensory Neurons

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Bastian, Joseph and Jerry Nguyenkim. Dendritic modulation of burst-like firing in sensory neurons. J Neurophysiol 85: 10–22, 2001. This report describes the variability of spontaneous firing characteristics of sensory neurons, electrosensory lateral line lobe (ELL) pyramidal cells, within the electrosensory lateral line lobe of weakly electric fish in vivo. We show that these cells’ spontaneous firing frequency, measures of spike train regularity (interspike interval coefficient of variation), and the tendency of these cells to produce bursts of action potentials are correlated with the size of the cell’s apical dendritic arbor. We also show that bursting behavior may be influenced or controlled by descending inputs from higher centers that provide excitatory and inhibitory inputs to the pyramidal cells’ apical dendrites. Pyramidal cells were classified as “bursty” or “nonbursty” according to whether or not spike trains deviated significantly from the expected properties of random (Poisson) spike trains of the same average firing frequency, and, in the case of bursty cells, the maximum within-burst interspike interval characteristic of bursts was determined. Each cell’s probability of producing bursts above the level expected for a Poisson spike train was determined and related to spontaneous firing frequency and dendritic morphology. Pyramidal cells with large apical dendritic arbors have lower rates of spontaneous activity and higher probabilities of producing bursts above the expected level, while cells with smaller apical dendrites fire at higher frequencies and are less bursty. The effect of blocking non-N-methylD-aspartate (non-NMDA) glutamatergic synaptic inputs to the apical dendrites of these cells, and to local inhibitory interneurons, significantly reduced the spontaneous occurrence of spike bursts and intracellular injection of hyperpolarizing current mimicked this effect. The results suggest that bursty firing of ELL pyramidal cells may be under descending control allowing activity in electrosensory feedback pathways to influence the firing properties of sensory neurons early in the processing hierarchy.

INTRODUCTION

A commonly observed characteristic of neuronal spontaneous activity as well as stimulus-driven activity is that clusters of spikes separated by short interspike intervals (ISIs) or “bursts” occur more often than expected given the average firing rate of spikes separated by short interspike intervals. This characteristic is thought to convey important information about the stimulus. Burst-like firing patterns may also be important characteristics of neurons involved in the initial stages of sensory processing systems where brief clusters of spikes may signal the presence of specific stimuli. For example, apical dendritic morphology is important in determining the properties of a cell’s firing pattern. Recent in vivo studies of the electrosensory system of South American weakly electric fish showed that these neurons signal the occurrence of critical features of a stimulus and that different neurons signal different features of a stimulus. One example of this is the weakly electric fish used in this study, Apterodonotes leptorhynchus, which generates a quasi-sinusoidal electric organ discharge (EOD) having a frequency ranging from about 600–1,000 Hz depending on the individual. Electroreceptors scattered over the body surface encode the amplitude (P-type receptors) and timing (T-type receptors) of this discharge. Two broad categories of sensory stimuli are thought to be encoded by this system. Electrolocation stimuli are thought to consist of relatively localized changes in the amplitude and timing of the EOD. Burst-like firing patterns may convey information about these stimuli by signaling the occurrence of critical features of a stimulus. Two broad categories of sensory stimuli are thought to be encoded by this system. Electrolocation stimuli are thought to consist of relatively localized changes in the amplitude and timing of the EOD. The costs of publication of this article were defrayed in part by the payment of page charges. The authors must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
discharge resulting from the presence of objects in the animal’s environment that have an impedance different from that of the surrounding water. Electrocommunication stimuli also consist of changes in EOD amplitude and timing, but these result from the interaction of the EOD of an individual with that of conspecifics, and these are typically spatially extensive influencing the activity of electoreceptors over large regions of the body surface (see Turner et al. 1999 for recent reviews of electoreception). Electoreceptor afferents terminate within a medullary nucleus, the ELL, and the P-type or amplitude encoding afferents provide excitatory synaptic input to basilar pyramidal cells (E cells). A second category of pyramidal cells, nonbasilar or I cells, are driven by inhibitory interneurons that receive excitatory afferent input (Maler 1979; Maler et al. 1981; Saunders and Bastian 1984).

In addition to receiving electoreceptor afferent inputs, ELL pyramidal cells receive, via elaborate apical dendrites, massive synaptic input descending from higher centers (Maler et al. 1981; Sas and Maler 1983, 1987). One subdivision of the descending pathways is thought to be involved in gain control (Bastian 1986a,b; Bastian and Bratton 1990; Nelson 1994) while another may provide positive feedback to accentuate important stimulus features (Berman and Maler 1999; Berman et al. 1997; Bratton and Bastian 1990; Maler and Mugnaini 1994). Recent studies have also demonstrated robust synaptic plasticity at the pyramidal cells’ apical dendrites (Bastian 1999; Bell et al. 1997).

Studies in which single ELL pyramidal cells were intracellularly labeled and reconstructed showed that several physiological characteristics of these neurons were strongly correlated with neuronal morphology (Bastian and Courtright 1991). Apical dendritic size is highly variable, and both spontaneous firing frequency as well as rate of adaptation to changes in stimulus amplitude are negatively correlated with the size of a cell’s apical dendritic arbor. This report extends these observations showing that pyramidal cells are also highly variable in terms of their tendency to produce bursts of action potentials, that apical dendritic size is strongly correlated with a cell’s tendency to burst, and that pharmacological reduction of descending excitation to these dendrites greatly reduces bursty firing.

**METHODS**

The South American weakly electric fish *A. leptorhynchus* was exclusively used in these studies. Animals were housed in 50-gallon population tanks at 26–28°C and with water conductivity ranging from 200 to 400 μS. Experiments were done in a 39 × 44 × 12-cm-deep experimental tank containing water from the animal’s home tank. Surgical techniques were the same as previously described (Bastian 1996a,b) and all procedures were in accordance with the University of Oklahoma’s animal care and use guidelines.

