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. Three 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 24.1 Hz/nA. During prolonged suprathreshold 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 most 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 aspiny or sparsely spiny stellate cells, with bitufted 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 somadendritic morphologies 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.
INTRODUCTION

An understanding of the integrative mechanisms of a neural circuit depends critically upon a detailed knowledge of the properties of each cell involved (e.g., Ref. 21). This process may be simplified when it is possible to show that cells fall into distinct categories. Neurons of the neocortex have traditionally been classified on the basis of a large variety of morphological distinctions (69). Cortical cell types have also been defined by physiological criteria, such as sensory receptive fields (38, 62), biophysical properties (95), and axon conduction velocities (2). Most recently, cell heterogeneities have been revealed immunocytochemically or by enzyme or neurotransmitter histochemistry (19). Each of these distinguishing features helps to define the functional role of a particular neuron. Unfortunately, there have been relatively few attempts to directly correlate the morphological, physiological, and biochemical characteristics of neocortical elements. A few notable achievements have been made by intracellularly injecting the dye horseradish peroxidase (HRP) into functionally defined cells in vivo. In cat striate cortex, visual receptive fields of single cells have been mapped and their soma-dendritic and axonal structures subsequently described in exquisite detail (25, 26, 44, 49, 55, 88). By a similar method, both morphological and electrophysiological distinctions have been demonstrated between slow and fast pyramidal tract neurons of the cat and monkey motor cortex (14, 31).

Most studies of the membrane properties of neocortical neurons have dealt only with presumed pyramidal cells, i.e., the principal cells of the cortex (11, 13, 46, 52, 90–92, 95, 98). This is understandable because pyramidal cells constitute the majority in all cortical areas, are relatively large, and some can be identified by antidromic activation after stimulation of specific subcortical sites. Among the pyramidal tract cells it has been possible to demonstrate distinct electrophysiological differences between identified subtypes (4, 95). 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 somadendritic shape, that seems to correspond to the subtypes of nonpyramidal neurons that utilize the inhibitory neurotransmitter γ-aminobutyric acid (GABA).

METHODS

Slicing procedure

In vitro neocortical slices were prepared by methods that have been described (11), 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 regions or parietal regions that correspond histologically to the sensorimotor area of rat cortex (17). 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 1000). Slices were immediately placed in a recording chamber and allowed to incubate for at least 1 h before recordings were begun.

Recording chambers held the slices on lens paper-covered platforms that were continuously superfused with physiological solution. Humidified gas of 95% O₂-5% CO₂ flowed over the slice surfaces; temperature was thermostatically held at 35–37°C. Normal bathing solution contained (in mM): NaCl 124, KCl 5, CaCl₂ 2, MgSO₄ 2, NaH₂PO₄ 1.25,
were measured in the chamber and compared to Nissl-stained sections corrected for shrinkage. In the quantitative analyses summarized in Table 1, recordings, data from these cells were not included. Because of their high resistance, yielded generally noisy intracellular recordings, electrodes were filled with a 5% solution of Lucifer yellow CH (Aldrich) in 0.1-2 M lithium acetate (93). Lucifer yellow was injected by passing hyperpolarizing current pulses (0.5-2 nA, 800 ms duration) at 1 Hz for 5-10 min (30). Electrophysiological data were recorded on magnetic tape (0-5 kHz); data were digitized off-line and analyzed with a Minc-23 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

Cortical slices 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.

Immunoreactivity to glutamic acid decarboxylase (GAD) was localized with a sheep antiserum (code no. 1440) that was raised to rat brain GAD by Oertel et al. (66) and graciously provided to us by Dr. Donald Schmechel. The immunocytochemical technique we have used is a slight variation of the one suggested by this group. Guinea pigs were perfused transcardially with 4% paraformaldehyde in periodate-lysine fixative, and cortical sections were cut 50 pm thick on a vibratome. Antiserum was diluted 1:1,000, and localization was carried out using the avidin-biotin-peroxidase system (37; Vectastain 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. Stained neurons were drawn with the use of a camera lucida attachment. Measurements of cell sizes were carried out on a digitizing tablet (MOP 3; Zeiss) and are uncorrected for tissue shrinkage caused by dehydration.

