Comparative Electrophysiology of Pyramidal and Sparsely Spiny Stellate Neurons of the Neocortex

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SUMMARY AND CONCLUSIONS

1. Slices of sensorimotor and anterior cingulate cortex from guinea pigs were maintained in vitro and bathed in a normal physiological medium. Electrophysiological properties of neurons were assessed with intracellular recording techniques. Some neurons were identified morphologically by intracellular injection of the fluorescent dye Lucifer yellow CH.

2. Two distinct neuronal classes of electrophysiological behavior were observed; these were termed regular spiking, bursting, and fast spiking. The physiological properties of neurons from sensorimotor and anterior cingulate areas did not differ significantly.

3. Regular-spiking cells were characterized by action potentials with a mean duration of 0.80 ms at one-half amplitude, a ratio of maximum rate of spike rise to maximum rate of fall of 4.12, and a prominent afterhyperpolarization following a train of spikes. The primary slope of initial spike frequency versus injected current intensity was 241 Hz/A. During prolonged supra-threshold current pulses the frequency of firing adapted strongly. When local synaptic pathways were activated, all cells were transiently excited and then strongly inhibited.

4. Bursting cells were distinguished by their ability to generate endogenous, all-or-none bursts of three to five action potentials. Their properties were otherwise very similar to regular-spiking cells. The ability to generate a burst was eliminated when the membrane was depolarized to near the firing threshold with tonic current. By contrast, hyperpolarization of regular-spiking (i.e., nonbursting) cells did not uncover latent bursting tendencies.

5. The action potentials of fast-spiking cells were much briefer (mean of 0.32 ms) than those of the other cell types. This was accomplished by an unusually high rate of fall; the ratio of the rate of spike rise to fall was 1.39. Fast-spiking cells had a mean primary frequency-current relationship of 549 Hz/nA, and no displayed very little or no frequency adaptation. Local synaptic activation powerfully excited these neurons, and inhibition was usually weak or absent.

6. Injections of Lucifer yellow CH revealed that both regular-spiking and bursting cells were spiny pyramidal neurons. Regular-spiking pyramids were found in all layers below layer I, whereas bursting pyramids were observed mainly in layer IV or upper layer V. Fast-spiking neurons were invariably apical or sparsely spiny stellate cells, with bifurcated or radial dendritic arrangements, and were found in layers II–VI.

7. Guinea pig neocortex was reacted immunocytochemically for glutamic acid decarboxylase (GAD), the synthetic enzyme for the inhibitory neurotransmitter γ-aminobutyric acid (GABA). GAD-positive cells were distributed among all cortical layers. Their somatodendritic morphology and size distribution were very similar to those of the Lucifer yellow-labeled fast-spiking cells, implying that fast-spiking cells may use GABA as their neurotransmitter.

8. We suggest that a minority of neocortical pyramidal cells possess intrinsic burst-generating mechanisms. These cells are most densely localized in the middle cortical layers. Further, we suggest that GABAergic nonpyramidal neurons have electrophysiological properties that are distinctly different from pyramidal cells. Physiological evidence from subthreshold and action potential behaviors have been seen in different regions (cf. Refs. 20, 43, 56). It has also been possible to distinguish pyramidal from nonpyramidal cell types by physiological criteria (43, 45, 47). Most of the hippocampal studies were aided by the technical advantages of the in vitro preparation.

In a previous electrophysiological study of the guinea pig sensorimotor cortex in vivo we observed neurons with two qualitatively different modes of action potential generation (11). The most common type generated single spikes to a just-threshold stimulus and was distributed in all layers from I to VI. A rarer type generated a burst of spikes (≥3) when brief stimulus exceeded threshold, and was found only in layer IV and upper layer V. The morphology of these cells was not examined. In the present study we have used intracellular dye injections to identify electrophysiologically characterized neocortical neurons. We have found that neurons similar to both functional types described previously (11) have a pyramidal morphology. In addition, we report a third class of cells, identifiable by membrane physiology and somatodendritic shape, that seems to correspond to the type of nonpyramidal neurons that use the inhibitory neurotransmitter γ-aminobutyric acid (GABA).

METHODS

1. Slicing procedure

In vitro neocortical slices were prepared by methods that have been described in detail, with occasional exceptions that will be noted. Albino guinea pigs (250–400 g) were decapitated, and blocks of tissue were dissected from either anterior cingulate or parietal regions that correspond histologically to the sensorimotor area of rat cortex (17).

2. Tissue was immersed in ice-cold Ringer solution. Coronal slices (400–500 μm thick) were prepared with either a tissue chopper or a vibratome (Lancer series 1600). Slices were immediately placed in a recording chamber and allowed to incubate for at least 1 h before recordings were begun.

3. Recording chambers held the dyes on lens paper-covered plates that were continuously superfused with physiological solution. Humidified gas of 95% O2-5% CO2 flowed over the slice surface; temperature was thermostatically held at 35–37°C. Normal bathing solution contained (mM): NaCl 124, KC1 5, CaC12 2, MgSO4 2, NaH2PO4 1.2,
NaHCO₃ 26, dextrose 10; when saturated with 95% O₂-5% CO₂ the pH was 7.4.

