INTRODUCTION

Since the first reports indicating the presence of a small, noninactivating component of voltage-dependent Na\(^+\) currents in cortical structures (\(I_{\text{NaP}}\)) (French et al. 1990; Stafstrom et al. 1985), it has been discovered that it is involved in modulating the functions of cortical neurons. Early voltage-clamp studies (Stafstrom et al. 1985) demonstrated that it contributes toward sustaining recurrent firing in response to membrane depolarization in layer V neurons, and subsequent experiments showed that it takes part in generating depolarizing afterpotentials (Azouz et al. 1996) and action potential (AP) bursts in cortical structures (Franceschetti et al. 1995; Kim and McCormick 1998; Mantegazza et al. 1998; Mattia et al. 1997; Mendez de la Prada et al. 2003). It also significantly modulates postsynaptic inputs to dendrite arborization (Mittmann et al. 1997; Poznanski and Bell 2000; Schwindt and Crill 1995) and contributes in generating subthreshold membrane oscillations in neuronal subsets of the hippocampus, subiculum, and both entorhinal and sensorimotor cortices (Agrawal et al. 2001; Amitai 1994; Hu et al. 2002). Finally, the peacemaking effect played by \(I_{\text{NaP}}\) in subthalamic neurons (Do and Bean 2003) and its contribution to setting the AP threshold for spontaneous discharges in tuberomammillary neurons (Taddese and Bean 2002) are further recent examples of its primary role in specific physiologival functions.

A pathological enhancement of \(I_{\text{NaP}}\) may occur in case of dysfunctional events involving cortical neurons, such as epileptic discharges in acquired (Agrawal et al. 2003; Vreugdenhil et al. 2004) or genetically determined Na\(^+\) channelopathies (Lossin et al. 2002). The enhancement of \(I_{\text{NaP}}\) resulting from ischemia (Ju et al. 1996) may lead to neuronal damage and, more generally, increased Na\(^+\) flux throughout long depolarizations may play a role in neurodegenerative processes (Vajda 2002). Because \(I_{\text{NaP}}\) plays a role in sustaining epileptic discharges and long membrane depolarizations, it is a recognized target of antiepileptic and neuroprotective drugs. A number of traditional and new anticonvulsants or anesthetics (Chao and Alzheimer 1995; Gebhardt et al. 2001; Spadoni et al. 2002; Taverna et al. 1999) can reduce the persistent fraction of the Na\(^+\) current often at concentrations that do not inhibit the transient fraction of the current \(I_{\text{Na}}\) (Lampl et al. 1998; Segal and Douglas 1997).

These and other findings seem to have sufficiently established the role of \(I_{\text{NaP}}\) in triggering and sustaining physiological and pathological depolarizing events. Its involvement in shaping and sustaining such events certainly arises from its particular activation characteristics. In fact, \(I_{\text{NaP}}\) begins to activate at rather negative membrane potentials that are close to the resting potential and below the firing threshold (French et al. 1990; Stafstrom et al. 1985). However, its inactivation properties can also play a crucial role in determining its functional effect on membrane excitability. The results of previous experiments using mouse layer V neurons indicate that \(I_{\text{NaP}}\) actually inactivates with a time constant of about 2 s (Fleidervish and Gutnick 1996). In the entorhinal cortex, an even longer time constant characterizes its kinetics of inactivation, assessed by single-channel recordings (Magistretti and
Alonso 1999), thus suggesting complex inactivation kinetics (Magistretti and Alonso 2002).

The aim of this study was to characterize the voltage dependency of activation and inactivation and the kinetics of inactivation of $I_{\text{NaP}}$ in sensorimotor neocortical slices and to assess whether different properties distinguish $I_{\text{NaP}}$ in layer V and layer II/III pyramidal neurons, which are endowed with different firing properties and different functional implications.

**METHODS**

**Slice preparation**

Sprague–Dawley rats (Charles River, Florence, Italy) aged 13–18 days were anesthetized with ether and decapitated. Their brains were removed and placed in ice-cold artificial cerebrospinal fluid (standard ACSF) with (in mM): 124 NaCl, 26.5 NaHCO3, 2 CaCl2, 1.25 NaH2PO4, 2 MgSO4, 3.5 KCl, 10 glucose, and bubbled with 95% O2-5% CO2; the electrodes were filled with a solution containing (in mM): 3 KCl, 102 NaCl, 5 MgCl2, 15 NaHCO3, 10 HEPES-NaOH, 10 glucose, 0.2 CaCl2, 0.3 NiCl2, 0.4 CdCl2, 30 tetraethylammonium-Cl (TEA-Cl), and 2 kynurenic acid, and bubbled with 95% O2-5% CO2.

Coronal slices of 300 μm were prepared from the sensorimotor cortex, transferred to a submersion chamber kept at 35°C, and perfused with ACSF (see following text). All of the experimental procedures were carried out in compliance with the 86/609/UE law on animal research and the guidelines for animal care and management of the Ethics Committee of C. Besta Institute.

