Electrophysiological Properties of Neocortical Neurons in Vitro

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SUMMARY AND CONCLUSIONS

1. Intracellular recordings were obtained from neurons of the guinea pig sensorimotor cortical slice maintained in vitro. Under control recording conditions input resistances, time constants, and spiking characteristics of slice neurons were well within the ranges reported by other investigators for neocortical neurons in situ. However, resting potentials (mean of 75 mV) and spike amplitudes (mean of 93.5 mV) were 10–25 mV greater than has been observed in intact preparations.

2. Current-voltage relationships obtained under current clamp revealed a spectrum of membrane-rectifying properties at potentials that were subthreshold for spike generation. Ionic and pharmacologic analyses suggest that subthreshold membrane behavior is dominated by voltage-sensitive, very slowly inactivating conductances to K+ and Na+.

3. Action potentials were predominantly Na+ dependent under normal conditions but when outward K+ currents were reduced pharmacologically, it was possible, in most cells, to evoke a non-Na+-dependent, tetrodotoxin- (TTX) insensitive spike, which was followed by a prominent depolarizing afterpotential. Both of these events were blocked by the Ca2+ current antagonists, Co2+ and Mn2+.

4. A small population of neurons generated intrinsic, all-or-none burst potentials when depolarized with current pulses or by synaptic activation. These cells were located at a narrow range of depths comprising layer IV and the more superficial parts of layer V.

5. Spontaneous excitatory synaptic potentials appeared in all neurons. Spontaneous inhibitory events were visible in only about 10% of the cells, and in those cases apparently reversed polarity at a level slightly positive to resting potential. Stimulation of the surface of the slice at low intensities evoked robust and usually concurrent excitatory and inhibitory synaptic potentials. Unitary inhibitory postsynaptic potentials (IPSPs) reversed at levels positive to rest. Stronger stimulation produced a labile, long-duration, hyperpolarizing IPSP with a reversal potential 15–20 mV negative to the resting level.

6. Neocortical neurons in vitro retain the basic membrane and synaptic properties ascribed to them in situ. However, the array of passive and active membrane behavior observed in the slice suggests that cortical neurons may be differentiated by specific functional properties as well as by their extensive morphological diversity.

INTRODUCTION

A detailed understanding of the mechanisms of information processing in neocortical circuits will require precise measurements of the biophysical properties of their neuronal membranes and synapses. The rich diversity of cellular morphologies would seem to presage a similar profusion of electrophysiological behavior in single cortical neurons. The earliest examinations of this hypothesis included some of the first intracellular measurements in mammalian central nervous systems and were obtained from neocortical cells of the cat (2, 4, 59). Subsequent intracellular studies of neocortical neurons in vivo have helped define some of their active and passive membrane properties and pharmacological sensitivities. Although certain investigative issues require exploration of neocortex in situ, others have proven amenable
to simplified systems such as cell culture (16) or, as will be shown here, the neocortical slice preparation. Such approaches offer certain technical advantages, in particular, a relative mechanical stability, which improves the quality and duration of intracellular recordings, the option of producing a simple and defined experimental alteration of extracellular fluids, and visibility and accessibility of the structures under study to facilitate the precise placement of electrodes.

The introduction of methods for the maintenance of thin slices of brain tissue in vitro (51, 53, 67) has allowed detailed electrophysiological studies of paleocortex (49, 50) and archicortex (51, 63, 65); however, to date intracellular investigations of neurons in slices of neocortex have been limited to short reports concerning biopsied human material (45, 46, 52), penicillin-treated tissue of the guinea pig (13), and layer V cells of cat motor cortex (56). Recently we have utilized the guinea pig neocortical slice to present evidence for electrotonic coupling between cortical neurons (21) and glia (20). In the experiments reported here we systematically explored the membrane properties and synaptic activity of neurons in sensorimotor cortical slices of adult guinea pigs. The purpose of the studies was threefold: we wished to validate the neocortical slice model by comparing neuronal properties in vitro with those obtained in situ by other investigators, to investigate neuronal membrane properties by experimental procedures not feasible in vivo, and to provide control data for subsequent studies of abnormal cortical function (19). Some of these results have been presented in a preliminary communication (11).

METHODS

Techniques for maintaining neocortical slices in vitro (13, 20, 21) were similar to those described for slices of hippocampus (51, 53, 67). Guinea pigs of either sex (250–400 g) were decapitated and the brains were rapidly removed and placed in an ice-cold physiological saline solution. A small block of tissue was dissected from a region approximately corresponding to sensorimotor cortex. Slices were cut in the coronal plane on a McIlwain tissue chopper and placed in the recording chamber. Initial experiments were performed on slices with a nominal thickness of 350 μm, but it was subsequently discovered that successful recordings in middle and deep cortical layers were more easily obtained from slices of 500 μm thickness. Increasing the slice thickness to 500 μm did not otherwise noticeably affect the electrophysiological properties. Slices were supported by a platform of nylon netting and superfused with a control solution containing (in mM): NaCl, 124; KCl, 5; CaCl2, 2; MgSO4, 2; NaH2PO4, 1.25; NaHCO3, 26; dextrose, 10, and saturated with 95% O2, 5% CO2 (pH = 7.4). For experiments requiring addition of Mn2+, Co2+, or Ba2+, a modified control medium containing (in mM): NaCl, 132; KCl, 5; MgCl2, 2, CaCl2, 2, NaHCO3, 22; dextrose, 10 was substituted. For experiments using Na+-free solutions, HCO3- was omitted and Tris (10 mM) or N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES) (10 mM) formed the buffer (pH = 7.4); solutions were bubbled with 100% O2, and Na+ (150 mM) was included in the control solution while equimolar amounts of Tris or choline were substituted for Na+ in the test solutions. In test solutions, divalent cations were added as 1–4 mM of the Cl- salt, and CaCl2 was then reduced to 1 mM. All experiments were carried out at 37°C. After an initial incubation period of about 1 h, excellent intracellular recordings could be obtained for at least 8–10 h.