Electrophysiological techniques

Standardized recording and stimulation techniques were employed. Extracellular stimuli (200 μs duration) were delivered through mono- or bipolar sharpened tungsten wires situated in either deep cortical layers or underlying white matter. Glass microelectrodes were formed from either 1-mm thin-walled capillaries (MP Instruments) and used unbaffled, or from 1-mm standard-walled capillaries (Frederick Haer) that were beveled on an alumina-coated wheel (Sutter Instruments). When filled with 4.3 M potassium acetate the former electrodes had resistances of 35–45 MΩ, whereas the latter were 125–175 MΩ. For intracellular labeling, electrodes were filled with a 2% solution of Lucifer yellow CH (Aladdin) in 0.1–2 M lithium acetate (93). Lucifer yellow was injected by passing hyperpolarizing current pulses (0.5–5 nA, 800 ms duration) at 1 Hz for 5–10 min (30). Electrochemical data were recorded on magnetic tape (0.5-kHz data were digitized offline and analyzed with a Mini-computer (Digital Equipment). Since Lucifer yellow-filled electrodes, because of their high resistance, yielded generally noisy intracellular recordings, data from these cells were not included in the quantitative analyses summarized in Table 1. The laminar positions of intracellular recordings were measured in the chamber and compared to Nissl-stained sections corrected for shrinkage.

Histological methods

Cells labeled with Lucifer yellow-injected cells were fixed in 10% formalin in phosphate buffer, dehydrated in a graded series of alcohols, and cleared in xylene or methyl salicylate. Cells were viewed with a Leitz microscope equipped with epifluorescence. Immunocytochemistry to glial fibrillary acidic protein (GFAP) was performed by the avidin-biotin-peroxidase method using avidin-biotin-peroxidase complex (ABC kit, Vector Laboratories). The chromagen used was diaminobenzidine. Control sections were incubated with a preimmune sheep serum under identical conditions and showed no staining. Sections or areas were drawn with the use of a camera lucida attachment. Measurements of cell sizes were carried out on a digitizing tablet (MOP; Zeiss) and are uncorrected for tissue shrinkage caused by dehydration.

RESULTS

Analysis of several hundred intraneuronal recordings from anterior cingulate and somatosensory cortical slices revealed three distinct classes of electrophysiological behavior. There were no significant differences in neuronal properties between these two cortical areas. Thus, all data presented below were pooled, unless noted. Injections of Lucifer yellow CH into some of these neurons suggested that each physiologically defined group also has a characteristic set of morphological features. For convenience, we will subsequently refer to these classes as J) regular-spiking cells (after Mountcastle et al., Ref. 64), J) bursting cells, and J) fast-spiking cells (after Simons, Ref. 82). These designations are based on characteristic physiological properties that are summarized in Table 1 and described in detail in the following sections.

Regular-spiking cells

The vast majority of neurons encountered in these experiments had electrophysiological properties similar to those described in detail in a previous study of neocortical slices (11). We have further analyzed some of the characteristics of these neurons, and the data are summarized in Fig. 1 and Table 1. The regular-spiking cells are distinguished from fast-spiking cells by their action potential durations (0.6–1.0 ms measured at one-half amplitude), prominent prolonged afterhyperpolarizations (AHPs) after trains of spikes, and a marked adaptation of spike frequency during prolonged depolarizing current pulses. Careful grading of injected-current intensity near the one suggested by this group. Guinea pigs were perfused transcardially with 4% paraformaldehyde and 0.1% glutaraldehyde, and cortical sections were cut 50 μm thick on a vibratome. Anterior is defined as 1/1,000, and localization was carried out using the avidin-biotin-peroxidase complex (37; Vestastrum ABC kit, Vector Laboratories). The chromagen used was diaminobenzidine. Control sections were incubated with a preimmune sheep serum under identical conditions and showed no staining. Sections or areas were drawn with the use of a camera lucida attachment. Measurements of cell sizes were carried out on a digitizing tablet (MOP; Zeiss) and are uncorrected for tissue shrinkage caused by dehydration.

Table 1. Properties of three classes of neocortical neurons

<table>
<thead>
<tr>
<th>Class</th>
<th>Regular Spiking</th>
<th>Bursting</th>
<th>Fast Spiking</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>24</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>V_m (mV)</td>
<td>73.6 ± 5.1</td>
<td>72.2 ± 6.5</td>
<td>71.6 ± 7.1</td>
</tr>
<tr>
<td>(n = 11)</td>
<td>(n = 11)</td>
<td>(n = 11)</td>
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</tr>
<tr>
<td>R_m (Mohm)</td>
<td>39.9 ± 21.2</td>
<td>31.2 ± 8.5</td>
<td>55.6 ± 33.6</td>
</tr>
<tr>
<td>(n = 11)</td>
<td>(n = 11)</td>
<td>(n = 11)</td>
<td></td>
</tr>
<tr>
<td>t_on (ms)</td>
<td>20.2 ± 4.6</td>
<td>16.6 ± 5.9</td>
<td>11.9 ± 6.4</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>(n = 9)</td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>Spike amplitude (mV)</td>
<td>95.2 ± 9.5</td>
<td>85.5 ± 14.9</td>
<td>92.1 ± 9.7</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 9)</td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>Max rate of rise (V/µs)</td>
<td>37.4 ± 20.3</td>
<td>33.4 ± 10.5</td>
<td>40.3 ± 20.2</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td></td>
</tr>
<tr>
<td>Spike width (ms)</td>
<td>1.7 ± 0.41</td>
<td>1.5 ± 0.31</td>
<td>0.66 ± 0.19</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
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<tr>
<td>Spike width, 60 µs (ms)</td>
<td>0.00 ± 0.18</td>
<td>0.30 ± 0.14</td>
<td>0.32 ± 0.10</td>
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<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td></td>
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<tr>
<td>J/slope, primary, Hz/µA</td>
<td>2.01 ± 2.02</td>
<td>1.5 ± 0.14</td>
<td>0.66 ± 0.19</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td></td>
</tr>
<tr>
<td>Fast AHP</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Prolonged AHP</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Adaptation</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Evoked EPSP</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Laminar location</td>
<td>II-VI</td>
<td>IV-V</td>
<td>II-VI</td>
</tr>
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</table>

