Role of Voltage-Gated K⁺ Currents in Mediating the Regular-Spiking Phenotype of Callosal-Projecting Rat Visual Cortical Neurons

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INTRODUCTION

Although the functional roles of Ca²⁺-independent, depolarization-activated K⁺ currents have been explored in a number of mammalian central neurons (Bargus et al. 1989; Budde et al. 1992; Gean and Shinnick-Gallagher 1989; Halliwell et al. 1986; Hammond and Crepel 1992; McCormick 1991; Nisenbaum et al. 1994; Schwindt et al. 1988a,b; Segal 1984; Spain et al. 1991; Storm 1987, 1988; Wu and Barish 1992; Zhang and McBain 1995), few studies have been completed on cortical neurons (but see Hammond and Crepel 1992; Schwindt et al. 1988a,b; Spain et al. 1991), and none have investigated the functional roles of K⁺ currents in identified cells. In the experiments described here and in the preceding paper (Locke and Nerbonne 1997), we employed in vivo retrograde labeling to permit the in vitro identification of callosal-projecting (CP) neurons from rat primary visual cortex (Giffin et al. 1991; Katz 1984; Katz and Iarocci 1990; Solomon et al. 1993). As discussed previously (Giffin et al. 1991; Locke and Nerbonne 1997; Solomon et al. 1993), CP cells were selected to correspond to the “regular-spiking” phenotype described previously in recordings from cortical neurons in vivo and in the in vitro slice preparation (Connors and Gutnick 1990; McCormick et al. 1985). In response to brief depolarizing current injections, regular-spiking cells fire single action potentials that are followed by prolonged, and often complex, afterhyperpolarizations (Connors and Gutnick 1990). In response to prolonged current injections, regular-spiking cells fire action potentials repetitively at a frequency that depends on the amplitude of the injected current and, over time, these cells adapt (Chagnac-Amitai et al. 1990; Connors and Gutnick 1990; Mason and Larkman 1990). In vivo and in vitro, therefore, regular-spiking cells are readily distinguished from “fast-spiking” and “intrinsically bursting” cortical neurons (Connors and Gutnick 1990).

In the preceding paper, three distinct, Ca²⁺-independent, voltage-gated K⁺ currents, termed I_D, I_A, and I_K, were identified in CP neurons from rat primary visual cortex, and the detailed time- and voltage-dependent properties of these currents were delineated (Locke and Nerbonne 1997). Importantly, although the voltage-dependent properties of I_D, I_A, and I_K were found to be similar, these currents were distinguished readily based on differing kinetic properties and pharmacological sensitivities (Locke and Nerbonne 1997). Although the relative densities of the currents varied among cells, I_D, I_A, and I_K were found to be expressed in all CP cells (Locke and Nerbonne 1997). The differences in the kinetic properties and the densities of the currents suggested that I_D, I_A, and I_K likely make distinct functional contributions to determining the firing properties of CP neurons. The experiments here were designed to test this hypothesis directly. The waveforms of single action potentials and the repetitive firing behavior of isolated, identified CP neurons were examined using the whole cell version of the patch-clamp technique. The pharmacological properties of I_D, I_A, and I_K (Locke and Nerbonne 1997) were exploited to examine the functional roles of these currents in determining the latency of firing, shaping the waveforms of single action potentials, and controlling the repetitive firing properties of these cells.
potentials, and controlling the repetitive firing properties of CP neurons. In addition, the C Clamp/V Clamp neuronal simulation program, developed by Huguenard and McCormick (Huguenard and McCormick 1992; McCormick and Huguenard 1992) to describe the firing properties of thalamocortical relay neurons, was used to develop a model of the electrophysiological properties of rat visual cortical CP neurons. \( I_{Na} \), \( I_{D} \), and \( I_{K} \) were simulated using the detailed time- and voltage-dependent properties determined in the preceding paper (Locke and Nerbome 1997) and incorporated into the model; the relative conductances of \( I_{Na} \), \( I_{D} \), and \( I_{K} \) in the model were matched to the experimental data (Locke and Nerbome 1997). The model then is used to simulate the firing properties of strongly adapting and weakly adapting CP cells and to examine the functional effects of reducing or eliminating \( I_{D} \), \( I_{Na} \), and \( I_{K} \) on the waveforms of individual action potentials and the repetitive firing properties of CP neurons.

**METHODS**

**Isolation and identification of CP neurons**

Whole cell voltage- and current-clamp recordings were obtained from identified CP visual cortical neurons isolated from postnatal day 7–13 (P7–P13) Long Evans rat pups. Methods for CP cell labeling, isolation, and identification were as previously described (Giffin et al. 1991; Locke and Nerbome 1997; Solomon et al. 1993).

**Electrophysiological recordings**

Whole cell patch-clamp recordings were obtained at room temperature (23–25°C) from identified CP neurons within 48 h of cell isolation (Locke and Nerbome 1997). Current- and voltage-clamp experiments were completed using a Dagan 3900 patch-clamp amplifier. Experiments were controlled and data were acquired using an IBM-compatible 486 computer with TL-1 interface to the physiological equipment and the PC clamp (Axon Instruments) software package. Series resistance compensation and pipette fabrication and resistances were essentially as described in the preceding paper (Locke and Nerbome 1997). Both current- and voltage-clamp recordings were obtained from all cells examined. Single action potentials and action potential trains were evoked routinely by 1-ms, 1,000–1,500-pA current injections and by 1.5–2.0-s, 10–120-pA current injections, respectively. Whole cell currents were evoked during 160-ms voltage steps to potentials between −60 and +60 mV from a holding potential of −70 mV.

**Solutions**

The procurement, handling and perfusion of the \( K^+ \) channel blockers 4-aminopyridine (4-AP), dendrotoxin (DTX), and tetraethylammonium chloride (TEACl) were as described in Locke and Nerbome (1997). For recordings, the bath solution routinely contained (in mM) 140 NaCl, 4 KCl, 2 CaCl\(_2\), 2 MgCl\(_2\), 10 N-2-hydroxyethylpiperazine-N\(_2\)-2-ethanesulfonic acid (HEPES), and 5 glucose (pH 7.4; 305 mosm). Importantly, because current-clamp and voltage-gated recordings were obtained from the same cells and, in several experiments, switching repeatedly between voltage- and current-clamp modes was required, all voltage-clamp recordings were obtained in the absence of tetrodotoxin and Co\(^{2+}\) or Cd\(^{2+}\) (blocks of voltage-gated Na\(^{+}\) and Ca\(^{2+}\) channels, respectively). Voltage-clamp records, therefore, reflect the sum of voltage-gated, as well as any Na\(^{+}\)- and Ca\(^{2+}\)-dependent, inward and outward K\(^{+}\) currents. The pipette solution contained (in mM) 135 KCl, 10 HEPES, 5 glucose, 3 MgATP, 0.5 NaGTP, 2 ethylene glycol-bis(β-aminooxy ethyl)aminomethylene #N,N,N',N'-tetraacetic acid, and 1.2 CaCl\(_2\) (pH 7.4; 305 mosm); the intracellular free Ca\(^{2+}\) concentration was 10\(^{-7}\) M.

**Data analysis**

Data were compiled and analyzed using Clampfit (Axon Instruments), Excel (Microsoft) and CSS Statistica (Stat Soft). All data are means ± SD. Resting membrane potentials were determined immediately after achieving the whole cell configuration, and only those cells that had resting membrane potentials up to −55 mV and overshooting action potentials were selected for experiments. Leak currents were always <100 pA at −70 mV and were not corrected. Input resistances and the amplitudes of the total outward (Ca\(^{2+}\)-independent and Ca\(^{2+}\)-dependent) and inward (Na\(^{+}\) and voltage-gated Ca\(^{2+}\)) currents were monitored during all recordings, and only cells in which these parameters remained constant were selected for analysis. The mean input resistance of analyzed CP cells was 1.3 ± 0.6 GΩ (n = 60), and the mean whole cell membrane capacitance of these cells was 31 ± 10 pF (n = 60). Note that the membrane capacitances of the cells examined were on average, larger than those reported in the previous paper. This reflects the fact that the cells studied here were in culture longer, and, as a result, in many cases, had elaborated processes. Action potential durations at 50% and 90% repolarization were determined by measuring the widths of the action potentials when the membrane voltage had returned halfway (or 90% of the way) back to the resting potential; values are reported in milliseconds. The peak outward current was defined as the maximum value the current attained during 160-ms voltage steps; the plateau current was defined as the current remaining 150 ms after the onset of the depolarizations. \( I_{D} \) amplitudes were determined by subtraction of currents recorded in the presence of 50 μM 4-AP (or 50 nM α-DTX) from controls, as previously described (Locke and Nerbome 1997). \( I_{K} \) amplitudes were estimated from the peak currents remaining in the presence of 50 μM 4-AP, and \( I_{Na} \) amplitudes were estimated from the plateau currents (measured 150 ms after the onset of the depolarizations) remaining in the presence of 50 μM 4-AP.