Control recording electrodes were pulled from 1 mm OD, thin walled capillary tubing with internal microfibers (Frederick Haer & Co.) and backfilled by capillarity with 4 M K acetate (neutralized to pH 7.0). Resistances (measured with 1 nA DC) were 30–50 MΩ, and most electrodes could pass ±1–2 nA without significant changes in resistance or noise when tested extracellularly. In some experiments electrodes were filled with test substances: 1 M Cs acetate, 1 M tetraethylammonium Cl (TEA), or 100 mM QX-314 dissolved in 4 M K acetate. Injection of Cs+ or TEA was facilitated by repetitive depolarizing current pulses (0.5–1 nA for 500 ms at 1 Hz); QX-314 was allowed to diffuse into the neuron passively (cf. Ref. 12). Successful neuronal impalements were made at all cortical depths from 150 to 1,700 μm (measured from the pial surface) and were signaled by a rapid negative shift of 40–60 mV. This steady potential either deteriorated rapidly or gradually increased (over 1–3 min) to a relatively constant level usually in excess of −65 mV. Once the latter condition had been achieved, stable resting potentials could often be maintained for 1–2 h, and only cells in this stable state are reported on here. Neuroglia were also impaled frequently and could be easily distinguished from neurons by the usual electrophysiological criteria (54), which we have corroborated anatomically in this preparation (20). An active bridge circuit was used to allow simultaneous passage of current and measurement of voltage with a single microelec-
trode. Negative capacitance feedback was optimally adjusted and the bridge was then balanced to remove the very rapid voltage transient at the onset and offset of the current pulses (17). Sharpened monopolar tungsten electrodes were used to stimulate the cortical slices focally. Single cathodal pulses (200 μs duration ≤100 μA) were applied either to the underlying white matter or to cortical layers deep or superficial to the point of recording.

Signals were stored on magnetic tape (0–5 kHz) and some data were digitized off-line and analyzed on a Minc 23 computer (Digital Equipment Co.).

RESULTS

Passive electrical properties

Impalements with microelectrodes often elicited an initial high-frequency injury discharge, which usually subsided in those cells that gradually hyperpolarized to a stable resting potential (see METHODS). Spontaneous firing during stable impalements was seen very rarely, and then only in some of the very small number of cells with bursting characteristics (see below). Resting membrane potential (Vm), determined by measuring the amplitude of the abrupt deflection obtained on withdrawal from the cell, was −75.3 ± 7.8 mV (mean ± SD, n = 40).

Input resistance (RN) at resting membrane potential was estimated by applying a square hyperpolarizing current pulse (≥100 ms) whose amplitude was sufficient to evoke a voltage deflection of 10 mV or less. Measurements were taken at the plateau of the voltage response. This method was used to keep the membrane potential within a linear range and minimize the influence of membrane rectification (see below). The mean RN for 40 neurons was 24.3 ± 13.7 MΩ, with a range from 6.7 to 78 MΩ. Among the sampled population, RN was not correlated with resting potential, as might have been the case if the microelectrode had introduced large, nonspecific current leaks (1).

Membrane time constant (τm) was estimated from semilogarithmic plots of the voltage deflection at the onset of a small (∼10 mV) hyperpolarizing pulse. Although many cells showed clear indications of one or more fast equalizing time constants (cf. Refs. 37 and 48), it was usually possible to fit a final, slower time constant to the voltage deflection. In 20 cells the mean τm (±SD) was 8.2 ± 4.7 ms (range of 2.0–19.5 ms). This value corresponds well with that reported by Lux and Pollen (37) for unidentified neurons of cat motor cortex. If it is assumed that specific membrane capacitance (Cm) is about 1 μF/cm², as determined for all rigorously studied biological membranes (10), then the mean specific membrane resistance (Rm) is ∼8,200 Ω/cm², since τm = RmCm.

Table 1 summarizes the passive membrane properties of 18 representative neocortical neurons from six experiments, as well as the cortical depth of each recording. There is no correlation between depth and either resting potential or RN among a population of 40 cells so examined. However, cells with calculated τms > 10 ms were found only at depths more superficial than 350 μm.

Current-voltage relationships

Results of measurements from neocortical neurons in vivo are somewhat conflicting, indicating that current-voltage relationships may be generally linear (37) or may display a hyperpolarizing increase in conductance (58). Current-voltage curves in our experiments were constructed in two ways. In the first (Fig. 1A, inset), square current pulses ≥100 ms in duration and of variable amplitude were applied in the hyperpolarizing and subthreshold depolarizing directions. Voltage deflections were measured at 25 ms after pulse onset (triangles) and just before current offset (filled circles) and were plotted against current intensity (Fig. 1A). The current-voltage curves of most neurons displayed distinct nonlinear behavior, as evidenced by the differences in slope resistance at various points along the current-voltage curves at latencies of 100 ms (Fig. 1A, cf. depolarizing and hyperpolarizing directions). In Fig. 1B the second method was applied to the same cell. Hyperpolarizing current pulses of 125 ms duration and constant intensity were superimposed on a variable background level of polarizing current (29, 40). Bridge balance was carefully monitored throughout. As seen in Fig. 1B (inset), depolarization in this neuron led to an increase in the amplitude of the voltage deflection as well as a distinct prolongation of the time constant. When an apparent cell input resistance (ΔV/ΔI) was calculated from the amplitude of the voltage deflection and plotted
TABLE I. *Electrophysiological properties of neocortical neurons*

<table>
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<tr>
<th>Cell</th>
<th>$R_N$, MΩ</th>
<th>$\tau_m$, ms</th>
<th>$V_m$, mV</th>
<th>Spike Amplitude, mV</th>
<th>Spike Width, ms</th>
<th>Spike Threshold</th>
<th>Voltage, mV</th>
<th>Rheobase, nA</th>
<th>Cortical Depth, μm</th>
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<td>Mean</td>
<td>25.4</td>
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<td>76.5</td>
<td>93.5</td>
<td>1.8</td>
<td>25.0</td>
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<tr>
<td>± SD</td>
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<td>±4.3</td>
<td>±7.4</td>
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<td>±0.5</td>
<td>±9.0</td>
<td>±4.3</td>
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$R_N$, input resistance; $\tau_m$, membrane time constant; $V_m$, resting membrane potential. Spike amplitude measured from $V_m$ to peak; spike width measured at base. Spike threshold measured with square current pulse ≥ 100 ms long; numbers refer to the voltage difference between resting potential and threshold voltage. Cortical depth refers to distance from pial surface.

as a function of membrane potential ($V_m$), this neuron exhibited a large increase in slope resistance at all potentials above rest (Fig. 1B). This latter method was a more sensitive indicator of voltage-dependent changes in cell input resistance and was utilized in subsequent analyses in this study.