All data expressed as mean ± SD. Mann-Whitney U test performed to compare differences from regular-spiking cells (P < 0.05; T < 0.01). T_0, resting membrane potential; R_m, Input resistance; t_on, membrane time constant; J/s, ratio of maximum rate of rise to maximum rate of fall; AHP, afterhyperpolarization; EPSP, inhibitory postsynaptic potential; +, present; characteristic, +, lacks characteristic.

revealed a mean initial slope of 241 Hz/µA (Fig. 1C). Secondary slopes were evident in most cells at higher currents, and these averaged 93 ± 50 Hz/µA (n = 10). When neurons were activated sympathetically, by single 200-μs cathodal pulses with the underlying white matter or deep layers, all neurons displayed an initial excitatory postsynaptic potential (EPSP) followed, in most cells, by one or more forms of inhibitory postsynaptic potential (IPS), as described previously (11, 96). At high stimulus intensities the secondary or occasionally two spikes could be evoked by the short-latency EPSP.

Neurons with regular-spiking characteristics were encountered in layers II-VI. When labeled with Lucifer yellow they were invariably identified as pyramidal cells, with prominent spiny apical and basal dendrites. The somata of 15 labeled cells had vertical diameters of 24.6 ± 7.3 μm (mean ± SD) and horizontal diameters of 18.5 ± 4.3 μm. Axons, when they could be traced, extended toward or into the underlying white matter, and usually had numerous branches that arborized locally. Illustrations of Lucifer yellow-stained pyramidal cells of guinea pig neocortex are presented in previous publications (5, 29, 36).

Bursting cells

In a previous study (11) intrinsic burst-generating neurons were observed in layer IV and the superficial portion of layer V in guinea pig sensorimotor cortex. Similar observations have now been made in anterior cingulate cortex, and we report here some further characteristics of burst-generating cells from both
cortical areas. The primary physiological property that distinguishes bursting cells from the two other types is their ability to generate, at a distinct threshold, a complex of three to five action potentials. Both regular-spiking and fast-spiking cells, by contrast, generate only single action potentials when stimuli are poised near threshold, and their spike frequency is a monotonic function of injected current.

Intrinsic bursting behavior is illustrated in Fig. 2. In this cell a spike complex could be evoked with both synaptic (Fig. 2A) and injected-current (Fig. 2Al) stimuli. The burst waveform is similar in each case, consisting of three or four spikes riding upon a slow depolarizing envelope. During the course of a burst, spike amplitude and rate of rise decreased, while spike duration increased. Burst latency, but usually not waveform, was very sensitive to the intensity of the stimulus. Each burst was terminated by a prolonged AHP. Bursts were not always generated all-or-none. For example, the cell illustrated in Fig. 2B displayed, at threshold, single spikes with prominent depolarizing afterpotentials (Fig. 2B1). When the current was slightly increased, however, a complex of two spikes was evoked (Fig. 2B2), and a slightly larger current pulse elicited a more typical complex of three to four action potentials (Fig. 2B3).

Neurons of the inferior olive (50) and thalamic nuclei (15, 40) appear to have slow calcium currents that are inactivated near resting potential; hyperpolarization removes this inactivation and allows membrane potential oscillations and intrinsic spike bursts. We tested the possibility that a similar voltage-dependent current might underlie intrinsic burst generation in neocortical cells, and that cells without bursting characteristics at resting potential could be converted to a bursting mode by a preceding hyperpolarization. For cortical bursting cells the probability of generating a burst during a stimulus was indeed dependent upon the previous voltage of the membrane. When continuous current was used to depolarize the membrane to near or above firing threshold, pulses of current that had previously evoked an all-or-none burst (Fig. 3A, 75 mV) elicited only trains of regularly spaced spikes (Fig. 3C, 75 mV). This suggests that the burst-generating mechanism was inactivated at depolarized levels. Similarly, when began at resting potential, long pulses of suprathreshold depolarizing current in these cells always elicited only a single burst of spikes followed, after an AHP, by a train of single action potentials rather than additional bursts (Figs. 4A, 75 mV, 4A; cf. Figs. 3C and 6 of Ref. 11). By contrast, regular-spiking cells that did not generate spike bursts when rapidly depolarized from their normal resting potential also did not burst when resting potential was manipulated over a wide range with tonic current injection (Fig. 3B). These results suggest that
the conductance mechanisms underlying intrinsic burst generation are differentially distributed among the neurons of the neocortex.