Increases in action potential durations at 50 and 90% repolarization in the presence of 50 μM 4-AP or 10 mM TEA were measured in individual cells, and percentage increases with respect to control durations are presented; increases in action potential durations in the presence of 2–5 mM 4-AP were measured, and percentage increases over the durations measured in 50 μM 4-AP are presented. Decreases in latency in the presence of 50 μM 4-AP were determined as percentage latencies for the same stimulus amplitude in the same cell. For all experiments, statistical significance of observed differences among different populations was examined using Student’s t-test for paired and for unpaired data; P values are presented in the text.

**Modeling of voltage-gated K\(^{+}\) currents and simulations of the firing properties of CP cells**

The V Clamp/C Clamp software package, generously provided to us by Drs. David McCormick and John Huguenard, was used to model the voltage-gated K\(^{+}\) currents in CP neurons and to simulate the firing properties of these cells. The development and application of the model to simulate the properties of thalamocortical relay neurons has been described in detail (Huguenard and McCormick 1992; McCormick and Huguenard 1992). The model assumes a single compartment, and includes the following currents: the fast Na\(^{+}\) current, \( I_{Na} \); a persistent Na\(^{+}\) current, \( I_{NaP} \); the rapidly activating, rapidly inactivating (transient) K\(^{+}\) current, \( I_{Ks} \); a delayed
rectifier K current, $I_r$; a rapidly activating, slowly inactivating K current, $I_{k2}$; the low-threshold Ca current, $I_t$; the high-threshold Ca current, $I_h$; a fast, voltage- and Ca-dependent K current, $I_{c}$; a slow, voltage-independent, Ca-dependent K current, $I_{ack}$; the hyperpolarization-activated cationic conductance, $I_{h}$; a muscarine-sensitive, depolarization-activated K current, $I_{M}$; a Na leak current, $I_{leak}$; and a K leak current, $I_{k}$.

The currents (with the exception of $I_{leak}$ and $I_{k}$) are described by Hodgkin-Huxley-like formulations; each current reflects the activity of a population of channels with identical microscopic properties, and the activity of each channel type is controlled by gates that are opened, closed, and inactivated by membrane voltage. Using data obtained from previous voltage-clamp studies, $I_r$, $I_h$, $I_{c}$, and $I_{k2}$ were simulated, and hypotheses about the specific roles of these currents in controlling rhythmic firing in thalamic relay neurons were advanced (Huguenard and McCormick 1992). To simulate the firing properties of thalamocortical relay neurons, the mathematical descriptions of $I_r$, $I_h$, $I_{c}$, and $I_{k2}$ were incorporated, $I_{leak}$ and $I_{k}$ were determined from the passive properties and input resistances of thalamic relay neurons, and mathematical descriptions of the other currents in the model, i.e., $I_{Na}$, $I_{K}$, $I_{C}$, etc., were developed using the time- and voltage-dependent properties of similar currents in other cells (McCormick and Huguenard 1992). The model was shown to reproduce both the rhythmic and the tonic firing behavior of thalamic relay neurons (McCormick and Huguenard 1992).

To develop a model of the electrophysiological properties of CP neurons, we used a similar approach. The Ca-dependent, depolarization-activated K currents, $I_{c}$, $I_{t}$, and $I_{k}$ in CP neurons were modeled (see: RESULTS) using the parameters obtained in the voltage-clamp experiments described in detail in the preceding paper (Locke and Nerbonne 1997): mean values of all parameters were used. The time- and voltage-dependent properties of the remaining currents in the model, $I_{Na}$, $I_{K}$, $I_{C}$, $I_{M}$, $I_{AHP}$, and $I_{leak}$, were not altered; only the relative densities of these currents were varied. In previous studies, we have shown that $I_t$ is expressed only in a subset (18%) of CP neurons, and that, when expressed, the amplitudes/densities of $I_t$ are small, even when compared with $I_k$ amplitudes/densities in the same cell (Giffin et al. 1991). Similarly, $I_{Na}$ amplitudes/densities in CP neurons is small (Solomon et al. 1993). In preliminary attempts to model CP neurons, therefore, we set the conductances of $I_{Na}$ and $I_{K}$ equal to zero. Subsequent testing with the model confirmed that including low-density $I_{Na}$ and $I_{K}$ currents (such as recorded in CP neurons) had little effect on simulated action potential waveforms or repetitive firing properties. For simplicity, therefore, the amplitudes of these conductances were set to zero for all of the simulations presented here. $I_{Na}$ and $I_{K}$ amplitudes were estimated from analyses of voltage-clamp data from CP neurons (Giffin et al. 1991) and were not changed throughout the simulations. The densities of the remaining currents in the model, $I_{Na}$, $I_{K}$, and $I_{AHP}$, then were varied. The goal was to make the minimum number of changes necessary to produce simulated action potential waveforms and repetitive firing properties that resembled those in strongly adapting and weakly adapting CP neurons (see RESULTS). For all simulations, numerical solutions to the differential equations describing each of the currents used a one-step Euler integration method. Although the time step could be varied, in general, the increment was set at 0.01 ms, such that the individual gates changed by <1% during each step.

RESULTS

Action potential waveforms and repetitive firing properties of CP neurons

Single action potentials are evoked routinely in identified CP visual cortical neurons by 1-ms, 1,000- to 1,500-pA depolarizing current injections (Fig. 1). In all cells ($n = 60$), action potentials rise rapidly to a maximal potential of $+50$ mV, and repolarization is complete in 5–20 ms. Action potential durations, measured at 50% repolarization, ranged from 1.1 to 3.2 ms (Fig. 1A) with a mean of 1.9 ± 0.5 ms ($n = 60$); action potential durations at 90% repolarization ranged from 2.9 to 12.4 ms, with a mean of 5.5 ± 2.0 ms. During prolonged threshold depolarizing current injections (1.5 s; 10–120 pA), CP neurons fire repetitively at a rate that increases with increasing stimulus strength. Amplitudes of the current injections are noted beside the traces; the resting membrane potential of this cell was $-66$ mV.

![Figure 1](http://jn.physiology.org/)

**FIG. 1.** Callosal-projecting (CP) visual cortical neurons are regular spiking. A: action potential waveforms evoked by 1-ms, 1,000- to 1,500-pA depolarizing current injections in 3 different CP cells are displayed. Resting membrane potentials are noted beside the voltage traces, and action potential durations at 50% (APD$_{50}$) and 90% (APD$_{90}$) repolarization are indicated. B: during prolonged (1.5 s) depolarizing current injections, CP neurons fire repetitively at a rate that increases with increasing stimulus strength. Amplitudes of the current injections are noted beside the traces; the resting membrane potential of this cell was $-66$ mV.

