Quantitative Analysis of Firing Properties of Pyramidal Neurons From Layer 5 of Rat Sensorimotor Cortex

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Schwindt, Peter, Jennifer A. O’Brien, and Wayne Crill. Quantitative analysis of firing properties of pyramidal neurons from layer 5 of rat sensorimotor cortex. J. Neurophysiol. 77: 2484–2498, 1997. Quantitative aspects of repetitive firing evoked by injected current steps and ramps were studied in layer 5 pyramidal neurons in brain slices of rat sensorimotor cortex to answer the following questions. Do the tonic firing properties of burst-firing and regular-spiking (nonbursting) neurons differ significantly? Does burst firing denote a discrete class of neurons or represent a continuum of firing properties? Is firing rate during the burst of action potentials related to stimulus amplitude? What aspect of the stimulus might the initial firing rate code? How stable are a neuron’s firing properties over time? All recorded neurons fired tonically to a long-lasting current above a minimum value, and the tonic firing properties of most neurons were quite similar irrespective of their initial response to a current step. Only a group of high-resistance neurons had significantly different tonic firing properties. When slow current ramps (rising between 0.5 and 20 nA/s) were applied, the relation between firing rate and current during the ramp was very similar to the relation between tonic firing rate and current obtained from long-lasting current steps. Low-resistance cells exhibited three distinct initial responses to a current step: fast adaptation, high-threshold bursts, and low-threshold bursts, observed in 54, 28, and 10% of recorded cells, respectively. High-resistance cells exhibited a distinctive slow adaptation of firing rate. Slowly adapting, fast-adapting (FA), and high-threshold burster (HTB) neurons exhibited no adaptation near the minimum current that evoked repetitive firing ($I_o$). FA and HTB cells exhibited two-spike adaptation to a final tonic firing rate during currents up to 1.6 times $I_o$. Only a higher current (2.1 times $I_o$) evoked a burst in HTB cells, whereas a burst was evoked at $I_o$ in the low-threshold burster cells. In most cells analyzed, the initial firing rate, whatever its nature, increased monotonically with current step amplitude. The response to fast current ramps indicated that firing rate during adaptation or bursting may code rate of change of current. Repeated measurements during long-duration impalements indicated that both transient and tonic firing properties are stable over time. We discuss how the different tonic firing properties of large and small pyramidal neurons could be more important functionally than the different transient responses (burst/nonburst) of the large neurons. We conclude that the large neurons would perform a better linear transduction of time-varying synaptic current that reaches their somata. We compare the responses evoked by somatically injected current with those evoked by dendritic glutamate iontophoresis in previous studies.

INTRODUCTION

Studies of current-evoked repetitive firing in neocortical pyramidal neurons from several rodent species studied in vitro have revealed that some of these neurons possess an intrinsic ability to fire bursts of action potentials, a property that distinguishes them from nonbursting, regular-spiking pyramidal neurons (Connors and Gutnick 1990). This burst-firing capability has been of special interest because the burst of action potentials may tend to synchronize activity in the neurons on which the burst-firing neurons synapse (Chagnac-Amitai and Connors 1989; Connors 1984; Silva et al. 1991). Synchronous firing may be important in both physiological and pathophysiological cortical function. Subsequent studies have focused largely on whether the burst-firing neurons (‘‘intrinsic bursters’’) also have a special laminar location, morphology, axon trajectory, and pattern of synaptic input (Agmon and Connors 1992; Chagnac-Amitai and Connors 1989; Chagnac-Amitai et al. 1990; Mason and Larkman 1990; Tseng and Prince 1993).

A number of questions about the firing properties of neurons from rodent neocortex has remained unanswered, however. In many cells, bursts of action potentials are reported to occur only at the onset of the injected current step, and the initial bursts are followed by regular spiking (Agmon and Connors 1989; Chagnac-Amitai et al. 1990; Connors et al. 1982; Franceschetti et al. 1995; Mason and Larkman 1990; McCormick et al. 1985). Does the subsequent tonic firing differ in any significant respect between bursters and nonbursters, or does the output of these cells differ only in their initial response to a current step? Because a burst of action potentials can be evoked in an all-or-none manner by a brief current pulse (e.g., McCormick et al. 1985), it is natural to assume that the burst itself is a unitary, all-or-none event that simply signals (with several closely spaced spikes) that a sudden depolarization has occurred. But even regular-spiking cells fire faster at the onset of a current step than later, a phenomenon known as spike-frequency adaptation, and the initial spike frequency is a function of current step amplitude (e.g., Stafstrom et al. 1984). Is it possible that firing rate during a burst of action potentials is graded and codes for some aspect of the stimulus? Several investigators have remarked that the nature of the burst can vary widely among neurons, but the extent of this variability is not entirely clear. Some investigators have remarked that burst-firing neurons were the minority of those recorded, but their frequency of occurrence is not entirely clear. A related question is, how stable are repetitive firing properties over time? Can a nonburster “spontaneously” change into a burster? The answers to these questions require quantitative measurements of the firing properties of rodent pyramidal
neurons, but quantitative data on these questions are either sparse or nonexistent. It is important to answer these questions because the intrinsic properties of these neurons determine how synaptic input is converted to spike output and influence our ideas of cortical function.

During a recent series of experiments in which we iontophoresed glutamate on the apical dendrite while recording from the soma of layer 5 pyramidal cells (Schwindt and Crill 1995, 1996, 1997), we examined the repetitive firing properties of many recorded cells. These measurements of firing properties were performed partly to discover whether the transmission of the glutamate-evoked current from dendrite to soma might vary among cells and correlate with intrinsic firing properties and partly to perform quantitative measurements of firing properties in a sizable sample of layer 5 neurons from a delimited area of rat neocortex. Previous reports have suggested that firing properties vary in neurons from different cortical laminae, and the laminar location of bursters may vary among rodent species (Agmon and Connors 1989; Chagnac-Amitai et al. 1990; Connors et al. 1982; Mason and Larkman 1990; Montoro et al. 1988). Because intrinsic bursters were reported to be limited to the large pyramidal neurons of layer 5B of rat visual and sensorimotor cortex (Chagnac-Amitai et al. 1990; Mason and Larkman 1990; Tseng and Prince 1993), we used relatively large recording microelectrodes to bias our sample toward the large neurons, and we positioned our recording electrode in deep layer 5. We desired to investigate a nonvisual region and chose areas FL and HL of dorsal rat cortex, 0–3 mm posterior to bregma, which are said to possess both sensory and motor characteristics on the basis of cytoarchitectonic criteria (Zilles and Wree 1985). We find that the firing characteristics of neurons in this area are stable over time. The tonic firing characteristics of large neurons are similar whatever their initial, transient response, but they differ significantly from those of smaller neurons. Three distinct classes of transient responses were observed among the large cells. In most cells analyzed, the initial firing rate, whatever its nature, was related to stimulus amplitude.