The bursting capability of a cell did not seem to be a consequence of injury due to electrode impalement. Nonbursting neurons did not generate bursts when initially impaled, before hyperpolarization had stabilized, or after a previously stable recording had begun to deteriorate.

The passive membrane properties of bursting cells were similar to those of the more common regular-spiking cells (Table 1). When compared between these two cell types, the duration and maximum rates of rise and fall of the first action potential generated by a threshold stimulus were indistinguishable. Spike amplitudes in bursting cells were slightly lower, however. Synaptic activation in either cell type also yielded similar effects, including short-latency excitation and prolonged complex IPSPs (Fig. 4B). When synaptic inhibition was very prominent, it was usually impossible to evoke an intrinsic burst with orthodromic activation.

Sever burstng cells were injected with Lucifer yellow in anterior cingulate and somatosensory slices. All cells were spiny pyramidal neurons with prominent apical and basal dendrites (Fig. 5). Their somata were located in layer IV or upper layer V. The morphological characteristics of labeled bursting cells were indistinguishable from those of Lucifer yellow-stained regular-spiking (i.e., nonbursting) cells (see previous section). Bursting cell somata measured 23.1 ± 5.1 µm vertically and 17.0 ± 3.8 µm horizontally. These sizes were not significantly different from those of the nonbursting pyramids. In one case, injection of Lucifer yellow into a bursting cell yielded two well-stained pyramidal neurons. Dye coupling of neocortical pyramidal cells has been observed previously (9, 30) and may indicate the presence of dendrodendritic or dendroaxonic gap junctions (84).

Fast-spiking cells

A third distinct class of neuron, the fast-spiking cell, was encountered only rarely in all layers from II to VI. Impulses of these cells occurred much more frequently with high-resistance bevelled microelectrodes than with low-resistance bevelled ones. With both types of electrode, however, recordings were generally shorter and less stable than those from regular-spiking cells. Nevertheless, 25 fast-spiking cells with stable membrane potentials were recorded for periods from a few minutes to over 1 h; these form the basis for our quantitative analysis, as summarized in Table 1. Resting membrane potentials for these cells ranged from −60 to −87 mV (2 ± 71.5 ± 7.8 mV), and spike amplitudes ranged from 62 to 105 mV (2 ± 82.1 ± 9.3 mV). These values are somewhat lower than those of the regular-spiking cells (Table 1), but this may simply reflect the vagaries of recording from small neurons.

When activated with intracellular current pulses near threshold, each action potential was followed by a brief (10- to 15-ms) but prominent (8- to 15-mV amplitude) hyperpolarization undershoot (Fig. 6, 0.7 nA). Larger current pulses elicited sustained trains of spikes (Fig. 6, 0.35-1.1 nA), but not the slow AHPs (Fig. 6B) that are so characteristic of both slow-spiking and bursting cells (cf. Fig. 1, Rand 6B, and Fig. 3 of Ref. 11). All but two of the fast-spiking neurons lacked large, prolonged AHPs, regardless of their resting potentials.

Fast-spiking neurons were in general not spontaneously active at rest, and these were the highest resting potentials had spike thresholds 10–20 mV above the resting level. The primary slopes of their current-frequency plots were linear up to frequencies of 200–250 Hz (Fig. 6C), and had a mean value of 549 ± 261 Hz/ma. This value is significantly higher than that of the regular-spiking cells. During current pulses of 100–200 ms the frequency of fast-spiking cells did not adapt, even at high current levels (Fig. 6D). Indeed, unlike regular-spiking
Fig. 6. Electrophysiological properties of fast-spiking neurons. A: response to depolarizing intracellular current injection. Magnitude of current (in nA) is indicated to the left of each trace. B: illustration of the relative lack of a prolonged afterhyperpolarization (AHP) after a train of action potentials. Two different cells, whose resting potentials were -65 and -67 mV, are shown. C: frequency-current (f-I) plots for these fast-spiking neurons. In most cells, 3-5 separate slopes could be discerned. D: interspike interval (ISI) vs. interval number for cell illustrated in A. Magnitude of injected current is indicated to the right of graph. Note the initial increase in spike frequency (i.e., decrease in ISI) during the lower-intensity currents, followed by periods of sustained high-frequency firing (cf. Fig. 1D).

cells (cf. Fig. 1D), the initial interspike intervals were often longer than the sustained levels.

Fast-spiking cells could be unambiguously distinguished from the other types described here by the time course of their action potentials. Figure 7, A and B, illustrates representative spikes from the fast and regular categories. It is evident that there are significant differences in total action-potential duration, rate and duration of the falling phase, and afterpotential. When the distribution of spike widths was examined across all cells (Fig. 7C), there was a nonoverlapping bimodal distribution with a boundary at ~0.5 ms. The basis for this difference was examined by quantifying the rates of rise and fall from differentiated traces. As shown in Table 1, the maximum rates of spike depolarization were similar in all cell categories. The maximum rates of repolarization, however, were significantly greater in the fast-spiking neurons than in both regular-spiking and bursting classes. These data indicate that the brevity of the fast action potentials is due to a rapid repolarizing phase.