**Recording and stimulation**

Extracellular recordings were made with metal-filled micropipettes constructed as described by Frank and Becker (1964). Intracellular recordings were made with borosilicate or aluminosilicate sharp electrodes pulled with a Brown-Flaming P-87 pipette puller and filled with 3 M K-acetate. Initial electrode impedances ranged from 150 to 200 MΩ and were beveled (K. T. Brown BV-10 bevelor) until resistances fell to between 60 and 100 MΩ. For extracellular studies, electrodes were advanced with a Kopf 650 hydraulic micro-drive and signals with amplified with a WPI DAM50 preamplifier. For intracellular studies, electrodes were advanced with a Burleigh piezoelectric microdrive and preamplified with a WPI 767 electrometer. Spike times and times of EOD zero-crossings, and during intracellular recordings, membrane potential waveforms were acquired with Cambridge Electronic Design 1401plus hardware and SpikeII software. Spike and electric organ discharge (EOD) timing was measured with a resolution of 0.1 ms, and analog waveforms were A/D converted at a rate of 12.5 kHz. All subsequent data processing was done using Matlab (The Mathworks, Natick, MA).

The electric organ of *Apteronotus* is composed of modified motoneurons rather than muscle cells so the normal electric organ discharge remains intact during the neuromuscular blockade used in these experiments. The pyramidal cell spontaneous firing properties described therefore refer to activity in the presence of the normal baseline receptor afferent activity, which is very constant in the absence of modulations of the EOD amplitude. Electronically produced stepwise increases or decreases of the EOD, typically 1 mV/cm in amplitude and 300 ms in duration, were applied between electrodes straddling the fish and used as search stimuli. Stereotyped responses to this stimulus enabled categorization of cells as either basilar pyramidal cells (E cells) or nonbasilar pyramidal cells (I cells). The former respond to increased EOD amplitude with short-latency increases in spike frequency while the latter respond to increased EOD amplitude with reductions in firing frequency (Saunders and Bastian 1984).

**Data analysis**

Pyramidal cells were divided into subgroups, bursty or nonbursty, depending on whether or not autocorrelograms of spontaneous activity deviated significantly from that expected assuming Poisson spike trains (Abeles 1982). Autocorrelograms, 1-ms binwidth and 200-ms duration, were produced from records of spontaneous activity typically containing 1,500–2,000 spike times. For a few very low frequency cells, only 500 spike times were used. Spike trains were initially displayed as plots of instantaneous frequency versus time and only cells showing stable firing frequencies were studied. Expected correlogram bin contents \( y \) was determined as

\[
y = f \cdot N \cdot \Delta t
\]

where \( f \) = mean firing rate, \( N \) = the number of spikes in the list, and \( \Delta t \) = correlogram binwidth (1 ms). Autocorrelograms from Poisson spike trains are expected to be flat with bin contents approximately equal to \( y \), ignoring the depression at the origin due to the cell’s refractory period. Initial peaks in the correlogram, determined to be unlikely assuming a Poisson spike train, were taken as the indication of bursty firing. For each cell, a 99.9% confidence limit was calculated for the autocorrelogram expected bin contents as described by Abeles (1982).

Given an expected bin contents, \( y \), the probability of finding \( m \) spikes within a bin is

\[
P[m; y] = e^{-y}y^m/m!
\]

and these probabilities were accumulated to determine the smallest \( m \) such that

\[
\sum_{i=0}^{m} P[i; y] > 0.999
\]

Autocorrelograms of spontaneous activity having initial peaks with bin contents exceeding \( m \) were categorized as bursty, and examples of autocorrelograms from nonbursty and bursty cells are shown in Fig. 4, A2 and B2, respectively. The maximum ISI separating spikes considered to be within a burst was also determined from these correlograms. The time at which the falling phase of the initial peak crossed...
the 99.9% confidence limit was taken as the maximum ISI characteristic of bursts for that spike train, maximum intraburst interval (IBI<sub>max</sub>), and in cases where multiple early peaks remained above the 99.9% threshold, the time of the minimum between the first two peaks was taken as IBI<sub>max</sub>.

Pharmacological techniques and current injection

Micropressure ejection techniques were used to apply the non-NMDA glutamate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to local regions of the ELL molecular layer containing the apical dendrites of a recorded cell. Multibarrel pipettes were pulled to a fine tip and broken back to a total tip diameter of about 10 µm. Typically two barrels were filled with a 1 mM solution of disodium CNQX, two barrels were filled with 1 mM glutamate, and a fifth control barrel contained distilled water. After a well-isolated single-unit extracellular recording or a stable intracellular recording was established, the pressure pipette was slowly advanced into an appropriate region of the ELL molecular layer while periodically ejecting “puffs” of glutamate. Typically ejection duration ranged from 50 to 100 ms, and ejection pressure was usually 40 psi. As described earlier (Bastian 1993), proximity to the apical dendrite of the recorded cell was indicated by short-latency increases in firing rate following glutamate ejection. Following correct placement, CNQX was delivered as a series of pulses (e.g., 100-ms puffs at 0.5 Hz for 20 s), and this treatment typically resulted in tonic alterations in pyramidal activity lasting approximately 5 min. In experiments where the effects of tonic hyperpolarization on pyramidal cell activity were studied, negative current injection, typically −0.5 nA for 5 min, was applied via the bridge circuit of the electrometer. Unless indicated otherwise, sample means are given ±1 SE.

RESULTS

Spontaneous activity

Spontaneous firing frequencies of 126 pyramidal cells were recorded from the lateral and centrolateral segments of the ELL of 30 fish in which the animals’ neurogenic electric organ discharge was intact. Recording site within the lateral and centrolateral segments were estimated from surface landmarks and recording depth, and no obvious differences among cells from these regions were seen. Cells were categorized as either E or I type, excited or inhibited by increasing electric organ discharge amplitude, respectively, and the distribution of spontaneous firing frequencies is shown in Fig. 1A. Spontaneous firing rates for this sample of E and I cells were not different, averaging 18.32 ± 1.34, n = 62 and 17.72 ± 1.03, n = 64, respectively, and these values are similar to those seen in earlier studies of this and the related fish Eigenmannia virens (Bastian 1986a; Metzner et al. 1998).