RESULTS

Analysis of several hundred intraneuronal recordings from anterior cingulate and sensorimotor 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 1) regular-spiking cells (after Mountcastle et al., Ref. 64), 2) bursting cells, and 3) 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 nococortical 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 threshold evoked single spikes, even with current pulses of several hundred milliseconds duration. This contrasts with the defining characteristic of bursting cells (see next section). During the course of a long, square current pulse that exceeded action-potential threshold, spike frequency steadily declined at all current intensities (Fig. 1A). Trains of spikes were followed by AHPs several milliseconds in duration and several hundred milliseconds in duration (Fig. 1B). Graphs of spike frequency (calculated from the first interspike interval) versus injected-current intensity re-
Table 1. Properties of three classes of neocortical neurons

<table>
<thead>
<tr>
<th></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 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>(n = 11)</td>
<td>(n = 22)</td>
<td></td>
</tr>
<tr>
<td>$R_N$, MΩ</td>
<td>39.9 ± 21.2</td>
<td>31.2 ± 8.5</td>
<td>55.6 ± 33.6</td>
</tr>
<tr>
<td></td>
<td>(n = 11)</td>
<td>(n = 22)</td>
<td></td>
</tr>
<tr>
<td>$\tau_m$, ms</td>
<td>20.2 ± 14.6</td>
<td>16.6 ± 5.9</td>
<td>11.9 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>(n = 12)</td>
<td>(n = 9)</td>
<td>(n = 15)</td>
</tr>
<tr>
<td>Spike amplitude, mV</td>
<td>95.2 ± 9.5</td>
<td>85.5 ± 14.0*</td>
<td>82.1 ± 9.5†</td>
</tr>
<tr>
<td>Max rate of rise, V/s</td>
<td>374.0 ± 95.9</td>
<td>334.4 ± 105.0</td>
<td>403.1 ± 89.2</td>
</tr>
<tr>
<td>Max rate of fall, V/s</td>
<td>94.5 ± 28.1</td>
<td>95.5 ± 29.0</td>
<td>310.5 ± 110.3†</td>
</tr>
<tr>
<td>dV/dt ratio</td>
<td>4.12 ± 1.02</td>
<td>3.5 ± 0.78</td>
<td>1.39 ± 0.35†</td>
</tr>
<tr>
<td>Spike width base, ms</td>
<td>1.74 ± 0.41</td>
<td>1.5 ± 0.31</td>
<td>0.66 ± 0.19†</td>
</tr>
<tr>
<td>Spike width, 1/2 amp, ms</td>
<td>0.80 ± 0.18</td>
<td>0.80 ± 0.14</td>
<td>0.32 ± 0.10†</td>
</tr>
<tr>
<td>f-I slope, primary, Hz/nA</td>
<td>241 ± 102</td>
<td>549 ± 261†</td>
<td>(n = 10)</td>
</tr>
<tr>
<td></td>
<td>(n = 10)</td>
<td></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 IPSP</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Laminar location</td>
<td>II–VI</td>
<td>IV–V</td>
<td>II–VI</td>
</tr>
</tbody>
</table>

All data expressed as mean ± SD. Mann-Whitney U test performed to compare differences from regular-spiking cells (*P < 0.05; †P < 0.01). $V_m$, resting membrane potential; $R_N$, input resistance; $\tau_m$, membrane time constant; dV/dt ratio, ratio of maximum rate of rise to maximum rate of fall; AHP, afterhyperpolarization; IPSP, inhibitory postsynaptic potential; +, possesses characteristic; −, lacks characteristic.

revealed a mean initial slope of 241 Hz/nA (Fig. 1C). Secondary slopes were evident in most cells at higher currents, and these averaged 93 ± 50 Hz/nA (n = 10). When neurons were activated synaptically, by single 200-μs cathodal shocks to 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 (IPSP), as described previously (11, 96). At high stimulus intensities one 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 (9, 29, 30).