To determine whether the differences in action potential durations at 50 and 90% repolarization (Fig. 1A) reflect differences in outward current densities among CP neurons, voltage-clamp recordings were obtained from each cell on which current-clamp experiments were performed. Peak outward currents, evoked from a holding potential of $-70$ mV by 160-ms voltage steps to potentials between $-60$ and $+60$ mV, were measured and subsequently normalized to cell capacitance to obtain peak outward current densities. It is important to note here that voltage-clamp recordings in these (and in all subsequent) experiments were, by necessity, performed in the absence of Na$^+$ and Ca$^{2+}$ channel blockers. The voltage-clamp records obtained and analyzed here, therefore, reflect the sum of voltage-gated and Ca$^{2+}$-dependent inward and outward currents. Plotting action potential...
served in CP cells. Of the 49 cells in which repetitive firing patterns were examined, 12 cells (24%) exhibited a strongly adapting firing pattern (Fig. 3A). The most striking characteristics of the strongly adapting firing pattern are the clustering of action potentials at the stimulus onset, the pronounced afterhyperpolarizations at the termination of each spike, and the cessation of repetitive firing in spite of maintained depolarizing current injections. Importantly, strongly adapting cells never were observed to resume firing during maintained depolarizing current injections (see DISCUSSION). In 37 of 49 cells examined (76%), a distinct firing pattern, which we refer to as weakly adapting, was observed (Fig. 3B). In weakly adapting cells, firing was maintained throughout the duration of the current injection and afterhyperpolarizations are not pronounced. In addition, the interval between spikes in weakly adapting cells increased as a function of time after the onset of the current injection (Fig. 3B), whereas the interspike interval varies only slightly in strongly adapting CP cells (Fig. 3A). During recordings, cells never switched between displaying the weakly and strongly adapting repeti-

FIG. 2. Action potential durations at 50% repolarization (APD₅₀) vary as a function of peak outward current density. Single action potentials were evoked as described in Fig. 1, and voltage-dependent K⁺ currents (in the same cells) were evoked during 160-ms voltage steps to potentials between −60 and +60 mV from a holding potential of −70 mV. It is important to note that, because of the recording conditions employed (i.e., without Ca²⁺ and Na⁺ channel blockers), the voltage-clamp records reflect both inward Ca²⁺ and Na⁺ currents as well as outward K⁺ currents. Peak outward current amplitudes at +60 mV were measured, normalized to cell capacitance, and plotted (A) with respect to the APD₅₀ determined in the same cell (n = 60). B and C: current- and voltage-clamp recordings from 2 representative cells from the extremes of the data set in A. For the cell with low peak outward current density (B), the action potential is broad, whereas for the cell with the high peak outward current density (C), the action potential is relatively brief.

duration at 50% repolarization (APD₅₀) versus the peak outward current density revealed a correlation (R² = 0.38; Fig. 2A): broad action potentials are recorded from cells with low outward current densities and brief action potentials are recorded from cells with high outward current densities. Data from two cells, representing the extremes of the data set in Fig. 2A, are presented in Fig. 2, B and C. As suggested above, owing to the conditions employed in these experiments, inward and outward currents are evident in these records. Importantly, the action potential is broad (2.5 ms at 50% repolarization) in the cell with a low outward K⁺ current density (86 pA/pF), whereas in the cell with a high K⁺ current density (258 pA/pF), the action potential is brief (1.6 ms at 50% repolarization). Action potential durations in CP cells, therefore, are correlated outward K⁺ currents amplitudes/densities (Fig. 2A).

Interestingly, two distinct repetitive firing patterns, differing in the extent of spike-frequency adaptation, were ob-

FIG. 3. Two distinct firing patterns are evident in CP neurons. A and B: action potentials trains were evoked by 1.5-s depolarizing current injections of increasing amplitude. A: action potentials in strongly adapting cells occurred at the onset of the stimuli, and despite maintained current injections, these cells stop firing. B: continuous firing is observed at most stimulus intensities for weakly adapting cells. Examination of the waveforms of individual action potentials revealed that afterhyperpolarizations are more pronounced in strongly (C) than in weakly (D) adapting cells. The records in A and C and in B and D were from the same cells; the resting membrane potentials of these cells were −64 mV (A and C) and −60 mV (B and D).
tive firing patterns. In addition, firing patterns were not affected by variations of ±5 mV in the membrane potential around rest. Thus strongly and weakly adapting cells are distinct, nonoverlapping populations of regular-spiking CP cells (see DISCUSSION). In addition, it is of interest to note that the strongly adapting firing pattern was observed only in CP cells isolated from animals ≥P11; 12 of the 30 (40%) ≥P11 CP cells were classified as strongly adapting, whereas all of 19 ≤P10 CP cells were weakly adapting. Taken together, these results suggest that strongly adapting cells likely are not immature CP neurons (see DISCUSSION).

Analysis of the interspike-interval voltage trajectories in strongly and weakly adapting cells revealed that action potentials in strongly adapting cells have more prominent after-hyperpolarizations (Fig. 3C) than do action potentials in weakly adapting cells (Fig. 3D). The pronounced after-hyperpolarizations result in a marked shortening of the action potential duration in strongly adapting as compared with weakly adapting cells. Mean action potential durations at 90% repolarization were 4.4 ± 0.6 ms (n = 12) and 6.3 ± 2.1 ms (n = 37) for strongly and weakly adapting cells, respectively (P < 0.001). At 50% repolarization, however, action potential durations were indistinguishable in the two cell types: mean durations were 1.9 ± 0.2 and 2.0 ± 0.5 ms, in strongly and weakly adapting cells, respectively.

Comparison of the membrane properties of weakly adapting and strongly adapting CP cells revealed two important differences (Table 1): input resistances are significantly lower and plateau outward K⁺ current densities are significantly higher in strongly adapting, than in weakly adapting, CP cells. Because plateau current densities are higher and peak current densities are similar, peak-to-plateau current ratios are significantly lower in strongly adapting, than in weakly adapting, CP cells (Table 1). As demonstrated in the companion paper (Locke and Nerbonne 1997), detailed analysis of voltage-clamp records revealed the expression of three Ca²⁺-independent, voltage-gated K⁺ current densities (Iş, Is, and Iₖ) in CP visual cortical neurons. Iş activates rapidly, inactivates slowly, and is blocked by micromolar concentrations of 4-AP and nanomolar concentrations of DTX; Is activates and inactivates rapidly and is sensitive to millimolar, but not micromolar, concentrations of 4-AP; and Iₖ activates and inactivates slowly, is 4-AP insensitive, and is blocked by millimolar concentrations of TEA. As also demonstrated, the peak outward currents in CP cells reflect primarily Iş and Iₖ, whereas only Iş and Iₖ contribute to the plateau currents (Locke and Nerbonne 1997). The finding that the peak-to-plateau current ratios are lower in strongly adapting cells, therefore, suggests that the relative densities of Iş and/or Iₖ are larger in strongly than in weakly adapting cells and, further, that Iş and Iₖ likely play important roles in producing the strongly adapting phenotype. Subsequent experiments, therefore, were focused on assessing the functional roles of Iş, Is, and Iₖ in shaping the waveforms of action potentials and in controlling repetitive firing in (strongly and weakly adapting) regular-spiking CP neurons.

### Functional roles of Iş in regular-spiking CP neurons

One role attributed to Iş (or Iş-like currents) in other preparations is to control the latency to firing an action potential in response to a threshold depolarizing current stimulus (Hammond and Crepel 1992; McCormick 1991; Nisenbaum et al. 1994; Storm 1988). To determine whether Iş in CP neurons plays a similar role, action potentials (evoked by 400 ms, 20- to 70-pA depolarizing current injections) were recorded in control bath solution and after superfusion of 50 μM 4-AP (Locke and Nerbonne 1997). As illustrated in Fig. 4A, the latency to firing an action potential was reduced when Iş was blocked. Similar decreases in latency were observed in eight of nine cells examined (in 1 cell no change was observed), and the mean decrease in latency resulting from block of Iş by 50 μM 4-AP was 37 ± 11%. Voltage-clamp experiments completed in parallel revealed that the decrease in latency is correlated (R² = 0.68) with the amplitude of Iş (Fig. 4B), i.e., the greater the amplitude of Iş, the more the latency was affected when Iş was blocked. Similar results were obtained with 50 nM α-DTX (n = 3).