The coefficient of variation (C<sub>V</sub>) of each pyramidal cell’s record of ISIs was calculated as an initial estimate of spike train variability and, as described for Eigenmannia (Metzner et al. 1998), C<sub>V</sub>s were highly variable ranging from 0.45 to 2.24. The C<sub>V</sub>s were also negatively correlated with these cells’ spontaneous firing frequency as shown in Fig. 1B. The C<sub>V</sub> is expected to be reduced with increasing spike frequency since firing must become more regular as the ISI approaches the cell’s refractory period; however, effects of refractory period alone cannot account for the range of C<sub>V</sub>s seen in this sample of pyramidal cells. Given a cell with an absolute refractory period of 5 ms, a spontaneous firing rate of 5 spikes/s, and a C<sub>V</sub> of 1.5, increasing firing rate to 50 spikes/s is expected to decrease C<sub>V</sub> by about 25% (Gabbiani and Koch 1998, equation 9.12). The reduction in C<sub>V</sub>s seen across pyramidal cells having a similar range of firing frequencies was approximately 50% or twice as large as predicted given refractory effects alone.

Unlike most weakly electric fish, the neurogenic electric organ discharge in Apteronotus remains intact under neuromuscular blockade, hence the “spontaneous activity” referred to herein is activity in the presence of the normal discharge. Electroreceptor afferents’ time of firing is strongly phase-coupled to the EOD waveform (Bastian 1981a; Hagiwara et al. 1965; Hopkins 1976) while the ELL pyramidal cells generally show a weaker phase relationship to the EOD cycle (Bastian 1981b). Phase histograms of firing times within the period of the EOD cycle were produced for a subset of the cells studied. Phase coupling was measured by computing the mean vector length for each histogram (Batschelete 1981); this statistic ranges from zero for histograms of activity unrelated to the EOD period to 1.0 for perfectly synchronized firing. The strength of the phase coupling to the EOD varied significantly among pyramidal cells, and phase histograms of spontaneous
activity showing stronger (mean vector = 0.42) and nonsignificant (mean vector = 0.06) phase relationships to the EOD are shown in Fig. 2, A and B, respectively. The degree of phase coupling to the EOD was also found to be highly correlated with the cells’ spontaneous firing frequency (Fig. 2C). Cells with higher rates of spontaneous activity showed stronger phase coupling. A similar correlation was seen for both E and I cells although the highest frequency cells and those showing the strongest phase coupling were E cells (Fig. 2, ●). The higher-frequency pyramidal cells’ stronger phase coupling to the EOD contributes to the lower ISI variability seen for these cells since spikes preferentially occur during a restricted phase of the highly regular discharge waveform.

In a previous study in which individual ELL pyramidal cells were intracellularly labeled with either horseradish peroxidase or Lucifer yellow, it was found that spontaneous firing rate and other physiological properties were significantly correlated with cellular morphology (Bastian and Courtright 1991). In particular, the size of both basilar and nonbasilar pyramidal cells’ apical dendritic trees was found to be negatively correlated with spontaneous rate. Examples of reconstructed pyramidal cells that span the range of variation seen are shown in Fig. 3, A–C. The spike trains from 17 morphologically described cells of this earlier study were reexamined, and Fig. 3D shows the relationship between the summed length of all apical dendritic branches and each cell’s spontaneous firing rate and spike train $C_V$. Apical dendritic lengths are significantly correlated with both spontaneous rate and ISI coefficient of variation ($r$’s $= −0.81$ and 0.74, respectively). Hence the pyramidal cells with smaller apical dendritic arborizations fire at relatively high frequencies and their spike trains are more regular. Additionally, the higher frequency cells’ firing patterns shows relatively strong phase coupling to the EOD cycle, indicating that receptor afferent inputs predominantly drive these cells. Conversely, the lack of phase coupling between the firing times of the low-frequency pyramidal cells and the EOD waveform suggests that receptor afferent activity is less effective in driving these cells, at least on an EOD cycle by cycle basis. Instead descending inputs that terminate in the ELL molecular layers and provide synaptic input to the extensive apical dendrites of lower-frequency pyramidal cells may play a larger role in driving these cells, and the more irregular character of the low frequency cells’ spontaneous activity (high $C_V$) may be a consequence of the biophysical properties of the more extensive apical dendrites, a result of patterned inputs to these dendrites or a combination of both.

**Burst-like firing patterns**

ISI histograms (ISIHs) of three pyramidal cells are shown in Fig. 4, A1–C1. Spike trains from a subset of the pyramidal cells studied had simple exponential ISI distributions as expected for a Poisson spike train (Fig. 4A1); however, the ISIHs of most
cells consisted of two phases: an initial peak at short ISIs followed by a longer tail (Fig. 4, B1 and C1) as described for pyramidal cells of the related fish Eigenmannia virescens (Metzner et al. 1998). The latter ISIH pattern is typical for cells having a tendency to produce short high-frequency “bursts” or clusters of action potentials.

A variety of techniques have been used to determine whether or not to classify a cell as bursty and to define the ISI size considered to be characteristic of spikes within a burst. These range from identifying features such as the first trough or inflection point following the peak of ISI distributions or autocorrelation functions (Metzner et al. 1998; Turner et al. 1996) to statistical methods based on the occurrence probability of short ISI sequences (Legendy and Salcman 1985). A method described by Abeles (1982) was adopted for identifying bursty pyramidal cells and for determining the ISIs characteristic of bursts. Autocorrelograms were constructed, and the expected bin contents based on the cell’s average firing rate as well as the +99.9% confidence limit for the expected bin contents (— and - - -, respectively, Fig. 4, A2–C2) were determined. Cells showing initial peaks in the autocorrelogram exceeding the confidence interval were categorized as bursty.