METHODS

Repetitive firing properties were examined in subpopulations of the cells recorded in previous studies (Schwindt and Crill 1995, 1997a,b), and the methods were as described in those studies. Briefly, Sprague-Dawley rats (21–35 days postnatal) were anesthetized with ketamine (150 mg/kg) and xylazine (10 mg/kg) and killed by carotid section. A section of cortex 0–3 mm posterior to bregma was isolated, and slices 350–400-μm thick were prepared and maintained as described. Recorded cells lay 1.03–1.37 mm below the pial surface (mode: 1.18 mm) and 2.03–3.37 mm from midline (mode: 2.96 mm), corresponding to layer 5 of areas HL and FL of sensorimotor cortex. Fifteen recorded cells injected with biocytin (0.5% in 2.7 M KCl or 2 M KCH3SO4) were recovered and visualized after standard histological processing. The purpose of this staining was simply to determine whether the recorded cells were pyramidal neurons located in deep layer 5 and whether their apical dendritic tree reached the pial surface. All recovered cells had these features.

Recordings were made in both submerged and interface chambers maintained at 31–34 and 34°C, respectively. Slices were perfused with (in mM) 130 NaCl, 3 KCl, 2 CaCl2, 2 MgCl2, 1.25 NaH2PO4, and 10 dextrose, pH 7.4, saturated with 95% O2–5% CO2. Cells were impaled with sharp microelectrodes made from standard 1.0-mm-OD borosilicate tubing and filled with 2.7 M KCl or 2 M KCH3SO4 (DC resistance: 30–40 MΩ). An Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) was used to record membrane potential and inject current either in active bridge mode or in discontinuous current-clamp mode with the use of a switching rate of 4–7 kHz (30% duty cycle). Membrane potential and injected current were monitored, filtered at 1–10 kHz, amplified, and recorded on a multichannel video cassette recorder with pulse code modulation (Neuro-Data, New York, NY). Resting potential was taken as the difference between the intracellular and extracellular potentials recorded on a chart recorder. Recorded data were digitized to analyze subthreshold responses and evoked firing rates with the use of a computer program. Values in the text are given as means ± SE.

RESULTS

Cell properties

Repetitive firing properties were examined in 68 cells. The data from 41 of these cells were analyzed in detail to obtain quantitative measurements of repetitive firing parameters. Repetitive firing data from the remaining 27 cells were inspected visually to determine qualitatively the nature of the initial responses to a series of current pulses. These latter cells were included only for purposes of determining the frequency of occurrence of cells having different initial response properties. Impalements in many cells lasted ≥1 h because of the time required for associated iontophoresis experiments (Schwindt and Crill 1995, 1996, 1997). Cells were accepted only if they exhibited a stable resting potential and action potential during the entire impalement. Whether their repetitive firing properties also remained stable was an experimental question (see below). Statistics given below are from the 41 analyzed cells. Resting potential averaged −72 mV (same as mode; range: −67 to −82 mV). Spike height averaged 108 mV (mode: 100 mV; range: 96–120 mV). Spike duration, measured at spike threshold, averaged 1.0 ms (same as mode; range: 0.5–1.6 ms). Input resistance was determined from a plot of membrane potential versus injected current (V-I plot). Membrane potential was measured at the end of 1-s-duration injected current pulses. All cells exhibited a sag of membrane potential toward resting potential during hyperpolarization, and their V-I plots always were fit best by two straight lines, the slopes of which gave input resistance (see Fig. 1B of Schwindt and Crill 1997). In the recorded population, input resistance during depolarization was 1.90 times greater, on average, than during hyperpolarization in the same cell. Across the population, depolarizing input resistance averaged 35.5 MΩ (mode: 15.6 MΩ; range: 9.2–123 MΩ). Hyperpolarizing input resistance averaged 18.0 MΩ (mode: 10.8 MΩ; range: 6.1–57 MΩ). Thus the great majority of cells was large cells, as judged by input resistance. In fact, these averages are skewed by a distinct population of recorded cells with high input resistance (see below).

Repetitive firing was evoked by depolarizing current pulses of 1–2 s duration (Fig. 1, A and B). All cells fired for the duration of the current pulse starting at some minimum current strength (Fig. 1C, In) that averaged 0.54 ± 0.04 (SE) nA (range: 0.15–1.40 nA). After a variable initial spike response, all cells eventually exhibited tonic repetitive firing.
at an average firing rate that increased with injected current strength (Fig. 1, A–C). The minimum steady firing rate (Fig. 1C, \( F_o \)) averaged 10 ± 0.4 Hz (range: 5–16 Hz). We restricted the maximum injected current (\( I_{\text{max}} \)) to ≤3 nA (range: 0.8–3.0 nA). \( I_{\text{max}} \) (Fig. 1C) was 4.2 ± 0.3 times \( I_o \). All cells would have fired faster to larger currents. The maximum steady firing rate (Fig. 1C, \( F_{\text{max}} \)) evoked by \( I_{\text{max}} \) was 6.7 ± 0.4 times \( F_o \), which seemed to be a large enough range of firing rates to obtain an accurate idea of firing properties. As illustrated in Fig. 1C, the steady-state firing rate versus injected current amplitude (\( F-I \) relation) of about half the analyzed cells (22 of 41) was fit best by two straight lines, as observed previously for cat neocortical neurons in vitro (Stafstrom et al. 1984). The break in the \( F-I \) relation (Fig. 1C, \( F_o \)) occurred at a firing rate of 4.6 ± 0.4 times \( F_o \). For the whole population, the slope of the first linear portion (‘primary range’) of the steady-state \( F-I \) relation averaged 41.8 ± 3.5 Hz/nA (range: 19.4–128.4 Hz/nA), and the slope of the shallower second linear portion (‘secondary range’) averaged 25.5 ± 3.0 Hz/nA.