Current-voltage relationships in neocortical cells displayed a very wide range of behavior, as shown in Fig. 1C where data from four neurons are plotted by the method of Fig. 1B. Of 40 neurons tested under normal recording conditions, 50% showed clear increases in $\Delta V/\Delta I$ as the membrane was depolarized (Fig. 1B; 1C, filled symbols). However, the resistance increases could begin either at resting $V_m$ (Fig. 1B; 1C, filled squares) or, more commonly, extend as a monotonic function of $V_m$ increasing above the resting level and decreasing below it (Fig. 1C, filled circles). In 27% of the neurons, $\Delta V/\Delta I$ was clearly negatively correlated with $V_m$ (Fig. 1C, open circles). There was no discernable correlation between the type or degree of rectification and the resting potential, $R_N$, $\tau_m$, or cortical depth of the neurons recorded.

The ionic basis for subthreshold, voltage-dependent membrane properties was investigated by pharmacological means. When neurons were iontophoretically injected with Cs⁺, which is known to block K⁺ channels (7), the mean resting potential decreased (to −65.1 mV; $n = 24$) and the mean input resistance approximately doubled (to 47.4 MΩ; $n = 21$) relative to the control group. Both of these changes were highly significant ($P < 0.001$; Mann-Whitney $U$ test). To assess changes in the voltage dependence of membrane conductance, plots of the type shown in Fig. 1B and C were constructed. A rectification ratio was then calculated, which compared $\Delta V/\Delta I$ measured at −55 mV and −80 mV (cf. Ref. 29). The level of −80 mV was chosen because it is at the level of or slightly more negative than most resting potentials and −55 mV because it lies in the
FIG. 1. Current-voltage relationships of neocortical neurons. All data were derived from square current pulses delivered via an active bridge circuit. A: intensity and polarity of the pulses were varied (inset), and voltage deflections at 25 ms (triangles) and 100 ms (filled circles) following onset of the pulse were measured and plotted. At the longer latencies, this neuron showed a large increase in slope resistance in the depolarizing direction and a slight decrease at hyperpolarized levels. Absolute resting potential ($V_m$), -77 mV. B: in same cell as A a small, constant hyperpolarizing pulse was delivered as the resting level was altered with tonic current (inset). The amplitude of the voltage deflection (normalized to the size of the current pulse) was then plotted as a function of the steady voltage level. As in A, an increase in slope resistance is quite evident in the depolarizing direction. C: examples of current-voltage relationships from four neurons. Examples illustrated were plotted by the method shown in B; resting potentials are circled.

very interesting voltage range just below the typical action-potential threshold (see Table 1). Data of this type are shown in Table 2. Cs\+ injection caused a large increase in the rectification ratio, as compared to control neurons, implying that $\Delta V/\Delta I$ was larger at more depolarized levels. Blockade of Na\+ conductances with TTX application resulted in a significant decrease in the ratio relative to control. These results suggest the presence of a subthreshold, noninactivating or very slowly inactivating Na\+ conductance. Further evidence for such a conductance is illustrated graphically in Fig. 2A–C, which shows the effects on three different neurons of complete elimination of extracellular Na\+ (Fig. 2A), application of $10^{-6}$ M TTX (Fig. 2B), and application of intracellular QX-314,
TABLE 2. Ionic basis of depolarizing rectification

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cs⁺</th>
<th>TTX</th>
<th>Mn²⁺</th>
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</thead>
<tbody>
<tr>
<td>R_N (−55)</td>
<td>1.17</td>
<td>2.20</td>
<td>0.84</td>
<td>1.36</td>
</tr>
<tr>
<td>R_N (−80)</td>
<td>± 0.07</td>
<td>± 0.34</td>
<td>± 0.10</td>
<td>± 0.10</td>
</tr>
<tr>
<td>n</td>
<td>22</td>
<td>8</td>
<td>8</td>
<td>7</td>
</tr>
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</table>

Significance, P | <0.001 | <0.02 | NS |

Values are means ± SE. Significance measured by the two-tailed Student's t test. NS, not significant.

Action-potential characteristics

With square injected current pulses, voltage thresholds for activation of a single spike were quite high, averaging 25.3 ± 9 mV (n = 21) above resting potential. Rheobase current for these neurons was 0.63 ± 0.4 nA, and mean spike amplitude was 94.6 ± 11 mV (n = 20, Fig. 3A1, B1, C,; Table 1), a value 10–30 mV larger than reported for pyramidal tract neurons in vivo (14, 37, 51). However, both the amplitude and duration of the spikes could vary to a significant degree during trains produced by prolonged current pulses. A spectrum of behaviors was encountered, extending from neurons in which spike amplitude decremented smoothly during a train (Fig. 3A2) to cells that showed a large decrease in amplitude after the initial spike, followed by a return to a higher, relatively steady spike amplitude (Figs. 3B3, AB). During repetitive activation with a long current

a quaternary lidocaine derivative (Fig. 2C). In each case the experimental treatment led to a distinct decrease in input resistance at membrane potentials lower than the resting level. Similar results were obtained using either Tris or choline as Na⁺ substitutes. In contrast, application of Mn²⁺, a Ca²⁺-channel blocker, had no effect on the depolarizing rectification (Table 2; Fig. 2D).