The fact that activation of Iş is rapid (Locke and Nerbonne 1997) suggested that Iş also should play a role in action potential repolarization in CP neurons. To test this hypothesis directly, action potential durations recorded in control bath solution were compared with those evoked in the same cell after superfusion of 50 μM 4-AP or 50 nM α-DTX. As illustrated in Fig. 4C, action potentials are prolonged markedly when Iş is blocked, and the peaks of the action potentials are increased slightly. Although qualitatively similar results were obtained in all cells examined (n = 30), the effect of blocking Iş is qualitatively distinct in individual cells. In some cells, the action potential duration at 50% repolarization increased by >50% (Fig. 4C, top), whereas in other cells, action potential durations at 50% repolarization increased only slightly (Fig. 4C, bottom). For the cells in Fig. 4C, voltage-clamp experiments revealed that Iş contributes 39, 24, and 12%, respectively, to the peak outward currents. Similar to the effect on latency, therefore, the magnitude of the effect of blocking Iş on action potential duration is correlated (R² = 0.76) with the amplitude of Iş (Fig. 4D). A similar relation was observed when Iₖ amplitudes

### TABLE 1. Membrane properties of strongly and weakly adapting CP neurons

<table>
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<tr>
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<th>n</th>
<th>Cm, pF</th>
<th>APD₅₀, ms</th>
<th>APD₉₀, ms</th>
<th>Peak Current Density, pA/pF</th>
<th>Plateau Current Density, pA/pF</th>
<th>Peak-to-Plateau Ratio</th>
<th>Rₑₑ, GΩ</th>
<th>Vₑₑ, mV</th>
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<td>Strongly adapting</td>
<td>12</td>
<td>36 ± 13</td>
<td>1.9 ± 0.2</td>
<td>4.4 ± 0.6*</td>
<td>184 ± 49</td>
<td>188 ± 54†</td>
<td>0.98 ± 0.07‡</td>
<td>1.18 ± 0.75‡</td>
<td>−65 ± 6</td>
</tr>
<tr>
<td>Weakly adapting</td>
<td>37</td>
<td>31 ± 9</td>
<td>2.0 ± 0.5</td>
<td>6.3 ± 2.1*</td>
<td>171 ± 66</td>
<td>150 ± 51†</td>
<td>1.23 ± 0.41‡</td>
<td>1.86 ± 1.09‡</td>
<td>−63 ± 6</td>
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All values are means ± SD. *Values are significantly different at the P < 0.001 level. † Values are significantly different at the P < 0.05 level. ‡ Values are significantly different at the P < 0.005 level.
FIG. 4. \( I_D \) contributes to the latency to firing and to action potential repolarization. A: action potentials were evoked by 400-ms threshold current injections under control conditions and after superfusion of 50 \( \mu \text{M} \) 4-amino pyridine (4-AP). In the presence of 50 \( \mu \text{M} \) 4-AP, the latency to firing the 1st action potential is reduced relative to the control. Similar results were obtained on 7 other cells. Percent decrease in latency in the presence of 50 \( \mu \text{M} \) 4-AP is plotted for each cell with respect to \( I_D \) amplitude (in the same cell) in B; the increase in latency to firing when \( I_D \) is blocked is correlated \((R^2 = 0.68)\) with the amplitude of \( I_D \). For these analyses, latencies were measured from the stimulus onset to the inflection of the voltage trajectory at the base of the action potential \((\uparrow\) in A), and \( I_D \) amplitudes were obtained by subtraction of the voltage-clamp records obtained in the presence of 50 \( \mu \text{M} \) 4-AP from control records. C: single action potentials, evoked by brief current injections, were recorded under control conditions and after superfusion of 50 \( \mu \text{M} \) 4-AP. After blockade of \( I_D \), repolarization is slowed and action potential durations are prolonged. For the 3 cells shown, \( I_D \) contributes 39, 24, and 12%, respectively, to the peak outward currents. Percent increase in action potential duration at 50% repolarization (APD\(_{50}\)) after blockade of \( I_D \) is plotted vs. \( I_D \) amplitude \((n = 30)\) in D; the increase in APD\(_{50}\) when \( I_D \) is blocked is correlated \((R^2 = 0.76)\) with \( I_D \) amplitude \((D)\) and to the percentage contribution of \( I_D \) to the peak outward current \((n = 30)\). E: mean \pm SD \((n = 30)\) action potential durations at 50% \( (\square) \) and 90% \( (\Box) \) repolarization under control conditions and in the presence of 50 \( \mu \text{M} \) 4-AP.

and action potential durations at 90% repolarization were compared (not shown). No significant differences were observed, however, in the effects of 50 \( \mu \text{M} \) 4-AP on action potential durations in strongly and weakly adapting cells. When data from all cells are pooled (Fig. 4E), it is clear that blockade of \( I_D \) increases mean action potential durations at 50% repolarization significantly \((n = 30, \text{paired } t\text{-test } P < 0.001)\) from 1.9 \( \pm \) 0.5 ms to 2.5 \( \pm \) 0.6 ms; mean action potential durations at 90% repolarization also increased significantly \((n = 30, \text{paired } t\text{-test } P < 0.001)\), from 6.6 \( \pm \) 5.2 ms to 9.6 \( \pm \) 6.2 ms (Fig. 4E).

Subsequent experiments revealed that repetitive firing patterns in CP cells are altered when \( I_D \) is blocked (Fig. 5) and, in addition, that the effects of blockade of \( I_D \) in strongly and weakly adapting cells are distinct. In weakly adapting cells, blockade of \( I_D \) increases the firing frequency in response to the same current injection (Fig. 5B). In strongly adapting cells, however, blockade of \( I_D \) results in continuous firing throughout the duration of depolarizing current injections (Fig. 5A). In all cells, blockade of \( I_D \) permitted current stimuli too weak to evoke action potentials in control conditions to produce repetitive firing (Fig. 5B1). Taken together, these results demonstrate that \( I_D \) plays an important role in damping excitability in both strongly and weakly adapting cells and suggest that \( I_D \) contributes importantly to the strongly adapting firing pattern (see DISCUSSION).

**Functional roles of \( I_A \) in regular-spiking CP neurons**

The facts that activation of \( I_A \) is rapid and that \( I_A \) is expressed at high-density in CP neurons (Locke and Nerbonne...
Fig. 6. $I_K$ contributes importantly to action potential repolarization in CP cells. Single action potentials were evoked under control conditions, after application of 50 μM 4-AP, and again after superfusion of 2 mM (or 5 mM) 4-AP. Results obtained in experiments completed on 3 different cells are presented in A; the average of each that was blocked in each cell and the resting membrane potential of each cell are noted. B: percent increase in $\text{APD}_{50}$ (beyond that observed with 50 μM 4-AP) in the presence of 2 or 5 mM 4-AP is plotted with respect to the amplitude of $I_K$ that was blocked in the same cell ($n = 18$). Similar to the findings for $I_C$ (Fig. 4D), the prolongation of the $\text{APD}_{50}$ in each cell is correlated ($R^2 = 0.66$) with the amplitude of the current (in this case $I_K$) that was blocked. C: mean ± SD action potential durations at 50% (●) and 90% (○) repolarization under control conditions, in the presence of 50 μM 4-AP, and in the presence of 5 mM 4-AP are displayed.

1997) suggested that this conductance pathway likely also plays an important role in action potential repolarization in these cells. Because there is no selective blocker of $I_K$, however, assessing the functional role of $I_K$ is more complicated than was the case for $I_C$; i.e., the role of $I_K$ could only be examined after first blocking $I_C$. As illustrated in Fig. 6A, exposure to 2 or 5 mM 4-AP markedly slows repolarization and increases the duration of evoked action potentials in CP neurons. Parallel voltage-clamp experiments allowed direct determination of the amplitude of $I_K$ blocked in each cell. These experiments revealed that the percentage increase in the action potential duration at 50% repolarization is correlated ($R^2 = 0.66$) with the amplitude of $I_K$ (in the same cell) that was blocked by 4-AP (Fig. 6B). A similar correlation was observed when the percentage increase in action potential duration at 90% repolarization and the amplitude of $I_K$ that was blocked by 2 mM or 5 mM 4-AP were compared (not shown). When data from all cells ($n = 18$) are pooled (Fig. 6C), it is clear that applications of 2–5 mM 4-AP increased the mean action potential duration at 50% repolarization significantly (paired t-test $P < 0.001$) from 2.3 ± 0.6 ms (in 50 μM 4-AP) to 4.0 ± 0.9 ms. Mean action potential duration at 90% repolarization also increased significantly (paired t-test $P < 0.001$) from 8.4 ± 3.8 ms (in 50 μM 4-AP) to 15.9 ± 5.9 ms (in 2–5 mM 4-AP) (Fig. 6C).