We grouped the recorded cells into categories on the basis of the nature of their spike responses at the onset of a current step. Two distinct categories of regular-spiking (nonbursting) cells and two distinct categories of burst-firing cells were observed, as described below.

**Fast-adapting cells**

Figure 2 illustrates the transient response observed in 54% (37 of 68) cells in this study, 18 of which were analyzed in detail. These regular-spiking, fast-adapting (FA) cells exhibited no burst firing at any current strength tested up to \( I_{\text{max}} \). We classified these cells as FA because their tonic firing rate was attained essentially after a single interspike interval (ISI) when larger currents were injected. This behavior is best appreciated from the plot of instantaneous firing frequency (1/ISI) versus time (\( F-T \) plot) in Fig. 2C. The fast adaptation of these cells also was apparent from the \( F-I \) plots in Fig. 2D. In these cells the \( F-I \) relation for the first ISI was bilinear (Fig. 2D), and the secondary range always was much steeper than the primary range or steady-state relations (mean slopes: 148 Hz/nA for secondary range vs. 34 Hz/nA for steady firing). The point at which the primary and secondary relations diverged corresponded to the current at which adaptation first appeared. The \( F-I \) relation for the second ISI is nearly the same as for steady-state firing (also see Fig. 6A). Equally remarkable was the absence of adaptation during smaller current steps in these cells (e.g., at 0.3 and 0.5 nA in Fig. 2C). Only when current amplitude reached 0.9 nA in Fig. 2C was the first ISI clearly shorter than the subsequent ISI. On average, the injected current at which adaptation first became apparent was 1.6 ± 0.1 times \( I_o \).

**High-threshold bursters**

Thirty-eight percent (26 of 68) of recorded cells exhibited an initial burst of action potentials at some stimulus strength. Burst-firing layer 5 pyramidal neurons usually have been classified simply as intrinsic bursters. In this study we observed two distinct groups of burst-firing cells, low-threshold bursters (LTBs) and high-threshold bursters (HTBs), in which burst generation seemed equally intrinsic. In the HTBs, the current that first evoked an initial burst (\( I_{\text{th}} \)) was 2.06 ± 0.15 times \( I_o \). In the LTBs, an initial burst of action potentials was evoked at \( I_o \). The HTBs constituted 28% (19 of 68) of all recorded cells, and 11 of these cells were analyzed in detail.

The duration of the burst of action potentials varied among the burst-firing cells. Figure 3 illustrates both the behavior of the HTBs and the range of burst durations observed among the recorded cells. Generally, a burst refers to a group of spikes occurring at a high rate and separated from other spikes by a longer ISI. We therefore recognized a two-spike burst, as for the cell in Fig. 3A, when the ISI following the first two high-frequency spikes was longer than the subsequent steady-state ISIs. Supporting the concept of a two-spike burst was our observation that there was no significant difference in the duration of the postburst ISI that followed a two-spike or a multispike burst, either among the LTBs or the HTBs (see Fig. 6A and below). Half of the HTBs exhibited only a two-spike burst. Aside from this second, longer-than-normal ISI, the properties of the HTBs were very similar to those of the FA cells described above, as is...
FIG. 2. Example of a fast-adapting (FA) neuron. All records from same cell. Resting potential: −72 mV. A and B: examples of initial response to current steps of indicated amplitude. C: plot of instantaneous frequency [1/interspike interval (ISI) duration] vs. time (F-T plot) during injection of current steps of indicated amplitudes. D: plot of instantaneous firing rate (F) for indicated ISIs (1, 2) and average steady-state firing rate (SS) as a function of injected current amplitude (I).

best appreciated from the F-T plot of Fig. 3B. Like the FA cells, the HTBs exhibited no adaptation during the injection of currents at and just above I₀ (Fig. 3B, △). At some higher current strength they exhibited one-ISI adaptation (Fig. 3B, △). This behavior was so similar to that of the FA cells that we pooled data from both FA cells and HTBs to obtain the mean value of current at which adaptation first occurred (1.6 times I₀, as given above). At a higher current this cell exhibited a two-spark burst (Fig. 3B, □). This same type of burst was observed at all larger currents (e.g., Fig. 3B, △) up to Iₘₐₓ. The F-T plot of this cell differed from those of FA cells only in that the long second ISI caused a dip in instantaneous firing rate before the final, tonic rate was attained. Especially during larger currents, several ISIs occurred that were longer than the final, tonic ISI (e.g., Fig. 3B, △). The other six HTBs exhibited a burst consisting of three to five spikes. Otherwise, they had the same features as the cells giving a two-spark burst, namely, no adaptation during low current and fast adaptation during some current smaller than the one that first evoked the burst. Figure 3, D and E, shows records from one such cell.

In all HTBs, firing rate throughout the burst was related to current amplitude. This observation is illustrated by the F-I plots of Fig. 3C (from the 2-spark burster) and Fig. 3F (from the 5-spark burster). The F-I plot for the two-spark burster (Fig. 3C) is similar to that of the FA neuron in Fig. 2D. The steady-state relation is nearly attained by the second ISI. For convenience, two lines were fit to the F-I relations for the first ISI in both Fig. 3, C and F, but there are actually three sections of the F-I relations. The first three points for the first ISI in Fig. 3C lie on the steady-state relation because there was no adaptation at these currents. The relation actually starts to steepen significantly only at currents that evoked the burst. The first ISI relation of Fig. 3F is similar in the sense that there was no adaptation during the lowest current, a small amount during the next current, and the next evoked a burst. The relation for the first ISI actually is S shaped in this cell because firing rate starts to saturate during the first ISI at higher currents. A saturating, S-shaped relation for the first ISI was seen in several of the HTBs, but subsequent ISIs had linear or bilinear relations.