Autocorrelograms of approximately 20% of the cells studied (12 E cells and 13 I cells) were either flat, as in the case of Fig. 4A2, or had small initial peaks that did not exceed the threshold confidence interval. These cells typically had simple exponential ISI distributions, had relatively high rates of spontaneous activity, and their average $C_V$ was 0.84. These cells were categorized as “nonbursty.” The majority of cells (80%) were categorized as bursty and the maximum intraburst interval (IBI_{max}) of spike doublets, or longer bursts, was taken as the time at which the falling phase of the autocorrelogram’s initial peak crossed the confidence limit (arrow of Fig. 4B2). In cases where the correlogram contained multiple peaks that exceeded the threshold, as in Fig. 4C2, the time of the minimum following the first peak was taken as IBI_{max}. The value of IBI_{max} for each cell was then used to identify bursts of from 2 through 10 spikes in the records of spontaneous activity.

The values of IBI_{max} for 50 E cells and 51 I cells averaged 15.98 ± 0.58 and 15.29 ± 0.71 ms, respectively, and the values of IBI_{max} were negatively correlated with the cells’ spontaneous firing frequencies (Fig. 5A). After identifying the IBI_{max} for a given cell, burst probability was determined as the ratio of the total number of bursts, each burst considered as a single event, to the total number of events (burst events plus single spikes) in the sample. Burst probabilities for E and I cells averaged 0.25 ± 0.014 and 0.22 ± 0.012, respectively, and were not significantly correlated with pyramidal cell spontaneous firing frequency (Fig. 5B).

Pyramidal cells were categorized as bursty based on significant deviations from the properties of a Poisson spike train;
that is, bursty cells are, by definition, those that produce an excess of spike clusters over those expected from a Poisson spike train. The probabilities shown in Fig. 5B are overestimates of the “excess” bursts since some number ISIs shorter than $IBI_{max}$ would be expected to occur even in a Poisson spike train. Therefore an alternative measure of burst probability designed to show the proportion of a cell’s total bursts above or in excess of those expected was also calculated. This was done by removing the fraction of bursts expected given a Poisson spike train of an average firing frequency predicted from the tail region of the ISIH: first, data from the tail of the ISIH were fit with a single exponential, which was extrapolated to its intersection with the start of the initial peak of the ISIH. An example is shown by the smooth line of Fig. 5C. The peak of the histogram above this extrapolation, including bins through 2 times $IBI_{max}$, was then replaced with values predicted by the exponential fit to the tail. The solid line shown in the inset of Fig. 5C shows the altered initial phase of the resulting histogram, and the dotted line indicates values that were replaced. The mean spike frequencies ($f$) determined from histograms with the initial peaks removed in this manner were used as the basis for predictions of the number of bursts expected due to an underlying Poisson process. The probability of $n$ events $p(n)$ in a given time period, $T$, is given as:

$$p(n) = (fT)^n e^{-fT}/n!$$

The probabilities of 2 spikes occurring within a time $T = IB_{max}$ of 3 spikes occurring within $T = 2 IB_{max}$, . . . , through 10 spikes occurring within $T = 9 IB_{max}$ were determined and subtracted from the actually observed probabilities for bursts containing 2 through 10 spikes. Each probability determined from the Poisson distribution was itself corrected for the small effects of refractory period. No spikes occur during a cell’s refractory period; however, given the Poisson distribution, there is a small probability of 2 or more spikes occurring within the time equal to the refractory period. So, for example, the probability of two spikes occurring during $T = IB_{max}$ was corrected by subtracting the probability of two spikes occurring during the time equal to the refractory period.

The resulting corrected probabilities represent a cell’s tendency to fire bursts in excess of those accounted for by an underlying Poisson process. These “non-Poisson bursts,” expressed as a percentage of the total burst probability, are plotted in Fig. 5D, and this measure is strongly correlated with the cells’ spontaneous firing rates ($r = -0.73, P < 0.001$). The majority of bursts seen in low-frequency spike trains are non-Poisson, but for higher-frequency cells, approximately 50% of the bursts seen are expected given a Poisson spike train. Given the strong negative correlation between spontaneous firing frequency and apical dendritic size, this analysis indicates that cells with larger apical dendrites produce more bursts in excess of those expected.

**Intraburst spike interval distributions**

To determine if characteristic patterns of ISIs occur within bursts, successive intervals within bursts of six spikes were analyzed for 64 pyramidal cells. Ten such bursts were produced by the cell of Fig. 6A within a 3-min record, and the successive ISIs within each burst are shown by ○. The means of these intervals are shown by ●. No systematic pattern of ISIs was seen for this cell except for the relatively long interval at the end of the burst. Mean ISIs within bursts containing six spikes from a sample of 10 cells are shown by ○ of Fig. 6B. The second through fifth intervals are expressed as a percentage of the first, and no systematic pattern of successive intervals within bursts was seen for these cells. The ● shows the grand mean of within-burst intervals normalized in this manner for the 64 cells analyzed, and no statistically significant trend in interval length was found.

A second analysis was performed on all bursts containing three or more spikes. The differences between successive intervals within each burst were determined as described by Turner et al. (1996), and histograms of these interval differences were produced. Figure 6C shows the distribution of intraburst interval (IBI) differences for the cell of Fig. 6A. That the interval differences are approximately normally distributed with a mean (0.035 ms) not significantly different from zero indicates that there are no simple trends in interval lengths within bursts. Successive intervals neither increase nor decrease systematically; rather, for this cell, intervals vary randomly about a mean length of approximately 16 ms. The means of IBI difference distributions, like that of Fig. 6C, were determined for 99 cells and are summarized in the histogram of Fig. 6D. The mean of this distribution (−0.016 ms) is also not different from zero. Although, in vitro preparations, significant numbers of pyramidal cells showed trends or nonrandom patterns of IBI differences (Turner et al. 1996), in vivo, no consistent patterning of IBIs is seen.
Blockade of descending excitation reduces burst-like behavior