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. 2A1) and injected-current (Fig. 2A2) 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).
FIG. 2. Response characteristics of bursting neurons to intracellular injection of current and to activation of synaptic inputs. A: all-or-none bursts of action potentials (3 superimposed traces in each case). Single shocks to the underlying white matter generated an excitatory postsynaptic potential (EPSP) or, at a discrete threshold, an EPSP plus a complex of 3 spikes (A1). Increasing the stimulus intensity decreased the latency of the burst. Intracellular current injection in the same cell (A2) caused a similar burst of action potentials that occurred in an all-or-none fashion and outlasted the depolarizing pulse. Increasing the magnitude of depolarization decreased the latency to burst onset. B: response of a different bursting cell to steps of depolarizing current. At firing threshold one spike was generated (B1) and was followed by a small depolarizing afterpotential that could sometimes generate a second spike (B2). Slightly increasing the magnitude of current caused a more robust burst to occur at a shorter latency (B3).

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. 3A, -67 mV). This suggests that the burst-generating mechanism was inactivated at depolarized levels. Similarly, when begun 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. 3A, -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
FIG. 3. Effects of membrane potential on response characteristics of bursting and regular-spiking neurons. A: a depolarizing current pulse at resting potential (−75 mV) caused a burst of action potentials followed by an AHP, and then a train of single spikes. Depolarization of this neuron to −67 mV (just below firing threshold) with tonic current caused the cell to respond to the same current pulse with a train of single spikes; i.e., the burst was inactivated. B: depolarizing current pulse in a typical regular-spiking cell at resting potential (−82 mV) gave rise to single spikes only. Hyperpolarization (−110, −127 mV) did not cause this neuron to generate burst discharges in response to depolarizing current pulses.

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 membrane potential 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.

Seven bursting cells were injected with Lucifer yellow in anterior cingulate and sensorimotor 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
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FIG. 4. Synaptic response of a bursting neuron. A: response of cell to depolarizing current injection showing a typical burst of action potentials (2–4 spikes). B: response of the same cell to a hyperpolarizing pulse of 0.15 nA followed by an orthodromic stimulus. At the resting potential of −69 mV (arrowhead) the orthodromic stimulus caused an excitatory postsynaptic potential (EPSP) followed by a long-lasting inhibitory postsynaptic potential (IPSP). Depolarizing this cell to −56 mV with intracellular current injection caused the response to the hyperpolarizing pulse to increase in magnitude; the latter phase of the response to the orthodromic stimulus reversed polarity, revealing both early and late IPSPs.

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 non-bursting pyramids. In one case, injection of Lucifer yellow into a bursting cell yielded two well-stained pyramidal neurons. Dye coupling of neocortical pyramids has been observed previously (9, 30) and may indicate the presence of dendrodendritic or dendrosomatic 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. Impalements of these cells occurred much more frequently with high-resistance bevelled microelectrodes than with low-resistance unbevelled 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 (\(x = 71.6 \pm 7.8\) mV), and spike amplitudes ranged from 62 to 102 mV (\(x = 82.1 \pm 9.5\) 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) hyperpolarizing undershoot (Fig. 6, 0.3 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. Figs. 1B and 6B, and Fig. 3 of Ref. 11). All but two of the fast-spiking neurons lacked large, prolonged AHPs, regardless of their resting potential.

Fast-spiking neurons were in general not spontaneously active at rest, and those with 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/nA. 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. 5. Photomicrographs of two identified bursting neurons filled with Lucifer yellow. A: pyramidal neuron localized within layer IV of the sensorimotor cortex. *Inset* illustrates the bursting response of this cell to intracellular injection of depolarizing current. B: pyramidal neuron also located within layer IV of the sensorimotor cortex. *Inset* illustrates the bursting response of this cell to an orthodromic stimulus. *Calibration bars* for insets: 25 ms, 20 mV, 1.0 nA. *Calibration bar* for photomicrographs, 50 μm.
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 afterhypolarization (AHP) after a train of action potentials. Two different cells, whose resting potentials were −60 and −65 mV, are shown. C: frequency-current (f-I) plots for three fast spiking neurons. In most cells, 2–3 separate slopes could be discerned. D: interspike interval (ISI) vs. interval number for cell illustrated in A. Magnitude of injected current is indicated to 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: $I-V$ 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 an 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 base-line noise in the largest depolarizing response of the cell illustrated in B1.