Low-threshold bursters

Ten percent (7 of 68) of recorded cells were LTBs, i.e., a burst was evoked by I₀. All seven LTBs were analyzed in detail. As with the HTBs, the duration of the burst varied among the cells, but most common (4 of 7) was a two-spark
FIG. 3. Examples of high-threshold bursters (HTBs). A–C are from 1 cell (resting potential: −72 mV); D–F are from a different cell (resting potential: −70 mV). A: initial response to current step of indicated amplitude. Asterisk: longer ISI preceding tonic firing. B: F-I plot for current steps of 0.7, 0.9, 1.2, and 1.4 nA as indicated by different symbols. C: plot of instantaneous firing rate for indicated ISIs and average steady-state firing rate as a function of injected current amplitude. D and E: initial response of another cell to current steps of indicated amplitudes. Long ISI (asterisk in E) appeared only at the higher current. F: F-I plot (similar to C) for this cell.

FIG. 4. Examples of low-threshold bursters (LTBs). A–C are from 1 cell (resting potential: −72 mV); D–F are from a different cell (resting potential: −70 mV). A and B: initial responses to current steps of indicated amplitude. Asterisk in A: longer ISI preceding tonic firing. C: plot of instantaneous firing rate for indicated ISIs and average steady-state firing rate as a function of injected current amplitude. D and E: responses to current steps of indicated amplitude in another cell. Asterisks: bursts of action potentials. Inset: 1st burst of E, shown at faster sweep (bar: 20 ms). F: F-I plot for indicated ISIs. Time calibration: 100 ms (A and B); 400 ms (D and E).
burst (Fig. 4A). In such cells a higher current could result in a three-spike burst (Fig. 4B), but the longer ISI that separated the burst from the subsequent tonic firing became less apparent. That is, the cells started to resemble FA cells at higher current.

Only two cells in this study exhibited rhythmic, repetitive bursts throughout the duration of a 1- or 2-s current pulse. The response of one of these cells is shown in Fig. 4D. Both of these cells had a three-spike burst (Fig. 4D, inset). Spike frequency slowed somewhat during each successive burst (from 313 to 250 spikes/s from the 1st to 4th burst in Fig. 4D). In both cells, the rhythmic bursts were evoked only at $I_v$. Increasing current amplitude by 50 pA caused two or three initial bursts to be followed by tonic firing (Fig. 4E). Raising the current another 50–100 pA (not shown) resulted in a single initial burst followed by tonic firing, and this pattern was observed at higher currents tested. The steady-state $F-I$ curve for these cells, constructed from the late tonic firing, was not significantly different in its slope or other properties from those of FA cells or other bursters.

Firing rate throughout the burst was related to current amplitude in three of the LTBs, as illustrated by the $F-I$ plot of Fig. 4C for the two-spike burster. In three other cells, the first ISI showed no relation to current, as shown in the $F-I$ plot of Fig. 4F. Even at $I_v$ the first two spikes occurred at a very fast rate in these cells (e.g., ISI labeled 1 in Fig. 4F; also see Fig. 6A), which may be the fastest rate at which the cells are capable of firing, but subsequent ISIs decreased monotonically as current amplitude was increased (e.g., ISIs labeled 2 and 3 in Fig. 4F). In one cell, there was no relation between firing rate and current amplitude (as for ISI labeled 1 in Fig. 4F) through an entire five-spike burst.

The durations of postburst ISIs (expressed as a fraction of the average, tonic ISI) are indicated by the box plots of Fig. 6A for both the LTBs and HTBs. There was no significant difference between the mean postburst ISI of these groups (2-tailed $t$-test, $P > 0.2$). Furthermore, ISI durations following two-spike and multispike bursts were similar in each group (data not shown). The LTBs differed from the HTBs in one respect besides burst threshold, however, as shown by the box plots of Fig. 6B. The instantaneous firing rate during the first ISI (expressed as a fraction of the rate during the postburst ISI in Fig. 6B) was much greater for LTBs (mean value: 217 spikes/s) than for HTBs (mean value: 93 spikes/s). Again, values from two-spike and multispike bursters were similar in each group. This greater excitability of the LTBs is even more remarkable in that the burst was evoked by a current step that was about twice as small on average as that in the HTBs.

Slowly adapting cells

The 36 cells described above (which formed the FA, HTB, and LTB groups) did not differ significantly (1-way analysis of variance, $P > 0.3$) in any measured property except their initial response to a current step. In contrast, a few recorded cells ($n = 5$) differed from all the others in several properties. We performed a two-tailed $t$-test (taking $P < 0.01$ as significant) to test for significant differences in parameters between this group of 5 cells and a group consisting of the other 36 analyzed cells. The following differences were statistically significant: depolarizing input resistance (means: 79.7 MΩ for these 5 cells vs. 39.5 MΩ for the other 36); hyperpolarizing input resistance (means: 40.5 vs. 15.5 MΩ); spike width (means: 1.2 vs. 1.0 ms), and $I_v$ (means: 0.27 vs. 0.58 nA). These five high-input-resistance cells were the only group showing a significant difference in primary range slope during tonic firing (means: 88.6 Hz/nA for these cells vs. 35.9 Hz/nA for the 36 others). Thus the only parameter associated with different tonic firing properties among our recorded population was cell size as judged by input resistance.

These smaller cells also exhibited a distinctive transient response to a current step. During larger injected currents, spike-frequency adaptation occurred over many ISIs (Fig. 5, A and B). This slow adaptation, best appreciated from the $F-T$ plot of Fig. 5B, is in marked contrast to the one- ISI adaptation observed in the larger, regular-spiking, FA cells described above. At $I_v$, these slowly adapting (SA) cells exhibited no adaptation, however. In each of the SA cells, instantaneous firing rate throughout adaptation was a bilinear function of current amplitude (Fig. 5C) in which the slope of the $F-I$ relation for each ISI became shallower until the final steady-state $F-I$ relation was attained.

In all but one SA cell, the spontaneous firing rate was apparent at the first current strength at which adaptation could be detected, as indicated by the box plots of Fig. 6A. The boxes for the SA and FA cells indicate the duration of the second ISI (expressed as a fraction of the average tonic ISI) measured at the first current strength at which adaptation could be detected. The average value of the second ISI for SA cells was 0.8 (indicating faster firing than the tonic rate), whereas the average for the FA cells was 1 (i.e., same as average tonic ISI). The difference between these two averages is small but statistically significant (2-tailed $t$-test, $P < 0.0005$).

