Intrinsic firing patterns of diverse neocortical neurons

Barry W. Connors and Michael J. Gutnick

Neurons of the neocortex differ dramatically in the patterns of action potentials they generate in response to current steps. Regular-spiking cells adapt strongly during maintained stimuli, whereas fast-spiking cells can sustain very high firing frequencies with little or no adaptation. Intrinsic bursting cells generate clusters of spikes (bursts), either singly or repetitively. These physiological distinctions have morphological correlates. RS and IB cells can be either pyramidal neurons or spiny stellate cells, and thus constitute the excitatory cells of the cortex. FS cells are smooth or sparsely spiny non-pyramidal cells, and are likely to be GABAergic inhibitory interneurons. The different firing properties of neurons in neocortex contribute significantly to its network behavior.

It is axiomatic in neuroscience that the informational output of most neurons is completely defined by their temporal patterns of action potentials. This notion is implicit in all contemporary models of the functions of neocortex, making it essential to understand how each neuron transforms its input into output. Studies show that the intrinsic membrane properties of neurons in the neocortex are not homogeneous, but instead produce several categories of transform characteristics.

The fine structure of the neocortex varies from region to region and from species to species; however, unifying principles underlie the assembly of individual neocortical neurons into functional circuits. A fundamental unit of neocortex appears to be a group of diverse, radially organized neurons, each with its own morphological and physiological characteristics and its own pattern of synaptic connections. One classic investigational approach to this complex local circuitry is taxonomic classification. Neocortical neurons have been categorized according to various criteria such as location, morphology (i.e. size, soma shape and dendrite patterns), synaptic relationships locally and with distant cortical and subcortical regions, and biochemical properties (especially neurotransmitters and their associated enzymes). Nevertheless, in order to understand a particular neuron's functional role within a circuit, it is not enough to know only these characteristics. Its electrical fingerprint, as determined by its intrinsic membrane properties, is also important.

It has long been known that neuronal membranes do not all behave similarly. Neurons differ in the types and distributions of specific ion channels on their somata and dendrites. These intrinsic membrane differences are manifest as differences in the shapes of individual action potentials. They also lead to distinctive temporal patterns of repetitive firing, and thus determine to a large extent the way individual neurons transform synaptic input into spike output. These intrinsic physiological properties constitute a reasonable basis for neuronal classification, since studies from a large number of brain areas show that the electrical fingerprint can be extremely uniform from cell to cell within a particular neuronal class (e.g. among cerebellar Purkinje cells, thalamic relay cells, or dopaminergic cells of the substantia nigra; for a recent comprehensive review, see Ref. 4).

Neurons of the neocortex are not physiologically homogeneous. Three basic types of intrinsic physiology have been recognized, and for them there are: regular-spiking (RS), fast-spiking (FS) and intrinsically bursting (IB). For each type, classification is based on three general variables—the characteristics of individual action potential–afterpotential complexes, the response to a just-threshold intracellular current pulse, and the repetitive response to prolonged, intracellularly applied stimuli. While these neuron classes are based entirely on physiological differences, intracellular staining experiments suggest that there are also some distinct morphological correlates.

The general characteristics of the three classes of neurons, derived mostly from studies of rodent neocortex in vivo, of slices in vitro, and from tissue culture (both explants and dissociated cells), are summarized in Table I and discussed below. It is not our intention here to describe the specific ionic mechanisms underlying these neuronal behaviors, since only RS neurons have been examined in any detail. We also emphasize that these categories are not necessarily comprehensive, exclusive or definitive. On the one hand, it is very likely that subcategories exist, or that a continuum of variation better describes some cell populations; on the other,

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Physiological class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regular-spiking (RS)</td>
</tr>
<tr>
<td>Single-spike:</td>
<td></td>
</tr>
<tr>
<td>rate of rise</td>
<td>++</td>
</tr>
<tr>
<td>rate of fall</td>
<td>+</td>
</tr>
<tr>
<td>Single-spike afterpotential</td>
<td>Complex, AHPs and ADP</td>
</tr>
<tr>
<td>Frequency adaptation</td>
<td>+</td>
</tr>
<tr>
<td>Spike bursts during injected current</td>
<td>-</td>
</tr>
<tr>
<td>Laminar location of soma</td>
<td>II to VI</td>
</tr>
<tr>
<td>Presumed morphology</td>
<td>Pyramidal or spiny stellate</td>
</tr>
<tr>
<td>Presumed synaptic function</td>
<td>Excitatory (glutamate or aspartate)</td>
</tr>
</tbody>
</table>