FIG. 9. Response of 2 separate fast-spiking neurons to activation of synaptic inputs. Single shocks (dots) were applied to deep cortical layers below the recording sites. A: increasing the intensity of stimulation (left to right) increased both the magnitude and duration of the depolarizing response. No hyperpolarization or obvious inhibition was seen. Resting potential was $-76$ mV. B: response of a second cell to increasing intensities of stimulation. At higher intensities a train of action potentials was generated. Resting potential in this cell was $-71$ mV. At resting potential the cell in B was closer to its firing threshold than the cell in A.
tionship over the range tested (Fig. 8, cell 2), while three displayed an increase in slope (i.e., 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 three fast-spiking cells an anomalous increase in spontaneous voltage fluctuations occurred during depolarizations 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, A and B). At higher stimulus intensities these EPSPs triggered trains of spikes that could reach frequencies of 300–400 Hz. EPSPs ranged up to 120 ms 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 hyperpolarizing

![Figure 10](https://example.com/figure10.png)

**FIG. 10.** Variability of synaptic inhibition in fast-spiking neurons. A: response of cell to depolarizing input with and without superimposed activation of orthodromic synaptic input (dot). B: graph of interspike interval (ISI) vs. interval number during depolarizing pulse with and without activation of synaptic inputs. Data calculated from traces illustrated in A. Synaptic activation caused a transient increase in firing frequency but no detectable decrease, implying a lack of significant net inhibition. C: response of another fast-spiking cell to depolarizing current injection. D: response of cell illustrated in C to activation of synaptic input (dot). Note the prominent hyperpolarizing inhibitory postsynaptic potential. Morphology of the cell with responses in C and D is shown in Fig. 13C.
FIG. 11. Response of fast-spiking neuron to three consecutive orthodromic stimuli (1 Hz) in the presence of 10 \( \mu \)M bicuculline methiodide. Each stimulus caused a paroxysmal field potential (upper trace in each set) indicative of synchronized discharge 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.

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 \( \text{dV/dt} \) ratio of the action potentials in 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 bitufted arrangement of dendrites (cf. Ref. 72); their overall dendritic fields were hourglass shaped (Figs. 12A, 13A). The other labeled stellates had more spherical somata with radiating multipolar dendrites (Fig. 12B; 13, B and C). The dendritic fields of these cells extended a maximal detectable distance of 60 to 400 \( \mu \)m from the cell bodies, often crossing into adjacent cortical layers. It is possible that the maximal dendritic extent of some cells was underestimated because thin distal processes could not be visualized. The sizes of labeled somata are presented in Table 2.

GAD immunocytochemistry

Fast-spiking cells identified as aspiny or sparsely spiny stellates closely resemble neurons of rat, cat, and monkey cortex that have
FIG. 12. Photomicrographs of fast-spiking neurons injected intracellularly with Lucifer yellow. A: sparsely spiny neuron with vertically oriented bifurcated dendrites localized within layers II-III of the sensorimotor cortex. B: multipolar stellate cell with sparsely spiny dendrites located within layers II-III of the anterior cingulate cortex. At least 7 major dendrites can be seen radiating from the soma. Both A and B are oriented with the pia mater upward. Calibration bar, 50 μm.
FIG. 13. Reconstructions of three Lucifer yellow-filled fast-spiking neurons. A: multipolar stellate cell possessing beady dendrites that extended in an hourglass fashion for up to 300 μm in each vertical direction. A very fine process exhibiting multiple bifurcations and less beadiness was probably the axon (large arrow). One branch of this process crossed to the other side of the cell (small arrow). The axonal plexus roughly paralleled the dendritic field in its hourglass form. B: another multipolar stellate cell exhibiting four major dendrites extending radially. C: a sparsely spiny neuron with multiple thin dendrites originating directly from the soma. Calibration bar, 100 μm total length with a 10-μm subdivision.