Previous in vivo studies showed that localized blockade of excitatory inputs to pyramidal cell apical dendrites can be achieved via micropressure ejection of glutamate antagonists within the ELL dorsal and ventral molecular layers (Bastian 1993). This technique was used to determine if alterations of synaptic inputs to pyramidal cell apical dendrites influenced bursting behavior. As in previous studies, a multibarrel pressure pipette was positioned in close proximity to the apical dendrite of a pyramidal cell being recorded from. As the pressure pipette was advanced through the molecular layer, brief puffs of glutamate were periodically ejected, and short-latency responses to the glutamate were taken to indicate proximity of the pipette to the recorded cell. Previous pressure ejection experiments coupled with extracellular labeling of ejection site and intracellular labeling of the recorded cell verified that short-latency excitatory responses occurred when the pipette was very close to or within the cell’s dendritic arbor (Bastian 1993). Slightly longer-latency inhibitory responses were typically seen when the pressure pipette was further outside the dendritic arbor where glutamate preferentially activated inhibitory interneurons. Following recording of 200 s of spontaneous firing, the non-NMDA antagonist CNQX was ejected. Typically 50-ms puffs were delivered at a rate of one per 2 s, resulting in a reduction in the cell’s spontaneous firing rate and a decrease in the cell’s tendency to produce spike bursts. The reduction of spontaneous activity is not due to CNQX blockade of glutamatergic receptor afferent input to pyramidal cells. As shown earlier (Bastian 1993) and repeated in this study (data not shown), responses to changes in EOD amplitude are increased rather than decreased by CNQX application (thin lines) superimposed on that taken during application of the antagonist (thick line). Burst probability for this cell was initially 0.35 given an IBImax of 23 ms. In the presence of CNQX, this was reduced to 0.14 and IBImax was increased to 26 ms. Cells typically recovered within 3 min of the cessation of CNQX application, and this cell’s spontaneous rate returned to 10.2 spikes/s while burst probability recovered to 0.39 with an IBImax of 24 ms (Fig. 7C, I and 2).

The effects of CNQX application on the spontaneous firing patterns of 24 pyramidal cells (11 basilar and 13 nonbasilar) are summarized in Fig. 8. There was no significant difference in the behavior of these cell types so the data were pooled. On average, both total burst probability and non-Poisson burst probability were significantly reduced during CNQX application (Fig. 8, black and dark gray bars, $P < 0.005$, t-tests), and these measures returned toward their initial values following termination of CNQX application (Fig. 8, light gray bars). However, only 13 of the 24 cells were recorded through complete recovery. The coefficients of variation were also reduced by CNQX treatment in most cells (16 of 24); however,
the average $C_v$ of all cells during CNQX (1.04 ± 0.05) was not significantly less than that prior to treatment (1.09 ± 0.04). Although the reduction in burst probability during CNQX injection averaged approximately 45% for all cells studied, seven of these were only minimally affected by this treatment (burst probabilities changed by less than ±5%). It is not known if the lack of significant CNQX effects in these cells is a result of failure to deliver effective CNQX doses or if the firing characteristics of these cells are truly insensitive to this treatment.

The reductions in spontaneous firing frequency seen with CNQX application (compare Fig. 8, black and dark gray hatched bars) were not due to simply shifting the ISI distributions to longer values. Instead, as shown by Fig. 7B1, inset, this antagonist preferentially reduced the probability of short ISIs, and this loss of the higher-frequency spike bursts must contribute to the observed reduction in spontaneous rate. For comparison with the CNQX effects on firing rate, the bursts were artificially removed from the pre-CNQX spike trains and replaced with single spikes. The average frequency of these modified spike trains, which contained no bursts, is comparable to the frequency seen in the presence of CNQX (Fig. 8, hatched white bar). Although this manipulation of pre-CNQX spike trains indicates that loss of burst spikes alone could account for the reductions in firing rate seen with CNQX treatment, the application of CNQX also resulted in increased numbers of long (more than 300 ms) ISIs; this also contributes to the lower average firing rates seen.

**Hyperpolarizing current injection reduces burst-like behavior**

Possible mechanisms by which CNQX blockade of pyramidal cell apical dendritic inputs could reduce spike bursts include elimination of patterned synaptic inputs that directly evoke pyramidal cell bursting as well as reduction of tonic excitatory inputs resulting in hyperpolarization and reduced probability that a “burst-threshold” is exceeded. To determine if moderate hyperpolarization is sufficient to reduce bursts, intracellular recordings of spontaneous activity before, during, and after hyperpolarizing current injection were compared. Figure 9A1 shows two typical 1-s segments of intracellularly recorded basilar pyramidal cell spontaneous activity prior to hyperpolarizing current injection. Spontaneous firing rate was initially 15.3 spikes/s and the $C_v$ was 1.24. Constant current injection, −0.5 nA, initially silenced pyramidal cells but typically within 30 s spontaneous firing resumed at a lower but stable rate. Figure 9A2 shows 1-s epochs of spontaneous activity after the cell adopted a new steady-state firing frequency. This cell was hyperpolarized by 1.5 mV, which reduced spontaneous firing frequency to 8.6 spikes/s and $C_v$ to 1.07, and this small hyperpolarization reduced burst probability from an initial valued of 0.24 to 0.04. Interval histograms of this cell’s spontaneous firing before and during current injection are shown in Fig. 9A3 by the thin and thick lines, respectively, and, as shown for CNQX blockade of descending excitation, hyperpolarization preferentially reduced the initial peak corresponding to the shortest ISIs.

A pressure ejection pipette was also in place while recording from this cell so intracellular activity was monitored before and during CNQX blockade of inputs to the cell’s apical dendrite. Comparison of the records (Fig. 9B, 1 and 2) shows that CNQX blockade resulted in similar changes in pyramidal cell spontaneous activity and bursting as did hyperpolarizing current injection. The brackets above the spike records show bursts identified based on IBImax of 12 and 13 ms for the data of Fig. 9, A and B, respectively. Spontaneous firing rate was reduced from 15.5 to 8.4 spikes/s, $C_v$ was reduced from 1.3 to 0.74, and burst probability was reduced from 0.27 to 0.03. The interval

**FIG. 9.** Hyperpolarization of pyramidal cells mimics the effects of CNQX application to the vicinity of the apical dendrites. A1: 2 randomly selected segments of spontaneous activity recorded from a basilar pyramidal cell. A2: randomly selected 1-s segments of spontaneous activity during continuous −0.5-nA current injection. A3: ISIHs of spontaneous activity before (thin line) and during (thick line) −0.5-nA I injection. B, 1 and 2: randomly selected pairs of 1-s records of spontaneous activity before and during CNQX application, respectively. B3: ISIHs of normal spontaneous activity (thin line), of activity during CNQX (heavy line) and of activity with CNQX at a dosage reduced by 50% (dotted line).
histograms of Fig. 9B3 summarize this cell’s firing before and during the CNQX application (thin and thick lines, respectively), and the dashed line shows that the effects of CNQX were dose dependent; 50% of the original dose was applied in this case.