FIG. 2. Ionic basis of depolarizing rectification. Each graph is constructed by the method of Fig. 1B. A: control measurements (filled triangles) were made in normal solution, and slice was then bathed in choline-substituted, Na⁺-free solution for 6 min (open triangles); 10 min after return to control solution measurements were again repeated (diamonds). B: similar measurements, on different neuron, performed before (filled triangles) and after (open triangles) application of 10⁻⁶ M TTX to the bath. C: effect of intracellular QX-314. Neuron was recorded with an electrode containing 100 mM QX-314 + 4 M K acetate and measurements were made at 1 min (filled triangles) and 10 min (open triangles) following beginning of impalement. D: effect of extracellular Mn²⁺. Control measurements (open triangles) and 5 min after application of 4 mM Mn²⁺ (filled triangles).
FIG. 3. Characteristics of neocortical action potentials. Representative intracellular recordings from three different neurons are shown in A, B, and C. In A₁–C₁, spikes were elicited with short current pulses (≤2 ms). Each shows a phase of fast repolarization followed by a prolonged depolarizing afterpotential (DAP), which either decrements toward base line (A₁, B₁) or undershoots the base line to produce a slow afterhyperpolarization (AHP; C₁). Various modes of repetitive firing were observed in response to long current pulses (A₂–C₂), including a small population of cells that generated all-or-none burst potentials (C₃). Following such trains of action potentials, all neurons displayed a slow AHP (A₄–C₄), which decayed over several hundred milliseconds. Time scale for column 1 is given in C₁, time scales for column 3 are the same as those in column 2, and time scale for B₄ and C₄ are the same. Voltage scales for columns 1–3 are the same (C₁, 50 mV), and voltage scale for column 4 is given in C₄ (20 mV).

脉冲，成功刺激的脉冲总是变宽，但程度上以这种影响在初期的持续时间。图4说明了两个刺激选择以比较原因的非常相似RN和刺激高度。具有相同的去极化电流

脉冲，其是明显于图4B中显示的明显增加。在任何给定细胞，刺激变宽是取决于刺激电脉冲的大小和刺激间期，但不是简单的变化

脉冲后电位

当短电脉冲刺激时，动作电位通常显示三个复极化阶段（图3A₁，B₁，C₁）。首先，快速下降电位在0-25 mV相对静息水平终止，但没有下

scales in A and B (left) are the same; note different time scales for A and B (right).

FIG. 4. Action-potential broadening during repetitive activation. A and B are derived from two different neocortical cells with similar RN (18 MΩ for A, 24 MΩ for B) and presented with identical current pulses of 1.1 nA. Traces on left are voltage (upper) and current (lower) records. Traces on right are records of the same voltage traces, obtained by triggering the oscilloscope on the rising leg of each spike. Time scales in A and B (left) are the same; note different time scales for A and B (right).

When evoked by short current pulses, action potentials generally displayed three phases of repolarization (Fig. 3A₁, B₁, C₁). First, the rapid downstroke of the spike abruptly terminated some 10-25 mV positive to the resting level without ever undershooting it. Second, there was often an inflection, or "bump," on the postspike membrane trajectory, followed by a slow decay toward resting potential over the next 5-30 ms. This is termed the depolarizing afterpotential (DAP; cf. Ref. 28). Finally, in some cells the single-spike DAP was terminated and followed by an undershooting afterhyperpolarization (AHP), which could occasionally reach several millivolts in amplitude and last for 10-40 ms (Fig. 3C₁) but morc
typically was not seen at all. The absence of an AHP in most cells may have been due to the high resting potentials of neurons in the slice (Fig. 3A1, B1), since in injured or tonically depolarized cells the slow AHP was usually prominent. This sequence of afterpotentials is qualitatively similar to that seen in pyramidal tract cells in situ (cf. Refs. 14 and 43).

The AHP became much larger and longer when neurons were activated repetitively (Fig. 3A4, B4, C4), attaining a maximum amplitude of 3.0 ± 1.6 mV and a duration of several hundred milliseconds in 10 cells where these effects were measured. Most (Fig. 3B4, C4), but not all (Fig. 3A4) AHPs displayed a rapid followed by a slower rate of decay, with the slower one becoming prominent only following more intense activation. Although decreases in input resistance could usually be demonstrated when small current pulses were applied during AHPs, the maximum reduction was never more than 10% at the AHP peak. Polarization of cells during AHPs (Fig. 5) revealed a reversal potential 10–20 mV negative to the resting level. Addition of 2–4 mM Mn²⁺ or Co²⁺ to the bathing media greatly attenuated or abolished the AHP and its associated conductance change (not shown). These data are consistent with the notion that the AHP is due to an increased K⁺ conductance, possibly activated by the inward flux of Ca²⁺. This phenomenon has been repeatedly described in many types of cells (38), including pyramidal neurons of the mammalian hippocampus (25).

**Burst-generating neurons**

In distinct contrast to the great majority of recorded neocortical neurons, a small proportion (9 of 70 stable impalements under normal conditions) possessed the ability to generate burst potentials during intracellularly injected current pulses (Figs. 3C5, 6A, E, F) or, occasionally, following synaptic activation (Fig. 6B). Bursts were evoked at distinct thresholds (Fig. 6A, B) and consisted of
of multiple decrementing fast spikes riding on a relatively slow depolarizing envelope (Fig. 6A, B, F). Bursts could have widely shifting latencies of activation and often arose abruptly from the voltage base line (Fig. 6F), perhaps indicating a site of initiation remote from the recording electrode. The ability to burst was a somewhat fragile property that disappeared in three cells several minutes after impalement, concurrent with a large decrease in $R_N$ (cf. Fig. 6A and C) and a gradual hyperpolarization of the resting $V_m$. It was difficult to obtain stable, long-term recordings from burst-producing neurons. This feature together with their relative scarcity precluded detailed electrophysiological analyses. However, the mean (+SD) resting potential ($-69.3 \pm 9$ mV) and $R_N$ ($17.5 \pm 6$ MΩ) measured in six such cells were not significantly different from those of the non-bursting population (cf. Table 1).

Of considerable interest is the observation that burst-producing cells were impaled only between the subpial depths of 850–1,200 µm; non-bursting neurons were also encountered at the same sites with approximately equal frequency (Fig. 6G). Histological observations indicate that this corresponds to a region encompassing layer IV and the more superficial part of layer V. In contrast, non-burst neurons were recorded at all depths from 150 to 1,700 µm.