Symbols: +, the presence and relative strength of a characteristic; -, the absence of a characteristic. Abbreviations: AHP, afterhyperpolarization; ADP, afterdepolarization. Table adapted from Ref. 15, and compiled from data in the following Refs: 7–12, 16, 18, Gutnick, M. J. and White, E. L., unpublished observations, and Agmon, A., PhD Thesis, Stanford University, CA, 1988.

© 1990. Elsevier Science Publishers Ltd (UK) 0166-2236/90/$02.00
Fig. 1. Differences in intrinsic firing patterns between regular-spiking (RS) and fast-spiking (FS) neurons. (A) When stimulated with a suprathreshold step of depolarizing current, RS cells respond with an initial high-frequency spike output that rapidly declines to much lower sustained frequencies. In this and subsequent figures, intracellular voltages are displayed in the top trace, injected current steps in the bottom trace. (B) Under similar conditions FS cells generate high frequencies that are sustained for the duration of the stimulus. (C) Temporal patterns of typical RS and FS firing displayed graphically. The firing frequencies were calculated from the reciprocal of the interspike interval, and plotted as a function of time during a strong step pulse of intracellular current, as in (A) and (B). Although each cell initially generated similar spike frequencies (about 320 Hz), the RS cell (solid line) declined within 50 ms to <100 Hz, while the FS cell (dotted line) actually accelerated slightly to >350 Hz. [(A) taken, with permission, from Ref. 9; (B) and (C) modified from Ref. 10.]

it is possible that rare cell types have not yet been described. There is also a dearth of comparative data across both species and cortical areas.

**Regular-spiking (RS) neurons**

As their name implies, the neurons most commonly encountered in electrophysiological studies generate what Mountcastle and his colleagues were the first to call 'regular' action potentials. Most published intracellular recordings from neocortex in vivo have been from RS neurons (e.g. Refs 5, 6). Individual regular spikes of RS cells are relatively long-lasting, mainly because of a slow rate of repolarization. Each spike is usually followed by a complex set of intrinsically generated afterhyperpolarizations (AHPs) and afterdepolarizations (ADPs). When stimulated at threshold, an RS neuron generates only a single spike and, in contrast to IB behavior, as the stimulus amplitude increases, the first interspike interval decreases as a strong function of current intensity. When presented with prolonged stimuli of constant amplitude, RS neurons exhibit pronounced adaptation of the spike frequency (Fig. 1A, C).

**Fast-spiking (FS) neurons**

Mountcastle et al. also described rarely encountered neurons with relatively 'thin' (i.e. brief duration) extracellular spikes in monkey cortex. Nine years later, Simons made similar observations in rat neocortex and called these 'fast' spikes. Both groups remarked on how difficult it was to record thin/fast spikes with an extracellular electrode. Since the development of in vitro preparations, it has been possible to study the FS neurons using intracellular methods (Agmon, A., PhD Thesis, Stanford University, CA, 1988). However, recordings continue to be infrequent and technically challenging. Individual fast spikes usually last less than 0.5 ms. Although spikes of all three neocortical cell types have similar depolarization rates, those of FS cells are faster because of a more rapid rate of repolarization, and each spike is cut short by a deep, relatively brief AHP (Fig. 2B, C). The more prolonged hyperpolarizing (and depolarizing) afterpotentials that characterize RS and IB neurons are not prominent in FS cells. FS neurons are most impressively defined by their repetitive firing pattern; they undergo little or no adaptation during prolonged intracellular current pulses (Fig. 1B, C). Indeed, when strongly stimulated, they can sustain spike frequencies of at least 500–600 Hz for hundreds of milliseconds. FS cells are thus able to perform a relatively faithful conversion of input to output over a wide dynamic range; in strong contrast to RS and IB cells, the output of these neurons is likely to retain the precise temporal features of their input (Fig. 1C; cf. FS and RS firing patterns).