Subsequent experiments were aimed at determining the role of $I_K$ in controlling repetitive firing behavior of CP neurons. Similar to the experiments described above, it was only possible to explore the functional role of $I_K$ with $I_D$ also blocked. Nevertheless, these experiments did reveal that suppression of $I_K$ results in further increases (i.e., beyond those seen when only $I_D$ is blocked) in firing rates in both strongly (Fig. 7A) and weakly (Fig. 7B) adapting cells. These results support the hypothesis that $I_K$ functions to slow the rate of repetitive firing in CP neurons (see below and DISCUSSION).

**Functional roles of $I_K$ in regular-spiking CP neurons**

The fact that $I_K$ activates more slowly than $I_C$ or $I_A$ suggested that this conductance pathway should play a more prominent role during the later (rather than the earlier) phases of action potential repolarization. To test this hypothesis, single action potentials and action potential trains were recorded from isolated CP neurons before and after superfusion of 10 mM TEA. In the presence of TEA, action potential durations at 50 and 90% repolarization increased, although the effect on the $\text{APD}_{50}$ was more pronounced (Fig. 8). Importantly, afterhyperpolarizations were eliminated in both strongly (Fig. 8A) and weakly (Fig. 8B) adapting CP cells in the presence of 10 mM TEA. Exposure to 10 mM TEA also has profound effects on the repetitive firing properties of CP cells. At low stimulus strengths, 10 mM TEA increased firing rates in both strongly...
Modeling of strongly adapting and weakly adapting regular-spiking CP neurons

As discussed above, the lack of specific blockers for $I_H$ and $I_K$ complicated experiments aimed at investigating the functional roles of these currents. In an alternative approach, we sought to develop a model of CP neurons using the VClamp/CClamp software package and to use this model to probe the functional roles of the $\text{Ca}^{2+}$-independent, voltage-gated $K^+$ currents, particularly $I_A$ and $I_K$, in shaping the waveforms of individual action potentials and in mediating the repetitive firing properties of CP cells. The development and application of the model to simulate the properties of thalamocortical relay neurons has been described in detail (Huguenard and McCormick 1992; McCormick and Huguenard 1992) (see METHODS). The strategy employed here was similar to that of Huguenard and McCormick in that the currents of interest, i.e., $I_A$, $I_D$, and $I_K$, were first modeled with VClamp using the detailed time- and voltage-dependent properties (Table 2) determined experimentally (Locke and Nerbonne 1997). The CClamp program then was modified to reflect the properties and relative densities of $I_A$, $I_D$, and $I_K$, and the other conductances in the model were adjusted as described below.

To simulate voltage-gated $K^+$ current waveforms, mean values of the time- and voltage-dependent parameters describing $I_A$, $I_D$, and $I_K$ in CP neurons (Table 2) were used. As illustrated in Fig. 9, the simulated $I_A$, $I_D$, and $I_K$ waveforms are indistinguishable from the currents recorded in isolated, identified CP neurons. Simulations also were completed, however, in which the time- and voltage-dependent properties of $I_A$, $I_D$, and $I_K$ were varied over ranges encompassed by ±1 SD from the means (Table 2). Although the waveforms of the currents varied slightly, changing the time- and voltage-dependent properties of $I_A$, $I_D$, and $I_K$ over ranges encompassed by ±1 SD from the means did not appreciably change the waveforms of simulated action potentials or repetitive firing patterns. Mean values for the time- and voltage-dependent properties of $I_A$, $I_D$, and $I_K$ (Table 2), therefore, were used in subsequent simulations of action potentials and repetitive firing in CP neurons.

After modifying CClamp to reflect the properties of $I_A$, $I_D$, and $I_K$, the densities of the currents were adjusted consistent with the experimental data (Locke and Nerbonne 1997). $I_A$ amplitudes, for example, were set equal to four times $I_D$ amplitudes (in the same cell) and were equal in strongly and weakly adapting CP cells (Locke and Nerbonne 1997). $I_K$ amplitudes, however, were set 30% larger in strongly than in weakly adapting cells to reflect the fact that plateau current densities are higher in strongly adapting CP cells (Table 1). The time- and voltage-dependent properties of the other currents, $I_Na$, $I_Nap$, $I_T$, $I_L$, $I_H$, $I_C$, and $I_{AHP}$ (see METHODS) were not altered; only the relative densities of the currents were varied. In previous studies, for example, we have shown that $I_T$ is expressed only in a subset (18%) of CP neurons and that, when expressed, the amplitudes/densities of $I_T$ are small (Giffin et al. 1991). Similarly, $I_Na$ amplitudes/densities in CP neurons are small (Solomon et al. 1993). In preliminary simulations, therefore, $I_H$ and $I_T$ amplitudes were set equal to zero. After the models were developed fully, however, it was clear that including $I_T$ and/or $I_H$ at densities comparable with those recorded in CP neurons (Giffin et al. 1991; Solomon et al. 1993) had little effect on simulated action potential waveforms or repetitive firing properties. For simplicity, therefore, $I_T$ and $I_H$ amplitudes/densities were zero for all of the simulations presented here.
I

were not changed throughout the simulations. CP neurons. Specifically, model constrained by the known properties of CP neurons DISCUSSION).

Clearly, the goal was to produce simulated action potential waveforms and repetitive firing properties that resembled those in strongly adapting and weakly adapting CP neurons as simply as possible. Preliminary simulations with the model constrained by the known properties of CP neurons revealed that \( I_c \) was required to produce the brief action potentials and prominent afterhyperpolarizations evident in strongly adapting CP neurons (Fig. 3). Because, to our knowledge, there have been no previous reports documenting the presence of functional \( I_c \)-like currents in cortical neurons, it will be of great interest to test the predictions of this model that \( I_c \) is expressed in rat visual cortical CP neurons (see DISCUSSION). In all simulations here, \( I_c \) densities were equal in strongly and weakly adapting CP cells. The simulations also revealed that varying the densities of \( I_{AHP} \) and \( I_{Na} \) allowed the generation of the strongly and weakly adapting firing patterns observed experimentally in CP neurons. Specifically, \( I_{AHP} \) density in weakly adapting cells was twice that in strongly adapting cells; this difference was necessary to simulate the marked increase in the interval between spikes as a function of time during prolonged current injections that is observed in weakly (but not in strongly) adapting CP cells (Fig. 3). A low density \( I_{Na} \) (equal to 0.005–0.05% of the fast inactivating \( I_{Na} \)) was also necessary to reproduce the weakly adapting phenotype (see DISCUSSION).

Simulated action potential waveforms and repetitive firing patterns generated by the model are presented in Fig. 10. As is evident, the simulations closely resemble the experimental data (Fig. 3) for both strongly (Fig. 10A) and weakly (Fig. 10B) adapting CP cells. In particular, strongly adapting cells cease firing in spite of maintained depolarizing current injections, and the interval between successive spikes in a train is greater in weakly than in strongly adapting cells. In addition, single action potentials in strongly adapting cells have pronounced afterhyperpolarizations (Fig. 10C), whereas action potentials in weakly adapting cells do not (Fig. 10D).

**Simulations provide insights into the functional roles of \( I_{AHP}, I_{Na}, \) and \( I_c \) in CP neurons**

Similar to results obtained experimentally (see Fig. 4) when \( I_{D} \) is “blocked” (i.e., the conductance is reduced to zero) in modeled CP neurons, the latency to firing an action potential is reduced (Fig. 11Aa). Because removal of \( I_{D} \) produced similar effects in strongly and weakly adapting modeled CP neurons, results are illustrated only for strongly adapting cells. Removing \( I_{D} \) also increased action potential durations at both 50 and 90% repolarization and slightly increased action potential amplitudes (Fig. 11Ab). The simulations also revealed that reductions in \( I_{AHP} \) after removal of \( I_{D} \) leads to further increases in action potential durations at both 50 and 90% repolarization (Fig. 11Ac), and the magnitudes of these increases are similar to those observed experimentally on application of 2–5 mM 4-AP to CP cells (see Fig. 6A).