In Fig. 7 the afterpotentials following a single spike are analyzed graphically. These data were obtained from a burst-generating neuron of layer IV. A plot of the voltage decay following a hyperpolarizing current pulse (Fig. 7D, filled circles) was fitted well by a single time constant of 8.7 ms (dashed line). However, when the same current pulse was inverted (Fig. 7A), the peak voltage response was larger and the initial rate of decay (Fig. 7D, open triangles) was much slower ($\tau = 13$ ms). This suggests that, as in some molluscan neurons (60), a slow, voltage-dependent process, which may contribute to the DAP, can be significantly activated even at subthreshold potentials. The same would appear to be true of the AHP, since in this cell the potential consistently undershot the base line even in the absence of a spike (Fig. 7A). By very slightly incrementing the depolarizing pulse, a spike was activated (Fig. 7B); its decay is plotted by the filled triangles (Fig. 7D). The presence of a spike clearly added components to both the DAP and the AHP. The curve represented by the filled squares in Fig. 7D is the algebraic difference between the subthreshold (open triangles) and threshold (filled triangles) voltage decays and demonstrates that an apparent peak of the DAP occurs at about 4 ms. Very occasionally the DAP was capable of triggering a second spike, even after the termination of the current pulse (Fig. 7C). This capacity was seen only in burst-firing neurons (cf. Refs. 60 and 64) and may arise from the ability of the DAP to hold the voltage above threshold long enough for the refractory period of the first spike to dissipate. Thompson and Smith (60) argued that the inward current underlying the DAP does not arise with a lag following the spike but decays monotonically and is interrupted initially by a fast-repolarizing $K^+$ current (analogous to the voltage-dependent $K^+$ current of squid axons; Ref. 24). This gives the DAP its convexity. An additional
late K+ current seems to operate here, resulting in the AHP that signals the DAP termination. By this scheme, the typical neocortical DAP is temporally bounded by two distinct hyperpolarizing conductances.

The bursting properties of this small subpopulation of middle-layer cortical neurons are reminiscent of those observed in hippocampal pyramidal cells both in vivo and in vitro (51, 63, 64).

**Ionic basis of neocortical action potentials**

When neurons were recorded with K acetate-filled electrodes, the ability to produce regenerative spikes was invariably abolished by the addition of $10^{-6}$ M TTX, or the removal of extracellular Na+, or the application of intracellular QX-314 (not shown). These results suggest that Na+ is the predominant carrier of inward current during most somatic neocortical action potentials. However, because of the overwhelming distribution of active calcium conductances among examples of well-studied excitable membranes (22), we performed manipulations designed to enhance the probability of detecting them in neocortical neurons. Intracellular Cs+ blocks outward neuronal K+ currents (7), and our results suggest that in neocortical cells it indeed inhibits one or more subthreshold K+ conductances (see earlier section). The effect of Cs+ injection on action potentials is shown in Fig. 8. As intracellular Cs+ levels increased during the recording period, spikes progressively broadened 2- to 50-fold and developed a prominent shoulder on the falling phase. Concurrently, the AHP following a train of spikes became suppressed and quickly reversed in polarity, resulting in a large and prolonged DAP with no subsequent hyperpolarization (Fig. 8, bottom). Application of $10^{-6}$ M TTX to the slice suppressed spiking capabilities in 20% of the Cs+-injected neurons tested, but in the remainder it was possible to evoke a large-amplitude, broad spike whose rate of rise was <20% of the non-TTX treated cells (Fig. 8, right traces). The TTX-resistant spike exhibited a DAP similar to that of the control Cs+-injected cell. Essentially identical results were found when extracellular Na+ was totally replaced by choline or Tris (Fig. 9A, B). The addition of hatching media containing 3-5 mM Mn$^{2+}$ (Fig. 9C) or Co$^{2+}$ (not shown) resulted in a reversible loss of nonsodium-dependent spikes. After blockade of active Na+ conductances (i.e., in TTX or Na+-free media), spikes could also be evoked by pretreatment with 1 mM Ba$^{2+}$ or intracellular injection of TEA. Both conditions are known to reduce K+ conductances (23, 61), and Ba$^{2+}$ is a very effective charge carrier through active Ca$^{2+}$ channels (61). Our results are consistent with a presence of voltage-dependent Ca$^{2+}$ channels in the soma-dendritic membrane of most neurons recorded in the neocortical slice.

When Mn$^{2+}$-containing medium was applied to a Cs+-injected cell before TTX exposure, there was usually a distinct narrowing of the action potentials and loss of the DAP. However, in two neurons, prolonged washing in Mn$^{2+}$ (>30 min) failed to abolish

![FIG. 8. TTX-insensitive action potentials. Neuron was recorded with a Cs+-containing microelectrode and 150-m, 0.4-nA depolarizing pulses were delivered at a rate of 2 Hz. Voltage records are shown at fast (top) and slow (bottom) sweep speeds for the points 20 s, 2 min, and 5 min following initial impalement. Spikes in lower records are truncated. Progressive Cs+ injection resulted in marked broadening of spikes and a reversal of the AHP to a prominent and slow DAP. Application of $10^{-6}$ M TTX (right traces) slowed the rate of spike rise and increased the threshold but did not block excitability or the DAP.](http://jn.physiology.org/)
a high-threshold, very slowly rising and falling regenerative potential, which rose from a shoulder on the second spike of a train (Fig. 9E). Subsequent treatment with TTX then blocked all spiking activity (Fig. 9F). It is not clear what these rare, slow, Mn2+-resistant, TTX-sensitive events represent. It may be that the 1 mM Ca2+ still present in the bathing solution carried a current not entirely inhibited by Mn2+ or that the slowly inactivating Na+ conductance may have been capable of supporting regenerative electrogensis under conditions of reduced K+ conductance.