The absolute maximum rates of action potential rise and fall were found to be rather sensitive to action potential amplitudes, when compared between different neurons and
within the same neuron. The ratio of maximum rate of rise to maximum rate of fall (dV/dt ratio), however, was much less sensitive. For fast-spiking cells the mean value of this ratio was 1.39; the values of 4.12 for regular-spiking and 3.5 for bursting cells were very significantly higher (Table 1). The distribution of dV/dt ratio across all cells was distinctly bimodal (Fig. 7D). Among the measured physiological parameters, a small spike dV/dt ratio appears to be the most reliable criterion for inclusion in the fast-spiking class.

The current–voltage relationships of 10 fast-spiking cells were examined by applying square current pulses of variable amplitude through the recording electrode. The data of Fig. 8 are representative of this group. Seven of the cells had a linear current–voltage rela-

FIG. 8. Current–voltage relations for three different fast-spiking neurons. A: T-V plots for the 3 neurons with each point representing a change of 0.05 nA in injected current. Zero mV on the vertical scale corresponds to absolute level of −75 mV. B: Response of the 3 cells to hyperpolarizing and depolarizing currents of equal magnitude. In each case the largest depolarizing current resulted in a membrane voltage just below firing threshold. Top trace in each set represents current, while the bottom trace represents voltage. Numbers in B correspond to those in A. Change in voltage was measured just before the end of the current pulse. Note the increase of baseline noise in the largest depolarizing response of the cell illustrated in B.

FIG. 7. Characteristics of action potentials of fast-spiking, regular-spiking, and bursting neurons. A: action potential of typical fast-spiking neuron. Top: deviation in Vm during action potential. Bottom: voltage trace differentiated with respect to time. Positive slope is downwards. B: action potential of typical regular-spiking neuron. Note the slower rise to peak. C: distribution of spike widths measured at one-half amplitude for the 3 types of cells: fast-spiking neurons (n = 25; open bars), regular-spiking neurons (n = 24; solid bars), and bursting neurons (n = 13; hatched bars). Bars of bursting neurons in C and D are placed above bars of regular-spiking neurons. D: distribution of the maximum rate of spike rise over the maximum rate of spike fall (spike dV/dt ratio) for the 3 classes of cells studied. All data were obtained from the first action potential evoked by a suprathreshold current step. Group designations in C apply also in D.
tionship over the range tested (Fig. 8, cell 2), while three displayed an increase in slope (e.g., an increase in apparent input resistance) in the voltage range just below spike threshold (Fig. 8, cells 1 and 3). The latter property is common among regular-spiking neurons (11, 89), although it is not ubiquitous. In these fast-spiking cells an anomalous increase in spontaneous voltage fluctuations occurred during deactivation just below action-potential threshold (Fig. 8B, cell 1). Each cell was recorded with a different electrode in different slices. These events resembled spontaneous synaptic noise, but were not further investigated.

Fast-spiking neurons were further distinguished by their synaptic responses to single stimuli applied to the deep cortical layers or the underlying white matter. In 13 of 21 cells tested such stimuli evoked an EPSP of graded amplitude and duration with no visible hyperpolarizing components (Fig. 9, 4 and B). At higher stimulus intensities these EPSPs triggered trains of spikes that could reach frequencies of 100-400 Hz. EPSPs ranged up to 120 mV in duration and 15 mV in amplitude. To more sensitively test for the presence of hidden synaptic inhibition, strong orthodromic stimuli were applied during a current-evoked train of action potentials (Fig. 10A).

Compared to a control train, the orthodromic response only increased the frequency of firing (Fig. 10B), making it unlikely that significant net inhibition had been evoked. By contrast, in eight additional fast-spiking cells orthodromic activation elicited hyperpolarizings.

FIG. 11. Response of a fast-spiking neuron to three consecutive orthodromic stimuli (1 Hz) in the presence of 10 μM bicuculline methiodide. Each stimulus caused a paroxysmal field potential (upper trace in each set) indicative of synchronized discharges in the local population of pyramidal cells. Consecutive stimuli evoked successively larger responses (top to bottom). The paroxysmal field potential was accompanied by a high-frequency discharge reaching a maximum of 650 Hz in the fast-spiking neuron (bottom trace in each set). As the magnitude of the field potential increased, the duration of discharge in the neuron lengthened.

The pharmacology of the fast-spiking neurons was also studied. Bicuculline (10 μM), a potent GABA antagonist, leads to the generation of synchronous, synaptically driven depolarization shifts in most or all pyramidal cells (8, 28). The behavior of two fast-spiking cells was recorded under these conditions. As illustrated in Fig. 11, during an evoked synchronous epilepticiform discharge, monitored by the extracellular paroxysmal field potential (upper traces; cf. Ref. 8), the cell was depolarized and fired a sustained train of spikes. Action potential frequency reached a peak level of 650 Hz. When this slice was repetitively stimulated at 1 Hz the amplitude and duration of the paroxysmal field potentials were progressively facilitated (Fig. 11, top to bottom). The simultaneously recorded response of the fast-spiking cells was progressively lengthened without significant change in its peak amplitude or maximal spike frequency.