**Intrinsically bursting (IB) neurons**

IB neurons are distinguished by the tendency for their spikes to appear in a stereotyped, clustered pattern, the burst(9–11). Bursts are often the minimal
cells, most of which have smooth or sparsely spined dendrites, prominent apical dendrites, excitatory synaptic function, and axons that project out of the cortex as well as locally; and non-pyramidal neurons: pyramidal cells, which have a high density of excitatory synaptic function, and an axon that projects out of the cortex. Virtually all neocortical neurons can be placed into one of two categorizations of cortical neurons. Virtually all cortical neurons can be placed into one of the traditional classifications of the Golgi anatomists, as determined by light microscopy. There is now a relatively consistent and accepted scheme for the morphological classification of cortical neurons. Almost any neuron might produce clusters of spikes in response to phasic synaptic input. However, this response pattern does not, of itself, justify classification as an IB neuron. The distinction between intrinsic bursts and synaptic-driven bursts, which is essential for understanding a neuron's role within a circuit, cannot be made without methods that isolate the activity of a cell from its extrinsic connections. This may account, in part, for the fact that IB neurons were only recently recognized in neocortex. Moreover, IB neurons are relatively rare and are usually encountered only in certain laminae.

Correlations between intrinsic physiology and morphology

By impaling neocortical neurons with micropipettes that contain an intracellular dye (such as Lucifer yellow, horseradish peroxidase or biocytin) it has been possible to correlate intrinsic physiological properties, as described above, with the traditional classifications of the Golgi anatomists, as determined by light microscopy. There is now a relatively consistent and accepted scheme for the morphological categorization of cortical neurons. Virtually all neocortical neurons can be placed into one of two groups: pyramidal cells, which have a high density of dendritic spines, prominent apical dendrites, excitatory synaptic function, and an axon that projects out of the cortex as well as locally; and non-pyramidal cells, most of which have smooth or sparsely spined dendrites of various configurations, inhibitory (GABA-mediated) synaptic function, and axons that arborize only locally within the cortex. One major subtype, found in layer IV of many primary sensory areas, is a class of small neurons usually called spiny stellate cells. With their high spine densities and presumed excitatory synaptic function, spiny stellate cells are very similar to pyramidal cells. However, their axons usually do not leave the cortex.

Pyramidal cells constitute the majority of neurons in the neocortex (60–70%). Their sizes range from some of the smallest to the very largest cortical cells, and their somata can be found in layers II through VI.

**Fig. 2.** Neurons that elicit EPSPs have more prolonged action potentials than neurons that elicit IPSPs. Pairs of neurons were recorded intracellularly in microcultures of rat neocortical neurons. One cell was stimulated with intracellular current to generate an action potential, while the resulting synaptic response was monitored in the second cell. (A) In this pair, a spike in cell 1 yielded a monosynaptic EPSP in cell 2. Application of the excitatory amino acid antagonist γ-D-glutamylglycine (DGG) blocked the EPSP. (B) In a different pair of neurons from those shown in (A), a spike in cell 3 yielded a monosynaptic IPSP in cell 4. Application of bicuculline, a GABA receptor antagonist, blocked the IPSP. (C) Action potentials of seven excitatory (E) and seven inhibitory (I) presynaptic neurons superimposed to show the differences in spike duration. (Figure modified from Ref. 12.)
Fig. 3. Diverse firing patterns of intrinsically bursting (IB) neurons. (A) Single intrinsic bursts evoked by threshold pulses of intracellular current in a neuron from guinea-pig sensorimotor cortex. Slightly larger currents generated a very similar burst at much shorter latency. Figure shows three superimposed traces. Voltage and current calibrations in (A) apply also to (B) and (C). (B) Response of an IB cell in mouse SI cortex to prolonged current stimulus. Sequence of bursts and single spikes terminating with a train of single spikes. Arrowhead points to a prominent ADP following a single spike. (C) Repetitive intrinsic bursting in response to prolonged stimulus. Mean interburst frequency was about 9 Hz. (D) Transition from rhythmic bursting to single-spiking in a deep layer V neuron from rat SI cortex (top). Each burst consisted of three spikes firing at about 250 Hz. The graph plots the response: the neuron generated four bursts at 10–11 Hz (interburst frequencies plotted by broken line), then abruptly changed to single-spike firing at 15–20 Hz (plotted by solid line). Each triplet of vertical lines represents an interburst interval. Each single vertical line is an interspike interval. (A) taken, with permission, from Ref. 10; (B) and (C) taken, with permission, from Ref. 9; (D) provided by Y. Chagnac-Amitai, L. R. Silva and B. W. Connors, see Ref. 17.