The effects of membrane polarization on Ca2+-dependent spikes are shown in Fig. 10A, in a neuron injected with Cs+ and exposed to TTX. Consistent increases in spike duration occurred as the resting potential was made more positive than resting Vm. Concurrently, the DAP underwent a polarity reversal at 5–15 mV positive to rest. The latter finding suggests that the DAP is formed by a balance of K+ and Ca2+ currents, which inactivate relatively slowly following the spike. The mechanism for the Ca2+ spike broadening is less clear. It may be that tonic depolarization causes a partial inactivation of voltage-dependent K+ conductances (3), allowing inward Ca2+ currents to maintain depolarized levels longer. The slower rate of repolarization near the peak of the depolarized spikes supports this hypothesis (Fig. 10A). Polarization experiments never revealed signs of two separate Ca2+ conductances with different voltage dependencies, as was recently described in inferior olivary neurons (35, 36).

Ca2+-dependent DAPs and, to a much smaller extent, their associated spikes were quite sensitive to the frequency of activation. At frequencies >1 Hz (Fig. 10B, C), both were depressed in amplitude and rate of rise (Fig. 10B) despite the absence of changes in resting potential (Fig. 10C) or input resistance (not shown). This may represent a slow inactivation of Ca2+ currents, possibly due to intracellular accumulation of Ca2+ (8). The preferential depression of the DAP may indicate that the spike and the DAP represent two different types or distributions of Ca2+ channels (36).
Synaptic activity

Spontaneous and evoked postsynaptic potentials (PSPs) are a prominent feature of recordings from intact neocortical neurons in anesthetized cats (5, 14, 31, 42–44, 58) and would seem to be an important index of the viability of a slice preparation (9). Indeed, spontaneous, transient fluctuations of membrane potential occurred in every stable neuron recorded under normal conditions (Fig. 11A). At resting potential these events were consistently depolarizing in all cells and usually rose quickly to a peak and decayed more slowly to baseline with a time course roughly similar to $\tau_m$. This passive repolarization, and the finding that hyperpolarizing the membrane with injected current did not reduce their frequency, makes it unlikely that they represent the more rapid and uniformly sized fast prepotentials, which are intrinsically generated in hippocampal pyramidal cells (28) and immature neocortical neurons (47). It seems probable that these events are manifestations of spontaneous chemically mediated postsynaptic potentials (PSPs). In a given cell they exhibited a wide range of amplitudes, ranging from the limits of our detection level (generally 0.3–0.5 mV) to maxima of 1–5 mV (mean maximum amplitude ± SD of 2.1 ± 1.1 mV; $n = 17$ neurons). The mean frequency of PSP occurrence was 7.7 ± 4.8 Hz (range, 1.5–30 Hz; $n = 17$), although this is a biased estimate, since it counts only the PSPs rising clearly above the baseline noise. A striking observation was the complete absence of spontaneous, unambiguously hyperpolarizing PSPs in any neuron at resting potential. Furthermore, when neuronal membranes were depolarized with steady current to levels just below firing threshold, only 10% displayed spontaneous hyperpolarizing PSPs. The latter were presumably unitary inhibitory PSPs (IPSPs) with reversal potentials somewhere near resting $V_m$. It was difficult to characterize them further due to their low incidence and frequency (0.1–2 Hz). When neurons were treated with TTX, virtually all spontaneous PSPs were abolished. By analogy with the hippocampal slice preparation (9), it is likely that the great majority of the visible
Further increases of intensity led to a consolidation and narrowing of the depolarization, as well as a more rapid decay (Fig. 11B, second column), probably because of the recruitment of inhibitory synaptic influences. Strong stimuli could usually evoke one, or occasionally two, action potentials in nonbursting neurons. The higher current intensities also often led to long-latency (100–300 ms) hyperpolarizations of 1–5 mV (see below). Qualitatively similar PSPs could be evoked in the same neuron by surface or white matter stimulation (Fig. 11C, D). Although in the illustrated}

PSPs result from random, unitary firings of presynaptic neurons and do not represent true “miniature” PSPs resulting from release of single synaptic vesicles.

The synaptic organization of the neocortex has been extensively studied in vivo by selective stimulation of subcortical areas projecting antidromically or via “specific” or “nonspecific” afferents to the cortical areas being investigated (e.g., Refs. 31, 43, 44, and 47). Although our slice preparation does not allow us to activate such anatomically distinct pathways, focal stimulation of the pial surface or underlying white matter produced large-amplitude PSPs in all recorded neurons (Fig. 11C, D). At low stimulus intensities it was sometimes possible to evoke multiple all-or-none depolarizing PSPs, which arose at relatively fixed but disparate latencies (Fig. 11B, first column). These synaptic events resembled the larger spontaneous PSPs in both amplitude and time course. Further increases of intensity led to a consolidation and narrowing of the depolarization, as well as a more rapid decay (Fig. 11B, second column), probably because of the recruitment of inhibitory synaptic influences. Strong stimuli could usually evoke one, or occasionally two, action potentials in nonbursting neurons. The higher current intensities also often led to long-latency (100–300 ms) hyperpolarizations of 1–5 mV (see below). Qualitatively similar PSPs could be evoked in the same neuron by surface or white matter stimulation (Fig. 11C, D). Although in the illustrated
FIG. 13. Long-duration hyperpolarizing PSP. A: neuron was presented with a control injected current pulse sufficient to evoke four action potentials (con). Identical current pulses were then preceded by 70 ms with a single stimulus to the underlying white matter at 0.1 Hz, and current intensity was gradually increased (intensity shown above each trace; arbitrary units). At 0.1, short-latency depolarizing PSPs were evoked, without affecting action-potential generation. Stronger stimuli resulted in a long-latency hyperpolarizing wave, which greatly reduced excitability. B: in a different neuron from A, three strong white matter stimuli were delivered at a frequency of 0.33 Hz, followed by a depolarizing current pulse at a latency of 60 ms. Resulting recordings are shown superimposed and labeled; the first stimulus completely inhibited spike generation, the second allowed 1 spike, and the third resulted in two spikes. C: the experiment of B was repeated while depolarizing the neuron by 15 mV with tonic current. In this case the short depolarizing current pulse was eliminated. Stimulation at 0.33 Hz caused a significant reduction in PSP amplitude following the first stimulus and a much smaller reduction following the second, which is not clearly seen in C. Note slower sweep speed in C.

case the white matter-evoked PSP rose more quickly and decayed more slowly than that from the surface, despite a longer latency, this was not a consistent finding. Antidromic spikes could often be evoked by stimulating layers deeper than the recording site.