Figure 10 summarizes the effects of hyperpolarization on the spontaneous activity of 14 pyramidal cells (7 basilar, 7 non-basilar). Current magnitude was typically −0.5 nA, and the average hyperpolarization that resulted was 3.25 mV. As with CNQX treatment, overall burst probability as well as non-Poisson burst probabilities were significantly decreased by hyperpolarization as was spontaneous firing rate. The effects of removing bursts of from 2 through 10 spikes from spike trains recorded at normal membrane potential and replacing them with single events was also examined. As seen with CNQX treatment, the resulting reduction in firing frequency due to artificial burst removal closely matched that due to hyperpolarization (Fig. 10, dark gray and clear hatched bars). Following hyperpolarization burst probabilities recovered but, unlike the recovery from CNQX where probabilities and spike rates remained somewhat depressed, following hyperpolarization both burst probabilities and firing rates were larger than initially, although these increases were not statistically significant. The result that hyperpolarization mimicked the effects of CNQX treatment is compatible with the idea that the CNQX blocked tonic excitation and altered a threshold for burst generation. However, this result does not rule out the possibility that CNQX treatment also eliminates patterned dendritic inputs. The intracellular recordings from cells that were also treated with CNQX did not reveal any consistent effects of this drug on resting potential; hyperpolarization was not systematically observed in response to this treatment. Although it is possible that the slow onset of CNQX effects along with small changes in impedance quality and other artificial sources membrane potential drift precluded clear-cut indications of CNQX-caused hyperpolarization, the lack of changes in resting potential may indicate that patterned inputs are also blocked by this treatment.

**DISCUSSION**

The results of this and of previous studies (Bastian 1993) show that there is considerable variability among ELL pyramidal cells in terms of their physiology and morphology as well as in the distribution of their neurotransmitter receptor types (Bottai et al. 1997, 1998; Dunn et al. 1999) and in the presence of various intracellular signaling molecules (Berman and Maler 1999; Berman et al. 1995; Zupanc et al. 1992). The variability of pyramidal cell apical dendrite morphology is particularly striking (Fig. 3, A–C), and the strong correlation between spontaneous firing rate and dendritic structure (Fig. 3D) enables us to predict the morphological characteristics of cells studied with extracellular recording techniques. Cells with the smallest apical dendrites (deep basilar pyramidal cells) also lie deeper within the ELL laminae (Bastian and Courtright 1991) and exhibited the highest firing rates and the lowest probabilities of bursts in excess of those expected based on a Poisson spike train, and their spontaneous activity showed the strongest phase coupling to the electric organ discharge. This indicates that these cells are principally driven by receptor afferent inputs whose activity is also strongly phase-coupled to the EOD. Conversely, the lowest-frequency cells have the largest apical dendrites, they are found most superficially within the pyramidal cell lamina, and their activity shows little to no phase relation to the EOD waveform. These cells’ activity is likely to be more strongly influenced by apical dendritic inputs; their spontaneous activity is more irregular (high $C_V$), and these cells show the highest probability of producing unexpected spike bursts. The correlation between bursting and extensive apical dendrites has been previously demonstrated in mammalian cortical pyramidal cells in vitro (Mason and Larkman 1990) and modeling studies also showed that apical dendritic size alone, without differential distributions of ion channels across morphological categories, was correlated with a cell’s ability to produce spike bursts (Mainen and Sejnowski 1996).

The tendency of various ELL pyramidal cell types to produce bursts may not be constant; instead, as sensory processing requirements change, bursty behavior may be modulated optimizing the performance of these cells for the task at hand. The large reductions in burst probability seen following blockade of descending excitation to the pyramidal cell apical dendrites and ELL inhibitory interneurons supports the idea that bursty firing may be under descending control.

**Sources of apical dendritic inputs**

The pyramidal cell apical dendrites receive input from two distinct sources. Distal regions of the dendrites receive glutamatergic inputs, via AMPA as well as NMDA receptors, from typical cerebellar parallel fibers (Berman et al. 1997) and enormous numbers of these granule cell axons comprise the ELL dorsal molecular layer (DML) (Maler 1979; Maler et al. 1981). The granule cell bodies are found in a region superficial to the DML termed the posterior eminentia granularis (EGp), and the granule cells receive descending electroosensory inputs as well as proprioceptive information and, possibly corollary
discharges of motor commands (Sas and Maler 1987). The
descending electrosensory inputs to the EGp originate in the
rhombencephalic nucleus praeminentialis (nP), and since the
nP also receives ascending electrosensory inputs from the ELL
pyramidal cells, the nP to EGp to DML circuit comprises a
feedback loop allowing descending electrosensory as well as
nonelectrosensory signals to influence pyramidal cell activity.
The nP efferents carrying the descending electrosensory infor-
mation are tonically active and track longer-term changes in
EOD amplitude, and this feedback pathway is thought to be
involved in pyramidal cell gain control (Bastian 1986a,b; Bas-
tian and Bratton 1990; Nelson 1994; Shumway and Maler
1989). In addition, this pathway participates in mechanisms
that adaptively cancel reafferent patterns of sensory input that
arise, for example, as consequences of the animals’ body
movements (Bastian 1995, 1996a,b, 1998a,b, 1999).