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 cingulate 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>
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<tr>
<th>TABLE 2. Soma sizes of fast-spiking and GAD-positive cells</th>
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<tr>
<td>Major diam, μm</td>
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<td>(9.9–18.2)</td>
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<td>Area, μm²</td>
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Data are expressed as mean ± SD with the ranges in parentheses. GAD, glutamic acid decarboxylase.
FIG. 14. Examples of GAD-immunoreactive neurons within the guinea pig anterior cingulate and somatosensory cortical regions. Stained cells had proximal dendritic fields that were vertically bitufted (E), multipolar (D), or some intermediate form (A and B). GAD-immunoreactive puncta were juxtaposed to unstained pyramidal cells (F) and, in some cases, GAD-immunoreactive neurons (C, arrow). Calibration bars. 50 μm total length with a 10-μm subdivision. Bar in D applies to A–E.

In addition, it was possible to discern GAD-positive puncta that often appeared to surround the nonstained 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, 32, 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 aspiny and sparsely spiny bitufted 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.
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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 fine 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, 39) and vasoactive intestinal polypeptide (7, 23), 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 correlative 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 $dV/dt$ ratio; a value > 2.2 was always associated with a pyramidal morphology in this study.

By observing the firing properties of cells during currents injected through the microelectrode, we can infer something about the manner in which the cell transforms excitatory inputs into spike outputs. Closely associated with the slow rate of action potential repolarization in pyramidal cells was a strong adaptation of spike frequency during maintained depolarizations. Adaptation is a universally described feature of neocortical pyramidal cells (4, 13, 46) and in vitro (67, 90). Its ionic mechanism is not known. However, it appears that both a calcium-activated potassium conductance and a muscarine-sensitive potassium conductance (the M-current; Ref. 3) are involved in the spike-frequency adaptation exhibited by hippocampal pyramidal cells (34, 54). Similar conductances seem to reside in neocortical neurons (11, 58). An additional factor in determining the firing rate is likely to be the noninactivating, voltage-dependent sodium current of neocortical pyramidal cells (11, 89). This persistent inward current is strongly activated near spike-threshold potentials, and the time-, voltage-, and ion-dependent balances between it and the various outward potassium currents may ultimately determine the instantaneous firing rate (89).

Previous investigations have convincingly documented both electrophysiological (4, 46, 95) and anatomical (14, 31) differences between the fast and slow pyramidal tract neurons of sensorimotor cortex. Although for the purposes of this study we have classified all nonbursting pyramidal neurons with a single group, we do not mean to imply homogeneity among these cells. Indeed, in this and a previous study (11) wide variations were noted among the passive and active membrane properties of cells that fall into this single category. Further investigation is necessary to determine whether these physiological differences represent discrete subtypes or a continuum, and whether correlative structural variations exist.

Bursting pyramidal cells
The present results demonstrate that at least some neurons generating intrinsic bursts of action potentials are pyramidal neurons. Although variations in the size and dendritic structure of all of our Lucifer yellow-stained pyramids were evident, it was not possible to distinguish bursting from nonbursting cells by morphological criteria. Quantitative structural analyses (e.g., Ref. 80), 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 intrinsic events. When stained with HRP, all of these cells were layer V pyramidal cells. In contrast, Grace and Llinas (27) have reported that all pyramidal neurons of guinea pig prefrontal cortex have the ability to burst. Because we have not stained all of the major cortical cell types, the possibility remains that some burst-
ing cells have a nonpyramidal shape. Indeed, the recent studies of M. J. Gutnick and E. L. White (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 there was no correlation between this aspect of membrane physiology and cell structure. The high density of bursting cells we have observed in layer IV of guinea pig (11) suggests that some of them, recorded but not stained, could have been spiny stellate cells.

The vigor with which neurons could burst was quite variable. Our criterion level for the bursting class, i.e., three spikes, is necessarily arbitrary. A small number of cells falling near the border of the regular-spiking/bursting criteria were difficult to categorize with confidence, and were relegated to the nonbursting group. Furthermore, none of the other measured variables could reliably distinguish between the two physiological classes of pyramids. These data suggest that there is actually a continuous distribution of spiking modes among pyramidal neurons. The molecular mechanisms underlying the burst mode may thus be expressed to varying degrees in cells with similar somadendritic structure. These data suggest that there is actually a continuous distribution of spiking modes among pyramidal neurons. The molecular mechanisms underlying the burst mode may thus be expressed to varying degrees in cells with similar somadendritic structure. The distinct laminar distribution of bursting cells may imply that this trait is determined at an early stage of cortical development, since the laminar position of a neocortical neuron is strongly determined by its birth date (74).