Moderate stimulus intensities evoked PSPs that were exclusively depolarizing; however, when PSPs were evoked during membrane polarization, multiple reversal levels became apparent. In the cell of Fig. 12, depolarization uncovered an IPSP that was apparently concurrent with the early excitatory PSPs and extended throughout the duration of the evoked PSPs. The reversal levels were a consequence of the relative balance of excitation and inhibition at any point in time and varied greatly between neurons. There were often small fluctuations during the latter phases of the evoked PSPs, which appeared to reverse at a level between resting potential and about 20 mV depolarized from rest (Fig. 12, B, arrows). These potentials may be unitary IPSPs, i.e., the IPSPs resulting from the firing of a single presynaptic neuron.

In about 75% of cortical neurons it was possible to evoke a long-duration hyperpolarizing IPSP, which lasted 150–2,500 ms and had a peak amplitude of 3.0 ± 2.1 mV and a latency to peak of 122 ± 30 ms (n = 7). This potential could be elicited by stimulation of either the white matter or pial surface. As shown in the neuron of Fig. 13A, the inhibition always had a higher threshold of activation than the preceding excitatory postsynaptic potentials (EPSPs), yet when fully manifest could very effectively reduce the ability of the cell to fire current-evoked spikes. This long IPSP was extremely labile. When intense stimuli were delivered at rates faster than 0.2 Hz, the IPSP amplitude diminished and its inhibitory potency was greatly reduced following the second and third shock (Fig. 13B). In Fig. 13C, outward current was used to depolarize the neuron by 15 mV to enhance the visibility of the IPSP. Under these conditions it is clear that repetitive stimulation reduced the IPSP throughout its extent.

The voltage dependence of slow cortical inhibition is illustrated in Fig. 14A–C. Although there was wide variability in the time course of IPSPs between neurons, in most it was possible to distinguish two phases, which
FIG. 14. Polarization of long-duration hyperpolarizing PSPs. A: strong stimuli were delivered to the underlying white matter at 0.1 Hz, and $V_m$ was altered with tonic current. Small hyperpolarizing current pulse preceded stimulus. Resting $V_m$ is designated by arrowhead. B: recordings from A have been shifted along the voltage axis and superimposed on the same base line $V_m$. The two-peak phases of inhibition have been labeled $I_1$ and $I_2$ and correspond to latencies of 17 and 140 ms, respectively. C: graph of $I_1$ (squares) and $I_2$ (triangles) amplitudes ($V_r$) as a function of $V_m$. Data taken from neuron of A and B. IPSP reversal levels correspond to $V_m$'s of about +1 mV for $I_1$ and -17 mV for $I_2$. D: conductance and excitability during long-duration inhibitory PSPs were measured with short-current pulses presented at varying latencies. Control pulses (i.e., without preceding white matter stimulus) are shown on right. Same neuron as in A–C.

we have labeled $I_1$ and $I_2$ (Fig. 14B). $I_1$ is associated with the greater increase in conductance (Fig. 14D) but has a reversal level at or slightly positive to resting $V_m$ (Fig. 14C). $I_2$ identifies the late hyperpolarizing phase, which reverses some 10–20 mV negative to rest (Fig. 14C). The nonlinearities in the voltage dependencies of $I_1$ and $I_2$ at very negative potentials (Fig. 14C) are probably due to the decrease in input resistance at these levels (note voltage responses to small current pulse in Fig. 14A and B).

DISCUSSION

If the isolated neocortical slice is to serve as a useful experimental analogue to the intact neocortex in situ, a careful comparison of cellular physiological properties in the two preparations must first be performed. Fortunately, there are several quantitative in vivo intracellular studies extant, although almost all were derived from various cortical areas of anesthetized cats, and many dealt exclusively with the small population of neurons whose axons project through the pyramidal tract. Our measurements were taken from unanesthetized guinea pig neurons from all cortical laminae below I. Although pyramidal cells constitute about 70% of the neurons in rat neocortex (62), they are clearly not the only cell type making up our recorded sample, since we have observed nonpyramidal neurons stained intracellularly under identical conditions (M. J. Gutnick and D. A. Prince, unpublished observations). Despite anesthetic, species, and probable architectonic differences, quite good agreement has been found with the results of in vivo studies. In addition, the technical advantages
of the slice preparation have allowed the characterization of some aspects of neocortical physiology that have not been previously examined.

One striking discrepancy between our data and those of others is the unusually high level of resting potentials (mean of \(-75\) mV) we have observed. Although our mean is just within the range of values reported by some (42, 57), it is consistently \(10-25\) mV greater than has been generally encountered in vivo (14, 30, 32, 37, 42, 57, 66). This is so in spite of the higher level of \([K^+]_o\) (5 mM) used in the perfusing solution in these experiments compared to that of the extracellular fluid in mammalian brain (3 mM, Ref. 55). An increased stability of the isolated preparation may certainly contribute to the higher resting potentials. Similar values were reported for neurons of the guinea pig olfactory cortical slice (\(-75.4\) mV, Ref. 49), but neuronal membrane potentials are significantly lower in slices of hippocampus maintained under the same conditions (\(-63.4\) mV, Ref. 6) and in slices of cerebellum (\(-65\) mV, Ref. 38) and inferior olive (\(-64\) mV, Ref. 35). Thus, high resting potentials are not entirely accounted for by a nonspecific effect of the slicing procedure. Electrode artifacts are also probably not relevant since our recordings from glial cells under identical conditions gave values similar to those encountered in situ (20). A likely contributory factor was the removal of tonic afferent activity. Spontaneous firing rates and background synaptic activity are relatively low in the slice preparation, and possible alterations in the release of slowly acting neuromodulatory substances cannot be assessed. The larger amplitude action-potential sizes in the slice (generally \(10-20\) mV greater than those seen in vivo; Refs. 14, 42, 57) are compatible with the high resting membrane potentials.