Ten neurons filled with Lucifer yellow were found to be nonpyramidal in morphology (Figs. 12 and 13). These neurons possessed physiological characteristics that were very similar to the fast-spiking neurons detailed above, and distinctly different from both regular-spiking and bursting cells. The D/I ratio of the action potentials of these identified cells varied from 1.2 to 2.2 and therefore were within the normal range of fast-spiking cells (Fig. 7D). In general, the recordings with Lucifer yellow/lithium acetate-filled microelectrodes were of poorer quality than those obtained with potassium acetate-filled microelectrodes, presumably because of greatly increased electrode resistance, nonlinearities in current passing capabilities, and possible effects of lithium on voltage-dependent potassium currents (57). As illustrated in the photomicrographs of Fig. 12 and the tracings of Fig. 13, all of these labeled neurons were aspiny or sparsely spiny stellate cells. Four had a vertically oriented, fusiform cell body with a bifurcated arrangement of dendrites (cf. Ref. 72); their overall dendritic fields were boulanger shaped (Figs. 12A, 13A). The other labeled stellates had more spherical somata with radiating multipolar dendrites (Fig. 12B, 13B and C). The dendritic fields of these cells extended a maximal detectable distance of 60-80 μm from the cell bodies, often crossing into adjacent cortical layers. It is possible that the maximal dendritic extent of some cells was underestimated because this distal processes could not be visualized. The sizes of labeled somata are presented in Table 2.

GABA immunocytochemistry

Fast-spiking cells identified as aspiny or sparsely spiny stellate cells resemble neurons of rat, cat, and monkey cortex that have
previously been reported to stain positively for GAD immunoreactivity (22, 36, 75). This suggests that some or all fast-spiking cells may correspond to GABAergic interneurons, since high intracellular levels of GAD seem to be correlated with a GABA-mediated inhibitory function in some systems (76). To further examine this hypothesis we tested both circulate and sensorimotor cortex of guinea pig for GAD immunoreactivity. The sizes, laminar distribution, and somadendritic morphologies of the GAD-positive cells were examined.

GAD-positive neurons were distributed among all cortical layers in both anterior cingulate and sensorimotor areas (Fig. 14, A–E).

**TABLE 2. Soma sizes of fast-spiking and GAD-positive cells**

<table>
<thead>
<tr>
<th></th>
<th>GAD Positive</th>
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<tbody>
<tr>
<td></td>
<td>Anterior</td>
</tr>
<tr>
<td><strong>4</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Fast spiking</strong></td>
<td>E1</td>
</tr>
<tr>
<td><strong>Sensorimotor</strong></td>
<td>D2</td>
</tr>
<tr>
<td><strong>Major diam.</strong></td>
<td>14.1 ± 3.1</td>
</tr>
<tr>
<td><strong>μm</strong></td>
<td>14.7 ± 5.0</td>
</tr>
<tr>
<td><strong>Area, um²</strong></td>
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<tr>
<td><strong>(81–204)</strong></td>
<td>(20–370)</td>
</tr>
<tr>
<td><strong>e</strong></td>
<td>350</td>
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</tbody>
</table>

Data are expressed as mean ± SD with the ranges in parentheses. GAD, glutamic acid decarboxylase.
In addition, it was possible to discern GAD-positive puncta that often appeared to surround the unstained profiles of cells. In some cases the puncta clearly outlined pyramidal neurons, identifiable by the shape of their soma and a prominent apical dendrite (Fig. 14F). Electron microscopic studies of similar punctate structures have identified them as axosomatic and axodendritic synapses with symmetric profiles (22, 23, 75). Neurons with GAD-positive somata and proximal dendrites took a variety of forms. Some had a relatively spherical cell body with several radiating dendrites and no clear orientation (Fig. 14D), whereas others had a more fusiform soma with two or more dendrites projecting from each pole, usually oriented vertically (Fig. 14E). The morphology of GAD-positive cells is shown in more detail in the camera lucida drawings of Fig. 15. The cells illustrated in Fig. 15A closely resemble the asynaptic and sparsely spiny bristle cells identified in Golgi-stained neocortex (70, 72), as well as some of our Lucifer yellow-stained, fast-spiking neurons (cf. Figs. 12A, 13A). Figure 14B shows profiles of GAD-positive multipolar cells with round cell bodies, which mimic our other examples of labeled fast-spiking neurons (cf. Figs. 12B, 13B). The mean soma size of the GAD-positive neurons was also very similar to those of the Lucifer yellow-stained fast-spiking cells (Table 2). The range of somatic sizes was considerably larger for the GAD-positive cells than for the Lucifer yellow-labeled cells, however. Both groups were significantly smaller, on average, than the Lucifer yellow-stained pyramidal cells.

The close correspondence between somadendritic morphology, laminar distribution, and somatic size for GAD-positive cells and Lucifer yellow-labeled fast-spiking cells suggests that the two populations are either identical or strongly overlapping.

**Discussion**

We have observed three general types of electrophysiological behavior in neocortical neurons in vitro. The most distinguishing characteristics of these classes are: 1) relatively slow action potentials with no tendency for intrinsic bursting (regular-spiking neurons), 2) relatively slow action potentials that do tend to burst in groups of three to five spikes (bursting neurons), and 3) fast action potentials with a uniquely rapid rate of fall and a prominent postspike undershoot (fast-spiking neurons). Intracellular labeling with Lucifer yellow CH has allowed tentative correlations of these physiological characteristics with specific neuron morphologies. Both regular-spiking and bursting cell types were spiny pyramidal neurons. The only structural difference we discerned was that the somata of robust bursting pyramids were confined to middle cortical layers (11), whereas nonbursting pyramids were found in all laminae below I. Fast-spiking neurons were invariably aspiny stellate cells and could be found in all layers.