What is the mechanism for intrinsic burst-firing in neocortical cells? Direct experiments remain to be done, but the basic behavior strongly resembles that of some hippocampal pyramidal cells (43, 103). Burst-generating cells in the CA3 area of hippocampus have fast, sodium-dependent action potentials that ride upon a more slowly activating depolarization generated by a voltage-dependent calcium conductance (41, 103). Nonbursting cells of neocortex also possess significant calcium conductances (11), but these are apparently not large enough, or the outward potassium currents are not small enough, to evoke a burst under normal conditions. The bursting cortical cell may therefore be the relatively rare cell that has the combination of slow, voltage-dependent calcium and potassium currents favoring such a display. Recent experiments in other types of bursting central neurons (40, 50) have identified a slow calcium conductance that is strongly inactivated at depolarized membrane levels. Bursting neocortical cells may have a similar conductance, as the bursting capabilities of these cells switched to a tonic, nonbursting mode of firing during sustained depolarizations. On the other hand, the majority of recorded cells, which did not burst, could not be converted to a burst-firing mode by hyperpolarizing prepulses that might have been expected to deactivate such a calcium conductance.

The functional role of bursting cortical cells can only be inferred from their laminar positions. Anatomical studies of mouse somatosensory cortex imply that all neurons sending dendrites through layer IV receive direct thalamocortical terminations (100). Spiny stellate cells and pyramidal cells of layer IV and upper layer V are thus ideally situated to receive strong thalamic excitation (101). These neurons would respond vigorously but transiently to an abrupt new stimulus, but maintain a stable, sustained output to a tonic stimulus—unique transform properties that imply an important role in the integrative functions of the cortex. The anatomy of their axons remains to be defined. As spiny cells, they are presumed to have excitatory terminals (6). Indirect support for this comes from a recent study of the epileptic discharge in disinhibited neocortex, where it was concluded that neurons within the middle layers, those containing the bursting neurons, were the initiators of the synchronized activity (8). If bursting cells are indeed the pacemakers for some forms of paroxysmal discharge, then it is likely that they have local axons with widely diverging sets of excitatory terminations.

Fast-spiking stellate cells

The neurons that we have classified as fast-spiking are unambiguously distinguishable from the remaining cells by several criteria, most notably a short spike duration, low ratio of action-potential rate of rise to fall, and the lack of prominent frequency adaptation. When stained, cells with these properties were always morphologically distinct from the spiny pyramidal cells; they were invariably aspiny or sparsely spiny stellate cells with several dendritic organizations. 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 the previous section, the validity of this conclusion will rest upon a more comprehensive sampling of the various cortical cell types.

Extracellular recordings 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 (64, 83) 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 radially projecting dendrites, vertically oriented, bitufted 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 tritiated GABA (33, 86), exhibit strong immunoreactivity for the GABA synthetic enzyme GAD (36, 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, 87). The results of our GAD immunocytochemistry are consistent with the data gathered from other species. GAD-positive neurons of guinea pig 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 grossly 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 hippocampus (1, 45, 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 postspike 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 dearth of prolonged potassium conductances could account for the lack of adaptation.

Neurons in cortical regions that are adjacent to epileptogenic foci display large IPSPs in synchrony with each focal epileptic discharge (16, 71). This may be an important mechanism for containing the paroxysmal activity. Our results suggest a cellular substrate for such surround inhibition. Apparently the GABAergic neurons within the epileptic focus receive strong synaptic excitation during each synchronous discharge (Fig. 11), as has been demonstrated for presumed pyramidal cells (28). Some of these neurons, especially the so-called basket and chandelier cells, have widespread horizontal connections that synapse densely upon the somata and proximal axons, respectively, of pyramids (22). Thus, GABAergic cells near the fringe of a cortical focus would be expected to profoundly inhibit ad-
jacent cortical areas where GABA transmission was still intact.

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