Current-clamp experiments have revealed a range of distinct nonlinearities in the steady-state, subthreshold electrical behavior of neocortical neurons. The membrane potential-dependent changes in slope resistance (Figs. 1 and 2) arise from the summation of voltage-dependent, slowly inactivating or noninactivating conductances to \(Na^+\), \(Ca^{2+}\), and \(K^+\). The alterations in subthreshold electrical behavior (Table 2, Fig. 2) suggest strongly that this voltage range (roughly \(-75\) to \(-50\) mV) is dominated by activation of \(Na^+\) and \(K^+\) currents in most cells, while significant \(Ca^{2+}\) current activation occurs only at potentials \(<-50\) to \(-40\) mV (the threshold of nonsodium-dependent spikes). Stafstrom et al. (56) have described a similar \(Na^+\) current in large layer V cells of cat motor cortex. Very slowly inactivating sodium and calcium conductances are also present in mammalian hippocampal pyramidal cells (26, 27) and cerebellar Purkinje cells (33). Under voltage clamp, the hippocampal \(Ca^{2+}\) current is first activated at levels of \(-50\) to \(-40\) mV (27). The slow \(Na^+\) current probably occurs in most neocortical neurons, since blockade of \(K^+\) conductances with \(Cs^+\) injection invariably caused a large increase in \(Mn^{2+}\)-resistant, TTX-sensitive inward rectification within the presumed voltage activation range for this current. The precise nature of the active \(K^+\) conductances present is not clear, but our data are compatible with the existence of at least two such systems: a rapidly activating and inactivating voltage-sensitive one (cf. Ref. 24) and a more slowly inactivating one, which may correspond to the \(Ca^{2+}\)-activated \(K^+\) conductance seen in a wide spectrum of cells (38).

The unclamped voltage behavior of any individual neuron will be a function of the voltage and time dependence, equilibrium potential, density, and soma-dendritic distribution of each conductance. Obviously, it would be of tremendous interest to determine whether specific membrane properties are related to certain neuronal morphologies within the cortex. The findings that 20% of neurons did not develop TTX-resistant spikes after \(Cs^+\) and that the population had different classes of rectification and firing properties indicate that physiologically definable subsets of neurons may be identified in the neocortical slice. Further dissection of the specific ionic mechanisms involved in neocortical cellular behavior will require correlative anatomical and physiological measurements from the same cells (46). It is possible that the classical cell types harbor a myriad of functional properties. For example, the pyramidal cells projecting through the pyramidal tract can be readily divided into at least two clear subgroups, based on a variety of both physiological (57) and morphological (15) characteristics.
Our description of normal neocortical neurons with clear intrinsic bursting capacities (Fig. 6) is apparently unique. This is not very surprising when one considers that these neurons were restricted to a narrow range of cortical depths, were a small minority of all the cells encountered and, once impaled, were difficult to maintain even under the stable conditions of the slice preparation. The latter observation suggests that these bursting elements are quite small. In hippocampal pyramidal cells (65) and cerebellar Purkinje cells (34), burst-producing electrogenesis appears to be preferentially localized to the dendritic regions. The possibility thus arises that our burst recordings are derived from occasional impalements of large apical dendrites arising from pyramidal neurons whose somata lie in deeper layers. If this is the case, then the sites of burst electrogenesis must be electrotonically quite distant from the somata of these deeper neurons, since bursts were never evoked in deeper recordings even by intense current injection or synaptic depolarization. It seems unlikely that dendrites would be impaled only in such a restricted zone. Some of the bursting elements also generated large, fast action potentials at lower thresholds than the bursts (Fig. 6D-F); thus it would seem that recordings of this type were obtained from sites electrically nearer the spike-initiation zone than the bursting region or that the bursts had significantly higher thresholds of activation than fast spikes. Compatible with all of these observations is the hypothesis that bursting neurons comprise a distinct population of small cells restricted to the middle cortical layers. Unambiguous identification of the morphological substrate for this type of electrical behavior will require intracellular marking. A likely candidate would be one of the several types of nonpyramidal neurons so prevalent within layers IV and V (18). In any case, the existence of such intrinsic behavior raises questions about its role in normal, neuronal function and the genesis of certain pathological states (19).

Although there is an overlap of afferent and efferent pathways in the neocortex, the slice preparation may still be one of the current methods of choice for studying the physiology of intracortical connections. Our results demonstrate that robust excitatory and inhibitory PSPs are easily generated by focal stimulation. The evoked PSPs are quite complex, and a separation of excitatory and inhibitory potentials was not clearly possible, except perhaps for the long-duration IPSP. The lack of short-latency hyperpolarizing PSPs in any neurons with high, stable resting potentials suggests that, as in slices of olfactory cortex (50), neocortical neurons may possess some form of depolarizing IPSP. This possibility is supported by the observation of evoked unitary IPSPs (Fig. 12) and spontaneous PSPs that reversed polarity at potentials positive to resting $V_{m}$. The slow, labile hyperpolarizing potentials seen at high intensities of stimulation (Figs. 13 and 14) are intriguing and may derive from slow polysynaptic circuits, some slowly acting inhibitory transmitter, or even a synaptically derived calcium-activated potassium current (41). The use of the neocortical slice, together with a more defined stimulation protocol (cf. Ref. 5) and the ability to make multiple simultaneous intracellular recordings (19) should allow direct testing of some current concepts of cortical synaptic organization (39), which have necessarily been based largely on anatomical and extracellular electrical analyses.

Using in vitro methods, the detailed cellular properties of a variety of mammalian central neurons have been recently described, including hippocampal pyramidal cells (25-27, 41, 51, 64, 65), cerebellar Purkinje cells (33, 34), olfactory cortical neurons (49, 50), and inferior olivary cells (33, 34). When each is compared, one is struck by the heterogeneity of electrical behaviors. Neocortical neurons share some of the characteristics of many neurons, in different combinations, presumably because of the unique functional requirements of neocortical circuits.

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