It is therefore not surprising that this is the cell type most frequently encountered by dye-containing microelectrodes. In recordings from neocortex both in vitro and in vivo, almost all RS cells that have been morphologically identified have been pyramidal cells (e.g. Refs 10, 18, 19). Most IB cells have also been identified as pyramidal cells; however, their somata are restricted to layers IV and V. A preliminary study of layer IV spiny stellate cells indicates that they show the same spectrum of physiological properties as layer IV pyramidal cells; some are RS cells while others burst intrinsically (Gutnick, M. J. and White, E. L., unpublished observations).

That neurons of different morphological classes can show similar intrinsic physiological characteristics, and vice versa, implies that these parameters are not causally related. Nonetheless, there is recent evidence that they can be consistently correlated. Chagnac-Amitai et al. have found that although RS and IB cells in rat layer Vb are all pyramidal neurons, the IB cells have larger somata, more dendritic branches, and markedly different patterns of intracortical axons. This latter difference may be an important clue to the function of these IB cells within the cortical circuit; while the RS cells all had axon collaterals that ascended and branched profusely within supragranular layers, the axons of IB cells tended to stay within infragranular layers, and often extended far in the horizontal dimension.

Two primary lines of evidence imply that the elusive FS cells are GABAergic non-pyramidal cells. First, all neurons with the physiological properties of FS cells that have also been intracellularly stained have had the somatic size and shape, and the smooth or sparsely spiny dendritic morphologies that are peculiar to cells containing GABA or its associated enzyme systems. Second, paired recordings in cultures of dissociated neocortical neurons have
Fig. 4. Schematic summary of correlations between intrinsic physiology and anatomy of rodent neocortical neurons. RS neurons (open symbols) are spiny cells, either pyramidal or stellate, distributed through layers II through VI. FS neurons (filled symbols) are aspiny or sparsely spiny non-pyramidal cells, with presumed GABAergic inhibitory function, also distributed through layers II through VI. IB neurons (shaded symbols) are restricted to layers IV and V, and are also spiny cells of pyramidal or stellate morphology. Neurons of layer I have not been studied physiologically. Braces on the right summarize the laminar distributions of the three neuron types, and illustrate a typical firing pattern of each. WM, white matter.

shown that cells generating monosynaptic, GABA-mediated IPSPs onto follower neurons have significantly faster spikes than those cells generating monosynaptic EPSPs12 (Fig. 2). It is still quite possible that there exist types of neocortical GABAergic neurons that are not FS cells, as suggested for the hippocampus21. However, the data strongly suggest that every FS neuron encountered in the neocortex is a GABAergic inhibitory cell.

The data are too scant to ascertain whether classification into three types of neurons on the basis of intrinsic physiological properties is generally applicable to all cortical areas and all species. Analogous neuronal classes have been described for the dorsal cerebral cortex of turtles, where pyramidal-shaped cells generate RS-like or IB-like activity and non-pyramidal interneurons generate FS-like activity22. Thus it is likely that this separation arose early in forebrain evolution, and may now be widespread. All three classes have been repeatedly observed in rodent neocortex (i.e. mice, rats and guinea-pigs), as described above. Mountcastle’s original description of FS and RS neurons applied to monkey neocortex, and human neocortex also has both FS and RS cells (Ref. 23; McCormick, D. A., unpublished observations). The prevalence of IB cells across species is less well described. They have not been observed in extensive investigations of layer V neurons in cat sensorimotor cortex in vitro18; however, these studies targeted only the largest (presumed Betz) cells by using microelectrodes with large tip diameters. An earlier study of cat pyramidal tract cells in vitro described some features of the rhythmic IB cells seen in rodents (see Fig. 6C in Ref. 6). There are at least two preliminary reports of IB neurons in human neocortex23,24. Figure 4 schematically depicts the general distribution of neuron classes in neocortex, based largely upon studies in rodents.