This investigation is far from comprehensive. Several morphological classes were not observed among our stained neurons. Most
notably, these include spiny stellate and bipolar cells. Spiny stellate cells are small and abundant in layer IV of primary sensory areas; they receive a strong direct thalamic input, and their synaptic field structure implies that they are excitatory local circuit neurons (51). Bipolar cells, characterized by two principal dendrites oriented in a long, narrow vertical array, have recently been shown to be immunoreactive for the neuroactive peptides cholecystokinin (19, 30) and vasoactive intestinal polypeptide (7, 33), as well as the synthetic enzyme for acetylcholine, choline acetyltransferase (18, 35).

**Regular-spiking pyramidal cells**

The principal type of neuron in the neocortex is the pyramidal cell, which constitutes about two-thirds of all neurons in a variety of species and cortical areas (96, 102). While the membrane properties of cells in this class have been extensively examined, both in vivo and in vitro, their identification in the vast majority of studies has either been inferred from physiological criteria or simply assumed. Our correlational physiological and morphological data strongly suggest that most guinea pig pyramidal cells share a similar mode of action potential electrogenesis. The bursting neurons, which have also been defined as pyramidal cells in this study (see below), closely resemble the nonbursting pyramids except for their more explosive mode of firing. The most distinct property of both of these cell types is the D/V ratio, a value > 2.2 was always associated with a pyramidal morphology in this study.

**Bursting pyramidal cells**

The present results demonstrate that at least some neurons generating intrinsically bursting patterns of action potentials are pyramidal neurons. Although variations in the size and dendritic structure of all of these neurons were evident, it was not possible to distinguish bursting from nonbursting cells by morphological criteria. Quantitative structural analyses (e.g., Ref. 35), which were not attempted, might yet reveal identifying features. In a recent study of rat sensorimotor cortex, Landry et al. (48) identified a small number of neurons that generated slow depolarizing potentials that were apparently intrinsically generated events. When stained with FITC, all of these neurons were pyramidal cells. In contrast, Grace and Lilja (27) have reported that all pyramidal neurons of guinea pig prefrontal cortex and hippocampal pyramidal cells have the ability to burst. Because we have not stained all of the major cortical cell types, the possibility remains that some bursting cells have a nonpyramidal shape. Indeed, the recent studies of M. J. Guitton and E. L. Volman (personal communication) indicate that both bursting and nonbursting (i.e., regular-spiking) cells may be either pyramidal cells or spiny stellate cells. In their study, the relations between the various extracellular action potentials recorded in the various cat's neurones are complex and cannot ultimately determine the instantaneous firing rate (85).

**Fast-spiking stellate cells**

The neurons that we have classified as fast-spiking are analogous to neurons in the remaining cells by several criteria, most notably a short spike duration, low ratio of action-potential rate to rate of depolarization, lack of prominent frequency adaptation. When stained, cells with these properties were always morphologically distinct from the pyramidal cells; they were typically medium- to sparsely stellate cells with several dendritic orientations. The results suggest, therefore, that a simple assessment of certain
membrane properties is sufficient to distinguish spiny principal cells from some types of sparsely spiny nonpyramidal cells. As pointed out in a previous section, the validity of this conclusion will rest upon a more comprehensive sampling of the various cortical cell types. Observations from the primary somatosensory cortices of rat (82) and monkey (64) have demonstrated two classes of single-cell action potentials; the slow, or "regular" spikes (64) and the "fast" (82), or "thin" (64) spikes. Our results provide strong support for the suggestion (84, 63) that regular spikes correspond to pyramidal cells and fast spikes arise from smooth stellate cells. This identity should greatly facilitate the interpretation of physiological data from cortical studies in intact animals.

Fast-spiking neurons correspond to the cortical cell types that are thought to use the inhibitory neurotransmitter GABA. Thus, all of our Lucifer yellow-stained cells had either multipolar radial projecting dendrites, vertically oriented, bifurcated sets of dendrites, or some intermediate arrangement. Cells of similar morphology have been extensively described in many areas of neocortex in a variety of species (e.g., Refs. 42, 70, 72, 97, 99). The fine structure of their synaptic terminations suggests an inhibitory function (33, 85). More importantly, cells of these types selectively accumulate tetradibutyl GABA (33, 86), exhibit strong immunoreactivity for the GABA syn- thetic enzyme GAD (56, 71, 75), and for GABA itself (68, 79), and react strongly for the GABA degradative enzyme GABA-transaminase (65). In addition, recent immunocytochemical data have shown that some GAD-positive cells in the cortex may also contain either somatostatin or cholecystokinin (59, 60, 67). The results of our GAD immunocytochemistry are consistent with the data gathered from other species. GAD-positive neurons in visual cortex have shapes and sizes very similar to those of the Lucifer yellow-filled, fast-spiking cells. These similarities strongly imply an identity.