In addition to providing excitatory input directly to pyri-
danal cell apical dendrites, the DML parallel fibers excite mol-
ecular layer inhibitory interneurons including the DML stel-
late cells. The finding that large CNQX injections result in
decreased spontaneous activity indicates that the direct excita-
dory drive due to parallel fiber activity outweighs the disynaptic
inhibition mediated via DML inhibitory interneurons in the
absence of changes in electrosensory input. However, CNQX
treatment has the opposite effect on pyramidal cell responses to
electrosensory stimulation; it increases both the magnitude and
duration of responses to EOD increases (Bastian 1993). The
seemingly paradoxical finding that a cell’s spontaneous activity
is reduced by glutamatergic blockade while responses to elec-
trosensory stimuli are enhanced was recently suggested by
Berman and Maler (1999) to be due to differences in the
dendritic architecture of the pyramidal and stellate cells.
The large pyramidal cell apical dendrites receive excitatory inputs
from very large numbers of parallel fibers, and, in the absence
of electrosensory stimulation, assumed spatially diffuse activ-
ity across large populations of parallel fibers is expected to
provide significant excitatory drive to pyramidal cells. Hence
blockade of this diffuse excitation reduces spontaneous activ-
y. Dorsal molecular layer stellate cells, with far smaller
dendritic arborizations, are thought to be minimally driven by
this spatially diffuse activity. In response to electrosensory
stimulation, however, spatially restricted populations or
“beams” of parallel fibers are proposed to be activated that
could have a proportionally larger effect on the stellate cells.
Hence stimulus-driven DML stellate cells are capable of in-
hibiting pyramidal cells, and release from this inhibition occurs
when their parallel fiber inputs are blocked.

More proximal regions of pyramidal cell apical dendrites
receive synaptic input from a directly descending electrosen-
sory feedback pathway also originating in the nP (Sas and
Maler 1987). This pathway terminates in the ELL ventral
molecular layer (VML) and provides both glutamatergic and
GABAergic inputs (Maler and Mugnaini 1994; Wang and
Maler 1994). The excitatory portion of this pathway shows
reciprocal topographic mapping between the ELL and nP; that
is, subsets of ELL pyramidal cells project to stellate cells of the
contralateral nP and the axons of these stellate cells provide
descending excitation to the same population of ELL pyrami-
dal cells (Sas and Maler 1987). It has been proposed that the
positive feedback loop resulting from these reciprocal excita-
tory connections functions as a “sensory searchlight mecha-
nism” that highlights the representation of important elec-
trosensory stimuli (Berman and Maler 1998b, 1999; Bratton
and Bastian 1990; Maler and Mugnaini 1994).

The differences in pyramidal cells’ apical dendrites suggest
that those that have the lower spontaneous firing rates, the
weaker phase coupling to the EOD waveform, and the most
irregular patterns of spontaneous activity (large C \(_{V}\) and high
proportion of non-Poisson bursts) may also be most sensitive to
changes in dorsal molecular layer activity simply because their
more extensive apical dendrites are expected to receive input
from larger numbers of DML parallel fibers. Those with re-
duced dendritic arborizations are expected to be less influenced
by DML inputs and may have more static physiological prop-
erties. Other properties of the pyramidal cells also suggest that
they comprise a functionally heterogeneous population. The
more superficial cells with extensive dendritic arbors also show
more rapidly adapting responses to long-duration electrosen-
sory stimuli (Bastian and Courtright 1991). These cells have
high concentrations of both inositol trisphosphate and ryanod-
eine receptors (Berman et al. 1995; Zupanc et al. 1992) as well
as high concentrations of the NR2B NMDA receptor subunit
(R. J. Dunn and L. Maler, personal communication). That the
most superficial pyramidal cells also have the largest intracel-
lular Ca\(^{2+}\) stores while those with the smallest dendrites have
far less (Berman and Maler 1999) and that the pyramidal cell
plasticity is Ca\(^{2+}\) dependent (Bastian 1998b) leads to the
prediction that the superficial pyramidal cells should also show
more robust synaptic plasticity. Hence bursting behavior may
not only be modulated by changes in DML afferent activity,
but may also change with alterations in the strength of these
apical dendritic synapses.

Mechanisms of burst generation and functional implications

The mechanism of burst generation in ELL pyramidal cells
has been extensively studied in vitro (Lemon and Turner
as a consequence of prolonged somatic depolarizations, depo-
larizing afterdepolarizations (DAPs), that follow somatic
spikes. The somatic spikes can trigger broader dendritic Na\(^{+}\)
spikes that backpropagate within the apical dendritic tree. The
DAPs result from electrotonic conduction of the longer-dura-
tion dendritic Na\(^{+}\) spikes back into the soma, and if the DAP
rises above threshold, additional somatic spikes are initiated
and the process repeats producing spike bursts. The bursts are
terminated when the intraburst spike interval falls below a
critical value and dendritic spike failure occurs due to refrac-
toriness (Lemon and Turner 2000).

Neuronal membrane properties including ion channel types
and densities as well as morphological features are known to be
 correlates of both spontaneous and driven activity patterns
(Connors and Gutnick 1990), and dendritic morphology seems
to be a particularly important factor in determining bursting
characteristics. A recent modeling study (Mainen and Sej-
nowski 1996) showed that activity patterns ranging from reg-
ularly firing to intrinsically bursting could be demonstrated in
neurons having constant ion channel distributions and differing
only in dendritic structure. As originally described by Turner
et al. (1994) a delayed depolarization, or DAP, resulting from
activation of voltage-gated dendritic conductances, is critical
for the generation of bursts, and as seen in this study, bursting
was positively correlated with the size of a neuron’s dendritic arbor.

In addition to showing that burst probability is related to cellular morphology, our results suggest mechanisms by which dendritic inputs may modulate a cell’s firing pattern. In vitro studies showed that the pattern of ELL pyramidal cell activity evoked by depolarizing current injection could shift from a tonic to a bursty pattern contingent on the degree to which the cell was depolarized (Lemon and Turner 2000). In the present study, blockade of direct excitatory and disynaptic inhibitory inputs to the apical dendrites reduced bursting and hyperpolarizing current injection, resulting in membrane potential changes as small as \(-1\) to \(-2\) mV, mimicked this result. Hence modulation of descending inputs could bias pyramidal cells in a way that determines whether responses to electrosensory stimuli consist of bursts, enabling the cells to act as feature detectors (Gabbiani and Metzner 1999; Gabbiani et al. 1996; Metzner et al. 1998) or consist of more tonic changes in firing rate that are better suited to encoding detailed information about a stimulus.