Firing properties are stable

We investigated whether the nature of the transient response, as categorized above, or the steady-state $F-I$ relation might change over time. These questions were investigated in 20 cells by examining their firing characteristics as soon as membrane potential stabilized after impalement ($\pm 5$ min), and periodically thereafter over periods lasting up to 214 min in individual cells (mean duration of test period: 45 min). Two of the cells included in this test were HTBs, one cell was an LTB, two were SA, and the rest were FA. No cell was observed to change the nature of its transient response over the time periods tested. The steady-state $F-I$ relation also was remarkably consistent for all cell types. Figure 7A shows an example of the small (5%) change observed over a 30-min testing period in one cell. A summary of results for the population tested is shown in Fig. 7B. This figure plots the change of steady $F-I$ slope from its mean value (computed from every trial during the entire recording period) in 20 cells when sampled repeatedly at different times during the impalement. Time 0 starts at the onset of the first $F-I$ test after impalement. The vertical scatter of points at time 0 reflects the fact that the initial $F-I$ slope usually differed from the mean slope obtained over the whole time period. Points from three individual cells are shown (filled symbols) to indicate the degree of variation seen among individual cells. In most trials, the $F-I$ slope varied by $\pm 10\%$ from its mean value (average variation of slope
from mean: 3.7 ± 0.6%). Thus both the transient response and the steady-state F-I relation are stable over time.

Response to slow current ramps

Cells in vivo probably experience a time-varying synaptic current much more often than a constant current. To investigate whether the firing properties obtained from long-lasting current steps had value in predicting the response to a time-varying stimulus, we examined the response of 10 cells to ramps of current having slopes of 0.5–40 nA/s. In practice, we applied a ramp followed by a constant current (Fig. 8A) whose amplitude was 1.0–3.6 nA in different cells, and we varied the duration of the ramp portion of this stimulus to vary the rate of change of current. In Fig. 8B is plotted the instantaneous firing rates observed both during ramps of the indicated durations and during the postramp constant current for the cell of Fig. 8A. In the plots of Fig. 8B, time 0 marks the start of all ramps. The delay to the first ISI was caused by the time required for membrane potential to traverse the subthreshold region. Both this delay and the duration of the first ISI decreased as ramp rate increased. The points associated with firing during the postramp constant current define a line with a small negative slope. That is, the cell fired more slowly during the constant current following a slow ramp compared with a fast ramp. The firing behavior during the postramp constant current thus revealed a slow spike-frequency adaptation that was not observed during current steps of similar or longer duration.

In the cell of Fig. 8B, only one ISI occurred during the 100-ms-duration ramp, and it was far shorter than the subsequent ISIs during the postramp constant current. This behavior defined a limiting ramp rate, which varied between 10 and 40 nA/s among different cells, at which we stopped.
shortening the ramp. For ramps >100 ms in Fig. 8B, firing rate during the ramp could be fit by a straight line whose slope became steeper with ramp rate. In fact, firing rate during the linear portion of the response also overshot the rate attained subsequently during the postramp constant current, and this overshoot increased with ramp rate. The cell of Fig. 8B had a steady-state F–I relation that was fit by a single line (not shown), and as was true of all such cells tested with the ramp stimuli, the F–I plot during the ramps also was fit by a single line. The ramp response of cells whose steady-state F–I exhibited both a primary and secondary range was in all cases (n = 6) best fit with two lines (not shown). Of the 10 cells tested with ramps, 1 was a five-spike LTB. This cell fired a burst as its first response only at the onset of ramps rising ≥5 nA/s. During the remainder of the ramp, or throughout the entire ramp at slower rates, and during the postramp constant current, only regular spiking was observed. Another cell tested was a two-spike HTB. No burst was observed during ramp stimulation in this cell for ramp rates up to 25 nA/s. The remainder of the cells tested was FA.

Because current amplitude during the ramp increases linearly with time, it was possible to construct an F–I relation from the F–T relation obtained from the ramp stimulus. In constructing this F–I relation, we associated the instantaneous firing rate at a given time with the instantaneous ramp current existing during the second spike of each ISI. This ramp-evoked F–I relation was then compared with the steady-state F–I obtained from current steps. A typical result is shown in Fig. 9A. The slopes of the two F–I relations were usually quite similar, but the firing rate during the ramp was always higher than for the steady-state curve at a given current. During the postramp constant current, firing rate slowly decreased (adapted) until the data points lay on the steady-state curve at the corresponding current (Fig. 9A, ■). This behavior reflects the slow adaptation seen during the application of the postramp constant current in Fig. 8B. Thus firing rate during these slow ramps is not quite at steady state, even though the slope (the ‘‘gain’’) of the relation between instantaneous rate and instantaneous current is very similar to the steady-state value. The similarity of the slopes of the ramp-evoked and pulse-evoked F–I relations held over a range of ramp rates, as shown in Fig. 9B. In this plot, the ordinate shows the ratio of F–I relation slopes (ramp/steady state). For perfect correspondence, the data should be fit by a horizontal line through unity. The regression line to the data points has a slight negative slope and is slightly above the unity line. One interpretation is that during a stimulus change, the rate during the ramp could be fit by a straight line whose slope (the ‘‘gain’’ relation) code for rate of change of current, which is maximal during the adaptation. But tonic firing rate also codes current amplitude. What then is the functional meaning of spike-frequency adaptation? We hypothesized that firing rate during adaptation may code for rate of change of current, which is maximal during a current step. Perhaps the variation of firing rate with time during a stimulus can be expressed as a sum of two components, one (Fr) related to stimulus amplitude (I) and the other (Fh) related to stimulus rate (dl/dt), i.e., F(t) = Fr + Fh. Following a current step, Fr corresponds to the final tonic firing, and Fh corresponds to firing rate during adaptation minus the tonic rate. In an adapting neuron, for example, the firing rate following a current step might be described as F(t) = Kσ · I + (F0 − Kσ · I) · exp(−t/τ), where Kσ is the slope of the steady-state F–I curve and F0 is the instantaneous rate during the first ISI. For an FA cell, the time constant of adaptation (τ) would be very brief; it would be considerably longer for an SA cell, and a more complex formulation would be needed for a burst-firing cell. It might be possible...
FIG. 8. Responses to slow current ramps. All data from same cell (resting potential: −68 mV). A: response to stimulus consisting of current ramp followed by constant current. Spikes are truncated. B: F-T plot from start of ramp for ramps of indicated durations and same final postramp current as in A. Equations of regression lines fitted to linear portion of responses are also shown.

that the quantity \((F_1 - K_A \cdot I) = F^*\) increases monotonically, or even linearly, with \(dI/dt\), i.e., \(F^* = K_R \cdot (dI/dt)\), where \(K_R\) is the constant of proportionality between \(F^*\) and \(dI/dt\). To test the hypothesis that \(F^*\) (thus firing rate throughout adaptation) codes rate of change of current, we investigated the response of 10 additional neurons to fast current ramps. One was SA, two were HTBs, and the rest were FA.