The effects of removing \( I_{D} \) on repetitive firing in modeled CP cells were also similar to those observed experimentally (see Fig. 5). In strongly adapting modeled cells, for example, elimination of \( I_{D} \) results in continuous firing throughout the stimulus duration (Fig. 11Be). In weakly adapting modeled cells, however, the firing rate (relative to the rate observed for the same current stimulus under control conditions) is increased when \( I_{D} \) is removed (Fig. 11Bb). When \( I_{AHP} \) is attenuated after removal of \( I_{D} \), firing rates are increased (relative to the firing rates observed when only \( I_{D} \) was elimi-

<table>
<thead>
<tr>
<th>( I_{Na} )</th>
<th>( I_{D} )</th>
<th>( I_{K} )</th>
<th>( V_{1/2act} )</th>
<th>( V_{1/2inact} )</th>
<th>Density, pA/pF</th>
<th>( \tau_{act} -70 \text{mV} )</th>
</tr>
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<tbody>
<tr>
<td>14.7 ± 4.0</td>
<td>1.74 ± 0.49</td>
<td>0.83 ± 0.24</td>
<td>19 ± 5</td>
<td>6 ± 6</td>
<td>215 ± 84</td>
<td>38 ± 7</td>
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All values are means ± SD.

\( I_{Na} \) and \( I_c \) amplitudes were estimated from analyses of voltage-clamp data from CP neurons (Giffin et al. 1991) and were not changed throughout the simulations.

The remaining currents in the model, \( I_{Na}, I_c, \) and \( I_{AHP}, \) to date have not been characterized in CP neurons, and the densities of these currents were varied somewhat randomly. Formulations of voltage-dependent \( K^+ \) currents were recorded under voltage-clamp conditions with tetrodotoxin and Cd\(^{2+}\) in the bath to block voltage-gated Na\(^+\) and Ca\(^{2+}\) currents, as well as Ca\(^{2+}\)-dependent K\(^+\) currents. Individual current components were separated based on differential sensitivity to 4-AP, dendrotoxin, and TEA (see text and Locke and Nerbonne 1997 for details). Recorded and simulated currents were obtained at room temperature (22–23°C).
larization (Fig. 12Aa). In contrast, removal of $I_K$ has no significant effect on the waveforms of individual action potentials in either strongly or weakly modeled adapting cells (Fig. 12Ab). The latter result is in marked contrast to what was observed experimentally using 10 mM TEA (see Fig. 8, A and B) and suggests that application of TEA to CP neurons indeed blocks Ca$^{2+}$-activated K$^+$ currents as well as $I_K$ and, further, that Ca$^{2+}$-dependent K$^+$ currents are also important for action potential repolarization in CP cells (see DISCUSSION). The simulations also revealed that attenuation or removal of either $I_A$ or $I_K$ from modeled cells decreases the latency to firing (Fig. 11A, c and d).

In weakly adapting modeled CP neurons, removal of $I_A$ accelerates the rate of repetitive firing in response to the same current stimulus and substantially depolarizes the interspike membrane potential (Fig. 12Bb). When $I_A$ is removed from strongly adapting modeled CP cells, repetitive firing throughout the duration of the depolarizing stimulus is observed routinely (Fig. 12Bf). Interestingly, the simulations also suggest that $I_K$ makes no significant contribution to the waveforms of single action potentials, but that this conductance pathway does markedly influence the repetitive

![Figure 10](image-url) Action potential waveforms and repetitive firing properties of modeled strongly and weakly adapting regular spiking CP neurons (see text for details of the models and the simulations). Repetitive firing patterns generated in response to different depolarizing current injections in strongly (A) and weakly (B) adapting modeled CP cells are displayed; the resting membrane potential of each modeled cell was $-70$ mV. Consistent with the experimental data, action potentials in strongly adapting cells have pronounced afterhyperpolarizations (C), whereas those in weakly adapting cells do not (D).

![Figure 11](image-url) Removal of $I_D$ in modeled CP neurons decreases the latency to firing, slows the time course of action potential repolarization, and alters repetitive firing. In both strongly and weakly adapting modeled cells, removal of more than $\sim 30\%$ of $I_A$ (after removal of $I_D$) prevents repetitive firing. In strongly adapting modeled cells, however, repetitive firing continues even when $>50\%$ of $I_A$ (and all of $I_D$) are eliminated. Presumably, the difference in the responses of the modeled strongly and weakly adapting CP cells to the removal of $I_A$ reflects the facts that $I_K$ density is higher in strongly than in weakly adapting CP cells, whereas $I_{NaP}$ density is higher in weakly adapting cells (see DISCUSSION).

Although insights into the functional roles of $I_A$ and $I_K$ were obtained by examining the effects of millimolar 4-AP and TEA on the firing properties of CP cells, the lack of specific blockers for these currents clearly compromised interpretation of these experiments. As a result, it was of interest to use the newly generated models of CP neurons to examine the effects of “blocking” $I_A$ and $I_K$ on action potential waveforms and repetitive firing patterns. These simulations revealed that, in both strongly and weakly adapting modeled CP cells, removal of $I_A$ slows action potential repolarization (Fig. 12Aa). In contrast, removal of $I_K$ has no significant effect on the waveforms of individual action potentials in either strongly or weakly modeled adapting cells (Fig. 12Ab). The latter result is in marked contrast to what was observed experimentally using 10 mM TEA (see Fig. 8, A and B) and suggests that application of TEA to CP neurons indeed blocks Ca$^{2+}$-activated K$^+$ currents as well as $I_K$ and, further, that Ca$^{2+}$-dependent K$^+$ currents are also important for action potential repolarization in CP cells (see DISCUSSION). The simulations also revealed that attenuation or removal of either $I_A$ or $I_K$ from modeled cells decreases the latency to firing (Fig. 11A, c and d).

In weakly adapting modeled CP neurons, removal of $I_A$ accelerates the rate of repetitive firing in response to the same current stimulus and substantially depolarizes the interspike membrane potential (Fig. 12Bb). When $I_A$ is removed from strongly adapting modeled CP cells, repetitive firing throughout the duration of the depolarizing stimulus is observed routinely (Fig. 12Bf). Interestingly, the simulations also suggest that $I_K$ makes no significant contribution to the waveforms of single action potentials, but that this conductance pathway does markedly influence the repetitive
firing behavior of modeled CP cells. When \( I_K \) is removed from weakly adapting modeled CP cells, for example, a dramatic increase in the rate of repetitive firing is observed (Fig. 12Be). In strongly adapting modeled CP cells, removing \( I_K \) results in continuous firing throughout the duration of the depolarizing stimulus (Fig. 12Bf).

**DISCUSSION**

**CP visual cortical neurons are regular spiking**

The experiments here were undertaken to examine the firing properties of isolated, identified CP rat visual cortical neurons and to explore directly the functional roles of the \(Ca^{2+}\)-independent, voltage-gated \(K^+\) currents \(I_A\), \(I_B\), and \(I_K\) (Locke and Nerbonne 1997) in mediating the firing properties of the cells. As discussed previously (Giffin et al. 1991; Locke and Nerbonne 1997; Solomon et al. 1993), CP neurons were selected to correspond to prototypical regular-spiking cortical neurons (Connors and Gutnick 1990; McCormick et al. 1985). The results presented here reveal that, similar to regular-spiking cells characterized previously by others (Connors and Gutnick 1990; Huettner and Baughman 1988; Huguenard et al. 1988), isolated CP neurons fire single action potentials in response to brief current injections. During prolonged depolarizing current injections, CP neurons fire repetitively at rates that depend on the stimulus intensity, and these cells display varying degrees of spike-frequency adaptation (see also below). Taken together, these results confirm that CP neurons indeed can be classified as regular-spiking cortical cells.

Action potential durations at 50 and 90% repolarization are variable among CP cells, although there was no correlation between spike width either at 50 or 90% repolarization and the age of the animal from which the cells were isolated (P7–P13) or the time the cells were in culture before recording. Importantly, however, combined voltage- and current-clamp recordings revealed that the differences in action potential durations among CP cells are correlated with peak outward \(K^+\) current densities, i.e., the higher the peak outward current density, the briefer the action potential.