Bursts seen in vivo and in vitro also showed some important differences. In vitro burst durations were much longer and bursts contained many more spikes than seen in vivo. In the former case, the average numbers of spikes/burst ranged from 8 to 61 depending on the ELL subdivision or map recorded from (Turner et al. 1996), while in vivo the average was between 2 and 3 spikes/burst (Gabbiani et al. 1996; and this study). Spike intervals within bursts recorded from cells in vitro were typically shorter, ranging from 3 to 13 ms (Lemon and Turner 2000) compared with 7 to 25 ms in vivo, and this may indicate differences in the temporal characteristics of the DAPs under in vitro and in vivo conditions. In vitro burst ISIs also often show serial patterning; alternating long and short intervals as well as serially decreasing intervals are seen and, in vitro, the last ISI of a burst is typically the shortest since refractory effects terminate in vitro bursts (Lemon and Turner 2000; Turner and Maler 1999; Turner et al. 1996). No consistent intraburst spike interval patterning was seen in vivo, and the last ISIs within bursts were not systematically different from any other intraburst ISI. The significantly shorter burst durations seen in vivo plus the absence of indications that short ISIs lead to burst termination suggests that processes other than refractory effects terminate bursts in vivo.

One possibility is that inhibitory interactions within the ELL terminate bursts and detailed analyses of inhibition in the ELL have recently appeared (Berman and Maler 1998a–c, 1999). Dorsal molecular layer parallel fibers excite pyramidal cells as well as inhibitory interneurons, DML stellate cells, and ventral molecular layer neurons, and both of these provide GABA\(_A\) inhibition to pyramidal cells (Berman and Maler 1998c). Stellate inhibition primarily reduces the amplitude of individual parallel fiber excitatory postsynaptic potential (EPSPs), while VML neuron inhibition is of longer duration and may modulate persistent Na\(^+\) channel currents (Berman and Maler 1998c, 1999). Given the importance of the persistent Na\(^+\) current in burst generation, VML cell inhibition is especially attractive as a candidate mechanism for burst termination in vivo. Appropriately timed excitatory parallel fiber inputs to pyramidal cells could initially augment the somatic spike-evoked depolarizing afterpotentials initiating bursts, and the disynaptically evoked inhibition could repolarize the cell terminating the burst. In addition, to terminating individual bursts, the results of the hyperpolarizing current injection experiments suggest that inhibitory inputs to the proximal apical dendrites and somatic regions could also modulate overall burst probability by participating in the control of a cell’s membrane potential.

In addition to the possibility that DML parallel fibers modulate pyramidal cell burst probability in a tonic fashion by altering the cell’s membrane potential and therefore the probability that a burst threshold will be exceeded, it is also possible that excitatory ventral molecular layer inputs trigger individual bursts. Nucleus praeminentialis stellate cells project bilaterally to the ELL ventral molecular layers and provide powerful excitatory inputs to the initial segments of the pyramidal cells’ apical dendrites (Sas and Maler 1983, 1987). These cells have very low rates of spontaneous activity, but they respond with high-frequency bursts of activity to certain patterns of electrosensory stimuli (Bratton and Bastian 1990), and stellate cell evoked EPSPs studied in vivo and in vitro show extreme frequency-dependent posttetanic potentiation (Bastian 1996b; Berman and Maler 1998b, 1999; Wang and Maler 1997, 1998). The reciprocally topographic relationships between ELL pyramidal cells and the nP stellate cells and the nP stellate cells’ burst-like responses to appropriate electrosensory stimuli and the highly facilitating pyramidal cell EPSPs evoked by the descending stellate activity all support the idea that this circuit behaves as a positive feedback loop that greatly amplifies responses to certain patterns of electrosensory input perhaps by evoking pyramidal cell bursts.

Electrosensory information serves at least two separate categories of behavior. Electromodulation behavior involves an animal’s detection of conspecifics’ discharges followed by the generation responses such as the jamming avoidance response (Heiligenberg 1991) or chirps (Bullock 1969; Larimer and Macdonald 1968). The stimuli received during electromodulation consist of the sum of an animal’s own discharge plus that of a conspecific. This summation produces a beat waveform consisting of cyclic patterns of amplitude and relative phase modulations (Heiligenberg 1991). These EOD modulations are spatially extensive and influence pyramidal cell receptive field centers and antagonistic surrounds simultaneously. Previous studies demonstrated that pyramidal cell spike bursts encode the occurrence of EOD amplitude increases and decreases more reliably than either electroreceptor afferent spikes or pyramidal cell single spikes (Gabbiani and Metzner 1999; Metzner et al. 1998), and accurate representation of the timing of these stimulus features is critical for the control of these electromodulation behaviors (Heiligenberg 1991).

Electrolocation stimuli also generate EOD amplitude and phase modulations, but unlike electromodulation signals, these AMs are spatially localized and typically have a movement component. Hence electrolocation stimuli may affect subdivisions of pyramidal cell receptive fields sequentially. As in the case of electromodulation stimuli, increases or decreases in EOD amplitude comprise important electrolocation stimulus features and reliably encoding these as bursts of spikes produced by a subset of the somatotopically mapped pyramidal cell population could, for example, indicate the current position of a moving object. However, it also seems likely that detailed information about the time course of the AM waveform may be important for electrolocation and cells
with high firing rates are better suited for encoding this information (Metzner et al. 1998). Although the highest spontaneous firing rates seen for pyramidal cells, 40–50 spikes/s, are still well below that typical for receptor afferents (150–400 spikes/s), additional studies focusing on these nonbursty pyramidal cells are needed to determine their potential for encoding detailed information about the spatially localized EOD modulations that occur during electrolocation.

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