There are several morphological classes of neocortical neurons whose intrinsic physiological properties have not yet been examined2. These include the small population of non-GABAergic, non-pyramidal neurons (notably peptidergic bipolar cells) and the assorted enigmatic neurons of layer I, many of which are GABAergic. Also, neurons of layer VI have been only sparsely studied. Finally, it would be of great interest to know whether the diversity of anatomy and biochemistry among GABAergic neurons2 is paralleled by a diversity of intrinsic firing patterns21.

Significance of diverse intrinsic firing patterns in neocortex

The intrinsic physiological properties of a neuron’s membrane play a central role in determining (1) how it transforms the information it receives into an output pattern, (2) how these transformations are modulated by humoral or environmental factors, and (3) whether (and with what pattern) the neuron generates spontaneous activity. Since these properties can vary widely from neuron to neuron, knowledge of the quirks of each cell type is an essential step in unraveling the functions of a neural circuit25. In the neocortex, it is evident that RS cells will attenuate prolonged excitatory stimuli while favoring the transmission of phasic ones; by contrast, FS cells offer a wide-band responsiveness and, if necessary, sustained high-frequency output. The complexities of IB cell behavior suggest more varied possibilities. Near threshold for firing they have very high gains,
Generating either a very large output or none at all in response to small increments of input amplitude. When coupled to each other synthetically IB cells may serve as initiators of synchronized cortical activities. Those IB cells with a tendency to oscillate may play a pivotal role in cortical rhythogenesis. During different behavioral states, various neurotransmitters would be expected to modulate the intrinsic firing patterns of RS, and presumably IB and FS, neurons by altering their intrinsic membrane properties.

Many important questions remain. We are just beginning to understand the varied functional properties of neocortical neurons and how they correlate with other cellular features. The ionic mechanisms of intrinsic firing patterns have only been extensively examined in RS neurons, and there is an urgent need to extend these analyses to IB and FS cells. We also need more extensive comparative data, across both species and neocortical areas, to establish the generality of the principles outlined here. As computational approaches to the functions of neocortex advance, many realistic models will need to incorporate not only the morphological and biochemical aspects of cortical connectivity, but also the specific biophysical characteristics of each class of neuron.

Selected references

Hormonal control of neuropeptide gene expression in sexually dimorphic olfactory pathways

Richard B. Simler

An abundance of experimental literature has established that gonadal steroid hormones are responsible for the sexual differentiation of neural circuitry, mediating a variety of reproductive behaviors and physiological mechanisms. These same hormones regulate the expression of reproductive function in the adult and may influence the responsiveness of the brain to specific olfactory cues. The recent demonstration that the expression of the neuropeptide cholecystokinin is activationally regulated by estrogen at the mRNA level, within a sexually dimorphic population of neurons in the medial amygdala, suggests a possible cellular mechanism for the hormonal modulation of olfactory information relayed along the vomeronasal pathway to the hypothalamus.

Gonadal steroid hormones influence mammalian reproductive function in two fundamental ways. First, during the perinatal period these hormones can permanently alter the pattern of copulatory behavior and gonadotropin secretion expressed during adulthood, and second, the expression of these sexually dimorphic functions in the adult is dependent on adequate levels of circulating gonadal hormones. In the rat, perinatal exposure to differential levels of gonadal steroids determines, at least in part, whether the mounting behavior that is typical of male rats, or the lordosis response typical of females, will be displayed by adult animals. In a similar way, perinatal steroids determine whether the adult pattern of gonadotropin secretion will be cyclic (with the periodic surges of luteinizing hormone that lead to ovulation in female rats), or relatively constant as it is in male animals. Furthermore, gonadectomy reduces reproductive behavior in both mature male and female animals, and subsequent hormone treatment restores, or `activates' these behaviors in response to appropriate sensory cues. The discovery that the morphological organization of certain brain regions thought to mediate reproductive function is sexually dimorphic led to the now widely accepted idea that functional sexual differentiation has an anatomical basis. Thus, gonadal steroids permanently alter the morphology and connections of certain groups of...