**Strongly and weakly adapting regular-spiking CP neurons**

Interestingly, two distinct regular-spiking patterns, which we have termed strongly adapting and weakly adapting, were evident among isolated CP neurons. Strongly adapting cells fire repetitively at the onset of depolarizing current injections but cease firing despite maintained depolarization. Weakly adapting cells, in contrast, fire continuously throughout the duration of depolarizing current injections. Examination of the waveforms of single action potentials revealed that action potential repolarization is faster and afterhyperpolarizations are more pronounced in strongly than in weakly adapting cells (see Fig. 3 and Table 1). In addition, analyses of combined voltage- and current-clamp recordings revealed that plateau current densities, corresponding to the slowly inactivating \(K^+\) currents (i.e., \(I_D\) and \(I_K\)) are significantly higher in strongly than in weakly adapting CP cells (Table 1).

Previous studies have demonstrated that the waveforms of individual action potentials and the repetitive firing properties of early postnatal cortical neurons are distinct from those of mature cortical neurons (Kriegstein et al. 1987; Lorenzon and Foehring 1993; McCormick and Prince 1987). Maximum firing rates, for example, are higher in mature than in immature neurons, and immature neurons are observed to stop firing in spite of maintained depolarizing current injections (Kriegstein et al. 1987; Lorenzon and Foehring 1993; McCormick and Prince 1987). The observation of strongly adapting CP cells, therefore, might be interpreted as suggesting that these (strongly adapting) cells are simply immature CP neurons rather than a distinct phenotype. Several lines of evidence, however, suggest that strongly adapting CP cells likely do not reflect an immature phenotype of regular-spiking CP cells particularly when compared with weakly adapting CP cells. The input resistances, for example, of strongly adapting cells are significantly lower (rather than higher as would be expected for less mature neurons) than those of weakly adapting CP cells (Table 1). In addition, action potential durations at 90% repolarization are significantly shorter (rather than longer as would be expected for less mature neurons) in strongly adapting than in weakly adapting CP cells (Table 1). Finally, repolarization is faster and fast afterhyperpolarizations are more prominent in strongly than in weakly adapting CP cells.
In addition, we note that distinct types of regular-spiking firing patterns have been described previously in mature neocortical neurons. In cat association cortex, for example, regular-spiking (RS) cells were subdivided into fast- and slow-adapting subtypes similar to those observed in CP neurons. Fast-adapting cells (16% of all RS cells) fired trains of spikes only at the beginning of depolarizing current pulses, whereas slow-adapting cells (84% of RS cells) fired continuously during a maintained stimulus (Nunez et al. 1993). Chagnac-Amitai and Connors (1989) subdivided regular-spiking cells in (4–6 wk) postnatal rat primary somatosensory cortex into three distinct subgroups (RS1, RS2, and RS3) based on characteristic differences in fast afterhyperpolarizations, depolarizing afterpotentials and the extent of spike-frequency adaptation observed during maintained depolarizing current injections. In addition, it was demonstrated that RS1, RS2, and RS3 cells have distinct laminar distributions: RS1 and RS3 cells, for example, were found in all cortical layers, whereas RS2 cells were found only in lower layers (IV–VI). Two distinct regular-spiking phenotypes, termed RS1 and RS3, also have been described in postnatal mouse somatosensory cortex (Agmon and Connors 1992). Strongly adapting CP cells resemble RS2 cells, whereas weakly adapting CP cells more closely resemble the RS1 phenotype described by Chagnac-Amitai and Connors (1989) and Agmon and Connors (1992). During prolonged depolarizing current injections, for example, RS2 cells often stop firing, i.e., RS2 cells are “strongly adapting.” In addition, the waveforms of individual action potentials in RS2 and strongly adapting CP cells are remarkably similar, both being characterized by large, fast afterhyperpolarizations. Weakly adapting CP cells, in contrast, resemble RS1 cells in that, although the interval between successive spikes increases initially, the intervals between spikes later in a train do not, i.e., the cells are “weakly adapting”; in both RS1 and weakly adapting CP cells, a spike doublet is sometimes observed at the onset of the stimulus. In addition, the waveforms of individual action potentials in weakly adapting CP cells and RS2 cells are similar; afterhyperpolarizations are not prominent in either cell type. Because CP neurons are found throughout cortical layers II–VI (Olavarria and Van Sluyters 1985) and are morphologically heterogeneous (Peters et al. 1990; Voigt et al. 1988), it may not be surprising that more than one regular-spiking phenotype is seen.

Finally, we note that the strongly adapting cells have only been observed in recordings from ≥P11 CP cells: 12 of the 30 (40%) ≥P11 CP cells studied were classified as strongly adapting. The strongly adapting phenotype was not seen in ≤P10 (n = 0/19) CP neurons. Although it is possible that the strongly adapting phenotype is transient, the fact that strongly adapting CP cells are only seen at ≥P11, whereas weakly adapting cells are seen at all ages (P8–P13), suggests that strongly adapting CP cells are not immature CP neurons particularly when compared with weakly adapting CP cells.

The expression of the strongly or weakly adapting phenotype is expected to have important functional consequences in that weakly adapting cells will track prolonged depolarizing inputs more reliably than will strongly adapting cells. Indeed, strongly adapting cells would be expected to actually attenuate prolonged excitation. As noted, RS1 cells in mouse somatosensory cortex display an initial phase of rapid adaptation followed by firing at a fairly constant rate, whereas RS2 cells continue to adapt throughout the stimulus duration (Agnon and Connors 1992). Interestingly, RS3 cells were found in cortical layers II–VI, whereas RS1 cells were encountered only in cortical layers V and VI, suggesting the participation of RS1 and RS2 cells in different cortical circuits.

Simulated voltage-clamp currents and firing properties of CP neurons

VCclamp/CCclamp, developed to simulate the properties of thalamocortical relay neurons (Huguenard and McCormick 1992; McCormick and Huguenard 1992), was used here to develop models of the firing properties of strongly and weakly adapting CP neurons. The voltage-gated K+ currents IA, IP, and IK, were modeled first with VCclamp using the detailed time- and voltage-dependent properties determined experimentally in isolated, identified CP cells (Locke and Nerbonne 1997). As illustrated in Fig. 9, the simulated and the experimental current waveforms are indistinguishable. The CCclamp program then was modified to reflect the properties and relative densities of IA, IP, and IK in CP cells, and the densities of the other conductances in the model were adjusted. INa and IC amplitudes, for example, were estimated from analyses of voltage-clamp data from CP neurons (Giffin et al. 1991), and, for the reasons outlined previously (see RESULTS), INa and IC amplitudes were set equal to zero (Giffin et al. 1991; Solomon et al. 1993). The other three conductances in the model, ICA, IC, and IH, however, have not been characterized in CP neurons. As a result, the densities of these currents were, by necessity, varied arbitrarily in our effort to reproduce the observed firing properties of CP cells. Importantly, the goal was to make the minimum number of adjustments in the models necessary to allow the generation of the firing patterns observed in strongly and weakly adapting CP cells.

Subsequent simulations revealed that an additional, rapidly activating outward current (i.e., in addition to IA, IP, and IK) was required to produce action potential durations and afterhyperpolarizations similar to those observed experimentally in CP neurons, particularly in strongly adapting CP neurons. Specifically, inclusion of the fast, Ca2+- and voltage-dependent K+ current IC at a density equal to the density of IK in the same cell resulted in shortened action potentials and prominent afterhyperpolarizations (Fig. 10) similar to those observed experimentally in (strongly adapting) CP neurons (Fig. 3). These findings were initially somewhat surprising primarily because there have been no published reports documenting the presence of functional IC-like currents in cortical neurons. In preliminary experiments, however, we have found that exposure to 100 μM Cd2+ [which blocks voltage-gated Ca2+ channels (Giffin et al. 1991)] and, therefore, any Ca2+-dependent K+ channels present], markedly prolongs action potentials and reduces the amplitudes of afterhyperpolarizations in CP neurons (n = 10) (unpublished observations). In addition, in voltage-clamp experiments, 100 μM Cd2+ suppresses the amplitudes of outward currents recorded in response to membrane depolarizations (n = 10) (unpublished data). Taken together, these results...
clearly demonstrate that Ca\(^{2+}\)-dependent K\(^+\) currents contribute to determining action potential durations and afterhyperpolarizations in CP neurons. In addition, these observations, together with the results of the simulations, suggest the presence of an \(I_c\)-like current in CP cells, and that at least part of the effect of Cd\(^{2+}\) on CP neurons is due to effects on \(I_c\). Clearly, further experiments aimed at characterizing the detailed properties and the functional roles of the Ca\(^{2+}\)-dependent K\(^+\) currents in CP neurons are warranted.