In these tests we used ramp rates \((dI/dt)\) varying from 2 to 500 nA/s. The slower rates overlapped those of the slow ramps described above. As with the slow ramps, the fast ramps were followed by a constant current, and \(dI/dt\) was varied by varying ramp duration. As shown in Fig. 10, A1 and A2, the first ISI shortened as ramp rate was increased. If the initial firing rate depends on rate sensitivity, then the same ramp rate ought to give the same \(F^*\) independent of final current amplitude. Thus we repeated the tests in each cell with the use of two to three different postramp constant currents (Fig. 10, B1 and B2). Because firing rate did not adapt during currents near \(I_o\), the postramp current was constrained to be large enough to actually result in adaptation or even linearly, with \(dI/dt\), i.e., \(F^* = K_R \cdot (dI/dt)\), where \(K_R\) is the constant of proportionality between \(F^*\) and \(dI/dt\). To test the hypothesis that \(F^*\) (thus firing rate throughout adaptation) codes rate of change of current, we investigated the response of 10 additional neurons to fast current ramps. One was SA, two were HTBs, and the rest were FA.

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Typical results are shown for one experiment in Fig. 11A. The connected data points (diamonds labeled “adjusted”) represent \(F_1\) measured during each ramp minus the average tonic firing rate corresponding to the steady postramp current. It is apparent that \(F_1\) increases with \(dI/dt\) to an upper limit \((dI/dt_{\text{max}})\) that was identical to the \(F_1\) obtained during a current step having the same amplitude as the postramp constant current. All cells gave this maximal (step) response during a rate of rise that was far slower than during a step (mean value of \(dI/dt_{\text{max}}\): 29.1 ± 5.7 nA/s). The lower limit of the cell’s rate sensitivity was less clear. We judged that if \(F_1\) was slower than the tonic firing observed during the postramp constant current, then the amplitude sensitivity of the cell was dominating the
FIG. 9. Comparison of $F-I$ curves derived from slow current ramps and current steps. A: plots of instantaneous firing rate $F(t)$ and current $I(t)$ during a ramp-and-hold stimulus as in Fig. 7A (Ramp: ■), and the steady-state $F-I$ relation (Step: ◦) from same cell (resting potential: $-70 \text{ mV}$). Equations of regression lines fitted to initial linear segments of each relation are also shown. B: plot of ratio of slope of ramp-evoked $F-I$ curve (as in A) to slope of steady-state, step-evoked $F-I$ curve for different cells (denoted by different symbols) for different ramp rates.

Response rather than the rate sensitivity, i.e., $F_1$ reflected the instantaneous value of $I$ during the ramp rather than $dI/dt$. Data points (diamonds) obtained during the slowest ramps in Fig. 1A have negative values because $F_1$ was smaller than the tonic firing rate during the steady postramp current. Clearly, $F_1$ was dominated by amplitude sensitivity for these points. Therefore we selected the lower limit of rate sensitivity ($dI/dt_{\text{min}}$) as the point at which $F_1$ equaled the tonic firing rate during the postramp constant current (mean value of $dI/dt_{\text{min}}$: 6.8 ± 1.2 nA/s).

The data points between the maximum and minimum value of $dI/dt$ could be fit satisfactorily by a straight line in every cell. For these points we eliminated the amplitude-sensitive portion of the response with the use of the definition of $F_1^*$ given above. When the second spike occurred during the ramp, we subtracted the quantity $K_R \cdot I(t)$ from $F_1$, where $I(t)$ was the value of $I$ at the time of occurrence of the second spike. This correction for amplitude sensitivity naturally reduced the slope of the relation. The resulting data points (squares labeled “corrected” in Fig. 10A) were then fit with a line whose slope gives $K_R$ as defined above. The mean value of $K_R$ obtained from all cells and trials ($n = 26$) was $3.1 \pm 0.6$ spikes/nA. Thus discharge in excess of the tonic firing rate corresponding to a given current is proportional to rate of change of current, but this relation holds only over a limited range of $dI/dt$, and the cells cannot distinguish a rate of rise greater than $\approx 30$ nA/s from a step.

Results from all cells and trials are shown in Fig. 11B. To determine whether $K_R$ was independent of current amplitude, the $K_R$ values obtained in each trial in a given cell were expressed as a fraction of the mean $K_R$ obtained in that cell, which was set to unity. In Fig. 11B these normalized values of $K_R$ were plotted against the corresponding final postramp current. This current also was normalized by expressing it as a fraction of the mean ($\text{mean} = 1.0$) of the different postramp currents used in the cell. The slope of the regression line to the data points is not statistically different from 0 ($P = 0.8$), indicating no significant dependence of $K_R$ on current amplitude. Thus $K_R$ is a parameter that signifies a cell’s rate sensitivity, as the slope of the steady-state $F-I$ curve signifies its amplitude sensitivity.

 DISCUSSION

It would be difficult to predict the contribution of cellular firing properties to cortical function if those properties
changed spontaneously in the absence of experimental manipulation. Thus one important result of this study is that a cell's firing properties are stable over time in the absence of specific neuromodulation. This assumption is central to present ideas of cortical function but seems never to have been tested explicitly, at least not over long times in a population of neurons capable of variable responses. In terms of tonic firing properties, we could only distinguish two groups, low-input-resistance and high-input-resistance cells. The high-resistance cells had significantly steeper tonic F-I slopes and lower $I_o$ than the low-resistance cells. They also exhibited a wider spike and much slower adaptation during a current step. On the basis of their slow adaptation, high input resistance, and wider spikes, our SA cells correspond best to those cells classified as RS2 by Chagnac-Amitai and Connors (1989) and Agmon and Connors (1992) and to many of the cells classified simply as regular spiking in other studies (Agmon and Connors 1989, 1992; Connors et al. 1982; Franceschetti et al. 1995; McCormick et al. 1985; Montoro et al. 1988; Tseng and Prince 1993). We used much lower-resistance microelectrodes than these investigators, which probably accounts for the much smaller sample of high-resistance (presumably smaller) cells in our study.