The transmitter GABA is considered to be the most important inhibitory substance in the cortex (47). Local antagonism of GABA receptors weakly disrupts the normal integrative functions of the cortex (81), and can also lead to epileptic discharge (53). The fast-spiking, nonadapting mode of firing favored by the putative GABAergic cells may allow rapid, faithful, and sustained dissemination of inhibitory influences, as defined by the axonal arborization of each cell. In primary somatosensory cortex, fast-spiking units have a much more broadly tuned responsiveness to sinusoidal peripheral stimuli than do regular-spiking units (64, 82).

Electrophysiological behavior similar to that of the fast-spiking cells has also been observed in identified inhibitory neurons of mammalian hippocampal (1, 42, 61, 78) and olfactory cortex (77), and in the relatively simple visual cortex of turtles (12), suggesting that this general class of telencephalic neurons has been highly conserved through the evolution of higher vertebrates.

The ionic mechanisms of electrogenesis in fast-spiking cells are unknown. The rapid upstroke of their action potential is consistent with a sodium-dependent mechanism, as in pyramidal cells (11). Their uniquely fast spike repolarization could be due to an unusually high rate of sodium channel inactivation (cf. Ref. 5); however, the brief but prominent postsynaptic undershoot is most compatible with a rapidly activating and inactivating phase of potassium conductance. The absence of a long-duration AHP after a spike train in most (23/25) fast-spiking neurons implies that relatively little calcium-activated potassium current exists in these cells. A lack of prolonged potassium conductances could account for the lack of adaptation.

Neurons in cortical regions that are adjacent to epileptogenic foci display a high degree of synchrony with each focal epileptic discharge (16, 71). This may be an important mechanism for sustaining the paroxysmal activity. Our results suggest a cellular substrate for such synchronized inhibition. Apparently the GABAergic neurons within the epileptogenic focus display large IPSPs (81), and there is little evidence for the presence of other neurotransmitters. This may be further demonstrated for presumed pyramidal cells (28). Some of these neurons, especially the so-called baskt and chandelier cells, have widespread horontial connections that synapse densely upon the somata and proximal axons, respectively, of pyramidal cells (23). Thus, GABAergic cells near the fringe of a cortical focus would be expected to profoundly inhibit adjaent cortical areas where GABA transmission was still intact.

ACKNOWLEDGMENTS

We thank Dr. A. R. Kogevinas for comments on the manuscript. We also acknowledge the excellent technical assistance of J. Raido, S. Ljung, and E. Elnayet, and the secretarial skills of C. Lioo.

This study was supported by the National Institutes of Health (NIH) Grants NS12511, NS-19510, NS-06477, and the Morris Research Fund. D. A. McCormick was supported by postdoctoral fellowships from the Huntington's Disease Foundation of America, the Simmons Foundation and NS-07331 from the NIH.

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Electrotonic Parameters of Neurons Following Chronic Ethanol Consumption

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SUMMARY AND CONCLUSIONS

1. The electrotonic parameters of nerve cells in the dentate gyrus following long-term ingestion of ethanol were studied in vitro. The ethanol was administered in a liquid diet for a period of 20 wk followed by a 3-wk withdrawal period. A control group received a similar diet with the ethanol replaced by maltose-dextrins.

2. Intracellular recordings were obtained from 44 neurons, and the voltage defays following current injections were analyzed with a recent electrical model of granule cells to take into account a somatic shunt already detected in previous studies. The new model accurately accounted for the fast voltage transients and showed that the membrane time constant in the dendrites is, on average, five times larger than the somatic time constant.

3. Injection of horseradish peroxidase into the neurons for the morphological analysis showed that neurons in the ethanol group have a longer dendritic tree than neurons in the control group. Estimation of the membrane surface area showed that the membrane area in the dentines is at least 60% greater (in both control and ethanol groups) when membrane foldings and irregularities are taken into account.

4. The results of the modeling analysis showed that the membrane time constant and the input resistance are not affected by ethanol. However, the membrane resistance is significantly increased in the ethanol group (6.632 versus 18.480 (µS cm²), and the capacitance is significantly decreased (4.45 versus 1.71 µF/cm²). The electrotonic length is also increased by chronic ethanol treatment (0.85 versus 0.94).

5. Higher values of membrane specific resistance (Rm) occur in ethanol-fed rats compared to controls. However, since the neurons from the ethanol group are on average larger than neurons in the control group, it is suggested that the change in Rm compensates for the increase in the length of the dendrites, thereby maintaining a value of the electrotonic length under 1.0. The observed changes in the passive parameters are in opposite direction from the recently measured effect of acute doses of ethanol on hippocampal neurons. These results support a model of chronic alcohol intake where homeostatic adaptive changes lead to the development of long-term changes in cellular physiology.

INTRODUCTION

Chronic and excessive use of ethanol is widely recognized as producing brain damage correlated with functional deficits in animals (33). Morphological and psychological evidence point to neuronal loss, impaired motor performance, memory deficits, and many other symptoms in chronic alcoholics as resulting directly from ethanol abuse (8). Although the literature on the acute effects of ethanol is quite extensive (see Review in Ref. 22), detailed studies of the pathophysiologic and neurochemical effects of brain damage resulting from chronic alcoholism is fairly recent (1, 18, 33). Of particular interest is the effect of long-term ethanol...