After including \(I_c\) in the model, adjustments were made to the remaining two conductances, \(I_{\text{Nap}}\) and \(I_{\text{AHP}}\), in an effort to produce the weakly adapting phenotype. These simulations revealed that small variations in \(I_{\text{AHP}}\) and \(I_{\text{Nap}}\) densities were sufficient to allow the generation of firing patterns (Fig. 10) indistinguishable from those observed experimentally in CP neurons (Fig. 3). The presence of a low density (0.005–0.05%) of the fast \(I_{\text{Nap}}\) of the \(I_{\text{AHP}}\) conductance in the model of weakly adapting CP cells allows these cells to continuous firing during maintained depolarizing current injections. \(I_{\text{AHP}}\), on the other hand, underlies the more pronounced spike-frequency adaptation seen in weakly (but not strongly) adapting CP cells. Interestingly, Nunez and colleagues (1993) hypothesized that differences in outward K\(^+\) current, \(I_{\text{Nap}}\) and/or \(I_{\text{AHP}}\), densities underlie the fast- and slow-adapting firing patterns seen in RS cells in cat association cortex. It will be of interest to test the predictions of these models and, in particular, to determine if \(I_{\text{Nap}}\) and \(I_{\text{AHP}}\) expression levels are indeed higher in weakly than in strongly adapting CP cells.

**Functional roles of \(I_D\), \(I_A\), and \(I_K\) in regular-spiking CP neurons**

The fact that \(I_0\) is blocked selectively and completely by 50 µM 4-AP and by nanomolar concentrations of α-dendrotoxin allowed direct evaluation of the role of \(I_0\) in shaping the waveforms of action potentials and in controlling the repetitive firing properties of CP neurons. These experiments revealed that action potentials are prolonged when \(I_0\) is blocked. In addition, combined voltage- and current-clamp recordings demonstrated that the magnitude of the prolongation of the action potential is correlated directly with the relative amplitude (density) of \(I_0\). Thus in all CP neurons, \(I_0\) plays an important role in action potential repolarization.

The experiments here also revealed that \(I_0\) contributes to controlling first spike latency. Although the effect of \(I_0\) on latency in CP neurons is significantly smaller than observed in CA1 hippocampal neurons (Storm 1988), these differences are quantitative, rather than qualitative, and likely reflect differences in the detailed properties and/or densities of \(I_0\) in hippocampal and cortical neurons. In hippocampal neurons, for example, \(I_0\) begins to activate at approximately equal to −75 mV, whereas the threshold for \(I_0\) activation in CP neurons is approximately equal to −40 mV. In both strongly and weakly adapting CP neurons, \(I_0\) also functions to control repetitive firing. Changes in firing rates after low concentrations of 4-AP have been observed in rat CA1 hippocampal (Storm 1988), prefrontal cortical (Hammond and Crepel 1992), nodose ganglion (Stansfeld et al. 1986), and neostriall (Nisenbaum et al. 1994) neurons and in mouse hippocampal (Wu and Barish 1992) and guinea pig lateral geniculate relay (McCormick 1991) neurons. Importantly, the functional roles of \(I_0\) revealed in experiments with low concentrations of 4-AP or DTX (Figs. 4 and 5) were reproduced in the models of strongly and weakly adapting CP cells (Fig. 11).

In the studies here, hypotheses regarding the functional roles of \(I_A\) were tested experimentally using 4-AP (see Figs. 6 and 7). The results suggested that \(I_A\) contributes importantly to shaping the waveforms of individual action potentials and to controlling the rate of repetitive firing in CP neurons. Although similar functional roles have been suggested for \(I_A\) in rat lateral geniculate (Budde et al. 1992) and amygdala neurons (Gean and Shinnick-Gallagher 1989) and in rat CA1 hippocampal pyramidal (Storm 1987) and nonpyramidal (Zhang and McBain 1995) neurons, the lack of a selective blocker for \(I_A\) clearly compromised the interpretation of these experiments and our ability to draw conclusions about the role(s) of \(I_A\) in controlling the firing properties of CP neurons. After generating and validating the models of strongly and weakly adapting CP cells (Figs. 10 and 11), therefore, simulations were completed to explore the functional roles of \(I_A\). The simulations (see Fig. 12) clearly suggest that \(I_A\) plays an important role in action potential repolarization and in determining first spike latencies. In addition, the simulations reveal that \(I_A\) functions to slow the frequency of repetitive firing in weakly adapting cells and that suppression of \(I_A\) in strongly adapting cells results in continuous firing. Taken together, therefore, the results of the simulations suggest that the functional roles of \(I_A\) and \(I_D\) in strongly and in weakly adapting CP cells are qualitatively very similar. It is interesting also to note that the results here indicate that neurotransmitter modulation of \(I_0\) or \(I_K\) will have similar functional effects and that these effects will be distinct in strongly and weakly adapting cells.

Exposure of isolated CP neurons to TEA has a greater effect on the APD\(_{50}\) than on the APD\(_{50}\) and profoundly influences repetitive firing in both strongly and weakly adapting cells. It is known, however, that TEA blocks other K\(^+\) currents, specifically Ca\(^{2+}\)-dependent K\(^+\) currents (Blatz and Magleby 1987), and preliminary experiments indeed suggest that Ca\(^{2+}\)-dependent K\(^+\) currents contribute importantly to action potential repolarization and to afterhyperpolarizations in rat visual cortical CP neurons (unpublished observations). Although it certainly would be possible to examine the effects of TEA on action potentials and firing patterns in the absence of Ca\(^{2+}\) (and therefore of Ca\(^{2+}\)-dependent currents), such experiments would not allow one to assess directly the normal, i.e., the physiological, role(s) of \(I_K\) because action potential waveforms and firing properties are affected profoundly by the removal of Ca\(^{2+}\) alone (unpublished observations). Studies aimed at examining the functional role(s) of \(I_K\) certainly would be aided greatly by the identification of a specific blocker(s) of \(I_K\). In lieu of a specific blocker of \(I_K\), simulations of modeled CP neurons were completed to explore the likely role(s) of \(I_K\).

In marked contrast to the results obtained with TEA, the waveforms of single action potentials essentially were unaltered when \(I_K\) was removed from both strongly and weakly adapting modeled CP neurons (Fig. 12). The simulations also demonstrated that \(I_K\) contributes to setting first spike latencies and to regulating repetitive firing patterns: removal of \(I_K\) dra-
matically increased the firing rate in weakly adapting modeled cells and produced continuous firing in the strongly adapting modeled CP neurons. The functional roles of $I_K$, therefore, are partially overlapping with those of $I_Na$ and $I_F$. An importance difference is the lack of contribution of $I_K$ to single action potentials. Transmitter mediated modulation of $I_K$, therefore, unlike modulation of $I_Na$ and $I_F$, would be expected to selectively regulate repetitive firing in strongly and weakly adapting regular-spiking cells, without affecting the responses of these cells to brief depolarizing inputs. In addition, and similar to $I_Na$, modulation of $I_K$ will have more dramatic effects on repetitive firing than will modulation of $I_F$ due to the fact that $I_K$ is expressed at fourfold higher density than $I_Na$. Indeed, the simulations demonstrated that removing $I_K$ (Fig. 12B, c and f) or $I_Na$ (Fig. 12B, b and e) resulted in firing at a more rapid rate than did removal of $I_F$ (Fig. 11B, b and e). It will be most interesting to test these hypotheses directly should a specific blocker of $I_K$ become available.

We thank R. J. Martinez and J. M. Coates for expert technical assistance with the in vivo retrograde labeling and with the preparation and the maintenance of cortical glial cultures. In addition, we thank Drs. John Huguenard and David McCormick for providing us with the VClamp/CClamp simulation package and Dr. Andreas Burkhart for many helpful discussions. Finally, we acknowledge the financial support provided by the National Institute of Neurological Disorders and Stroke Grant NS-30676.

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Received 3 September 1996; accepted in final form 30 June 1997.

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