It is simplest to assume that our high-input-resistance (SA) cells were physically smaller than our low-input-resistance cells. All 15 cells that we recovered after biocytin staining (see METHODS) were low-input-resistance cells (3 LTBs, 3 HTBs, and 9 FAs); no high-input-resistance (SA) cells were recovered. All recovered cells had similar general features (see Fig. 1A of Schwindt and Crill 1997): a pyramidal-shaped soma, an apical dendrite with the first major branch point 400–600 μm from the soma, a terminal dendritic arborization reaching the pial surface, and numerous basal dendrites and apical oblique branches. These are the general morphological features seen in identified intrinsic bursters (Agmon and Connors 1992; Chagnac-Amitai and Connors 1989; Chagnac-Amitai et al. 1990; Mason and Larkman 1990; Tseng and Prince 1993). Our staining was done only to ascertain whether recorded cells with similar electrical properties were pyramidal neurons from deep layer 5, however; no detailed reconstruction was attempted. In terms of morphology, the studies cited above report that intrinsic bursters have larger somata, thicker apical dendrites, more basal and apical oblique dendrites, and a larger terminal tuft than regular-spiking cells. These characteristics also have been observed in two-spike bursters (Wang and McCormick 1993) as well as nonbursting FA cells (Chagnac-Amitai et al. 1990; Franceschetti et al. 1995; Van Brederode and Snyder 1992). In contrast, SA regular-spiking cells are reported to have smaller somata, smaller-diameter apical dendrites, and a less extensive dendritic tree than burst-firing cells (Chagnac-Amitai et al. 1990; Franceschetti et al. 1995; Mason and Larkman 1990; Tseng and Prince 1993). It seems likely, therefore, that the high resistance of our SA cells was caused at least in part by their smaller size.

We observed two classes of regular-spiking cells in this study, the SA cells mentioned above and FA cells. The FA cells correspond best to those cells classified as RS1 by Chagnac-Amitai and Connors (1989) and Agmon and Connors (1992). Examples of FA cells are apparent in records

FIG. 10. Duration of 1st ISI varies with current rate during fast ramps. All records from same cell (resting potential: – 68 mV). Spikes are truncated. A1 and A2: 1st ISIs of different durations are evoked by ramps rising at different rates to same postramp current. B1 and B2: 1st ISIs respond as in A1 and A2 to ramps that rise to a larger postramp current that evokes a faster tonic rate than in A.
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FIG. 11. Firing rate during 1st ISI codes rate of change of current (dl/dt). A: plot of instantaneous firing rate \(F_1\) during 1st ISI evoked by current ramps rising at different rates (dl/dt) in 1 cell (resting potential: \(-72\) mV). Diamonds ("adjusted" points) plot the quantity F1 minus average tonic firing rate during postramp steady current. Squares ("corrected" points) plot rate-sensitive portion of response \(F^*_1\), defined in text) and are fit with a regression line whose equation is shown. Slope of this line gives rate sensitivity \(K_R\) for this cell. Limits of rate sensitivity are indicated by dl/dt min and dl/dt max (see text).

B: plot of normalized rate sensitivity \(K_R\) against normalized final postramp current for all experiments. Two to 3 final current amplitudes were employed in each cell. For each cell the mean value of \(K_R\) and final current amplitude were both set to unity, and individual \(K_R\)s and final currents were expressed as fractions of these means. Corresponding regression line indicates little dependence of \(K_R\) on final current amplitude.

presented in several other studies (Chagnac-Amitai et al. 1990; Franceschetti et al. 1995; Mason and Larkman 1990; McCormick et al. 1985; Montoro et al. 1988; Silva et al. 1991; Tseng and Prince 1993; Van Brederode and Snyder 1992), but the relative preponderance of these cells was not clear. The FA cells were distinguished from the SA cells principally by their adaptation pattern but also by their lower input resistance, shorter spike duration, and higher \(I_o\). The FA cells were the most numerous (54%) in our study and may be the most common low-input-resistance, layer 5 pyramidal neurons in the area of cortex investigated. New findings concerning regular-spiking cells are that neither SA nor FA cells exhibit adaptation when the injected current is near \(I_o\), and the FA cells exhibit no adaptation until the injected current becomes \(>60% > I_o\).

Only the low-input-resistance cells recorded in this study exhibited burst firing. The number of bursters we observed (38%) is in line with most other investigations (Agnon and Connors 1989, 1992; Connors et al. 1982; Franceschetti et al. 1995; McCormick et al. 1985; Montoro et al. 1988; Tseng and Prince 1993). We observed two classes of bursters distinguished by the injected current at which the burst was evoked. The HTBs were so called because a burst was evoked by an injected current about twice as large as \(I_o\), whereas \(I_o\) evoked a burst in the LTBs. The cells we call LTBs correspond best to most examples of intrinsic bursters presented in other studies. Cells that fired a burst only at \(I > I_o\) are apparent, however, in records presented in several studies (Chagnac-Amitai et al. 1990; Mason and Larkman 1990; McCormick et al. 1985; Montoro et al. 1988; Tseng...
and LTBs was not clear.

In our study HTBs were the second most numerous class (28%) and were clearly related to the FA cells. At currents lower than that evoking the burst ($I_b$), the behavior of the HTBs was indistinguishable from that of FA cells. The number of spikes in the burst (2–5) made no difference in the behavior of these cells for $I < I_b$, but the two-spike bursters again became similar to the FA cells at high current strength, because the postburst ISI became more similar to the duration of the ISI during tonic firing. LTBs were least numerous in our study (10%). Most of these cells were related to FA cells insofar as they displayed a two-spike burst that became less noticeable (more similar to FA cells) at higher currents, and they displayed tonic firing almost immediately after the burst. They differed greatly from the HTBs and from both classes of regular-spiking cells in their much faster initial firing rate (Fig. 6B). We only recorded two LTBs capable of repetitive bursts. Corticotectal and corticopontine layer 5 pyramidal neurons exhibit the repetitive bursts (Wang and McCormick 1993), however, and cells having this projection may be relatively rare in the region of cortex that we investigated.

The bursts we observed were two to five spikes long, but two-spike bursts were most common in our study. It has been reported that bursts of more than two spikes are unique to layer 5 pyramidal neurons, whereas two initial high-frequency spikes (“doublets”) can be evoked in cells in all cortical layers (Agmon and Connors 1992; Connors 1984; McCormick et al. 1985). However, it is not entirely clear whether the doublet firing of the superficial cells simply represents the short first ISI of a regular-spiking cell similar to our FA layer 5 cells (see, e.g., Fig. 4A2 of Mason and Larkman 1990). We recognized a two-spike burst if the postburst ISI was longer than the ISI during tonic firing. In both LTBs and HTBs we could find no statistically significant difference between the duration of the postburst ISI or the firing rate during the first ISI when comparing two-spike and longer bursts in each group. The number of spikes in the burst seems, therefore, simply to reflect the range of burst durations available among the neuron pool rather than qualitatively different burst responses.

In only 1 of 41 analyzed cells (an LT) did firing rate throughout the entire initial response show no relation to current step amplitude. In the other cells, earlier ISIs were shorter than later ISIs, and the firing rate of most ISIs was a monotonically increasing function of current amplitude. With the use of short ramps, we found that firing rate during the initial response (actually, the excess discharge above the final, tonic rate) increased linearly with the rate of change of current, which is maximal during a current step. Admittedly, we only sampled two HTBs in the fast ramp test, and we need to see whether this rate coding holds in a larger sample of bursters, particularly LTBs. The fact that firing rate during the burst was related to current step amplitude in most recorded bursters suggests that it will hold. If so, one possible functional implication of the variety of transient responses (both bursts and adaptation) is that the population of large pyramidal cells is endowed with a variety of gains ($K_S$) and durations (1 to several ISIs) over which to signal $dI/dt$ to follower cells. On the other hand, our fast ramp data indicate that the range and sensitivity of rate coding is limited: the cells cannot distinguish a sufficiently fast $dI/dt$ from a step. Furthermore, it is difficult to see how subsequent bursts in those cells that exhibited repetitive bursts during a constant current could be signaling rate of change of current.

Perhaps burst firing is important for a different reason. A burst is distinguished by a long ISI following a group of rapid spikes (Fig. 6A). The long ISI represents a period of decreased excitability during which it is more difficult to trigger a spike. Perhaps the significance of burst firing versus adaptation would be more apparent if the stimulus consisted of a train of short-duration pulses instead of a single long-lasting pulse. It is possible that regular-spiking cells and bursters would respond best (e.g., with the greatest number of spikes per unit time) to different pulse frequencies because of the different durations of their postburst ISIs. We might expect that FA cells would respond best to fast pulse frequencies and bursters to slower stimulus frequencies according to the number of spikes in the burst. Furthermore, LTBs would fire much faster than the other categories to a given pulse amplitude (Fig. 6B), but which category would actually give the greatest spike density is unclear. The response to pulse trains may thus provide a clearer picture of why there is a variety of transient responses than is apparent with the use of long-lasting pulses.

Some investigators have reported that burst firing is abolished when the bursters are tonically depolarized (McCormick et al. 1985; Wang and McCormick 1993). We did not test this important idea explicitly, but the simple fact that bursting in most recorded cells occurred only at the onset of the depolarization is consistent with this idea. Our (limited) findings that bursters did not burst during slow ramp depolarizations and that the rhythmic bursters converted to initial bursters during small depolarization also are consistent with this view. This idea needs to be tested more extensively because, if it is correct, it means that the burst-firing capability evaporates during tonic depolarization. Tonic firing properties would then determine the response to both time-varying and steady synaptic current. In the present study, all low-input-resistance cells (both bursters and nonbursters) exhibited similar tonic firing properties, and these properties were good predictors of their response to slow, time-varying currents. Recordings of neuron activity in alert animals in vivo, at least in motor cortex of the monkey, suggest that pyramidal neurons normally are depolarized because they often fire spontaneously, and they engage in long-lasting firing during motor tasks (e.g., Cheney and Fetz 1980). In this situation firing properties associated with tonic or slowly changing synaptic current would be most relevant, and the most significant functional division would then be between high-input-resistance (small) and low-input-resistance (large) cells because of the differences in their tonic responses.

On the basis of their tonic firing properties, both the large and small cells are well suited to perform a linear transduction of synaptic input to spike output over a wide range of currents for $I > I_c$. The larger cells have a significantly smaller DC gain ($F-I$ curve slope) than the smaller cells and would thus fire fewer spikes per unit change in direct driving current. Our data suggest another important distinction between the large and small cells, namely, the ability of their
output (firing rate) to accurately code (“follow”) rapid changes of synaptic current. If the transduction process obeyed the rules for a linear system, the time course of adaptation would indicate how well a cell’s output can follow its input. SA, FA, and HTB cells exhibit no adaptation at low currents, indicating perfect frequency following in these cells for currents near $I_c$. At higher currents FA cells, and even most bursters, reached steady state rapidly following a current step (within 1 ISI for the FA cells). Consequently, these cells can accurately follow rapidly changing input of any amplitude. The smaller (SA) cells depolarization underlying the current-evoked burst (Franceschetti et al. 1995). This glutamate-evoked bursting in these cells for currents near $I_c$ in all cells examined during iontophoresis (Schwindt and Crill 1995, 1996, 1997), but it is possible that cells that burst during somatic current injection have a particularly large somatic nonactivating Na$^+$ current (or a small K$^+$ current) compared with nonbursters. If so, a burst-generating membrane current would be available to these cells whether or not dendritic spikes were evoked.

Above, we outline several questions about spike coding during somatically injected current that remain to be examined. The answers to these questions will reveal more fully how the cell can respond to the synaptic current that reaches the soma, but this information is unlikely to reveal the full story of input-output transformation in a cortical neuron. Integrating these results with the varied responses that may be evoked by dendritic stimulation will be quite a challenge.

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