**Electrophysiological recordings**

The whole cell patch-clamp recordings were made at 35°C using an Axopatch 200B amplifier (Axon Instruments, Union City, CA) in layers II/III and V. Pyramidal neurons were directly visualized in brain slices with infrared differential-interference contrast microscopy using an upright microscope (Zeiss Axioscope) and a CCD camera (Hamamatsu).

The neurons were bath perfused with an external solution containing (in mM): 3 KCl, 102 NaCl, 5 MgCl2, 15 NaHCO3, 10 HEPES-NaOH, 10 glucose, 0.2 CaCl2, 0.3 NiCl2, 0.4 CdCl2, 30 tetraethylammonium-Cl (TEA-Cl), and 2 kynurenic acid, and bubbled with 95% O2-5% CO2; the electrodes were filled with a solution containing (in mM) 132 CsCl, 2 MgCl2, 1 CaCl2, 10 HEPES, 10 EGTA-CsOH, 2 Na3ATP, 10 phosphocreatine-diTris, 0.3 Na-GTP, and 20 U/ml creatine phosphokinase, pH 7.2.

In some experiments, CsCl in the internal solution was substituted with KGluconate and slices were perfused with standard ACSF to evaluate physiological firing characteristics with current-clamp recordings before Ca2+ and K+ currents blockade and voltage-clamp recordings of $I_{\text{NaP}}$. In these experiments, we began the recording of $I_{\text{NaP}}$ after a long perfusion with extracellular blockers, when a satisfactory blockade of Ca2+ and K+ currents was achieved.

In control experiments, 1 μM tetrodotoxin (TTX) was added to the perfusing medium to rule out the contribution of TTX-resistant currents that could activate in the voltage range used to study $I_{\text{NaP}}$ kinetics.

The data were digitized using a Digidata 1320 interface (Axon Instruments); pClamp 8.0 software (Axon Instruments) was used to generate stimulus protocols and acquire the signals. After seal formation and cell membrane rupturing, capacitance currents were minimized using the amplifier circuitry and 70–80% series resistance compensation was routinely applied.

$I_{\text{NaP}}$ was evoked using slow (50 mV s−1) voltage ramps or, in control experiments, by means of depolarizing steps from a holding potential of −80 mV. Junction potential errors were not corrected. The sampling frequency was 5 kHz for the ramp protocols and current-clamp recordings and 10 kHz for the step protocol. The membrane currents were filtered at 1 kHz (voltage ramps) or 3 kHz (step protocol). The recordings with voltage-clamp errors (i.e., presence of unclamped action currents) were excluded from the analysis.

Conductance–voltage (g–V) relationships (activation curves) were calculated from the currents recorded using voltage ramps as $g = I_{\text{NaP}}/(V - E_{\text{Na}})$, where $I_{\text{NaP}}$ is the recorded Na+ current measured at potential $V$ and $E_{\text{Na}}$ is the calculated equilibrium potential. Normalized activation curves were fitted to Boltzmann relationships in the form: $G/G_{\text{max}} = 1/[1 + \exp(V_{1/2} - V/k)]$, where $G_{\text{max}}$ is the maximal peak conductance, $G$ is the peak conductance at each test voltage, $V_{1/2}$ is the voltage at which half-maximal activation is reached, and $k$ is the slope factor. The patch clamp data were analyzed using pClamp8 (Axon Instruments) and Origin 7.5 software (MicroCal, Northampton, MA).

The data, presented as means ± SE, were statistically analyzed using nonparametric (Wilcoxon or Mann–Whitney) or ANOVA tests. Fits were compared using the F test to evaluate statistically the number of exponentials needed for the best fit.

**RESULTS**

**Peak amplitude and characteristics of activation of $I_{\text{NaP}}$**

$I_{\text{NaP}}$ in both layer V and layer II/III pyramidal neurons was elicited using a ramp stimulus protocol (50 mV s−1), whereas step pulses were applied in control experiments aimed at evaluating the correspondence between the amplitude and activation properties of $I_{\text{NaP}}$ evoked by means of different stimulation protocols (Fig. 1, A and B). In both layers, $I_{\text{NaP}}$ started to activate at potentials slightly negative of −60 mV, and reached a broad peak at membrane potentials ranging from −33 and −30 mV. In the presence of 1 μM TTX, the ramp protocols evoked only a small outward current, which activated at more positive membrane potentials than $I_{\text{NaP}}$ (see Fig. 1A).

Boltzmann fitting of the activation curves showed similar properties in the two layers (Fig. 1, C and D, and Table 1), and the comparison of $I_{\text{NaP}}$ activation assessed on TTX-subtracted traces using slow voltage ramps and step protocols showed similar activation properties ($V_{1/2}$: −43.5 ± 0.4 mV and −43.2 ± 0.6; $k$: 4.1 ± 0.2 and 4.2 ± 0.1; $n = 6$).

---

**FIG. 1.** Activation of persistent sodium current ($I_{\text{NaP}}$). $I_{\text{NaP}}$ evoked by a slow ramp protocol (A) and squared depolarizing pulses (B) in a representative pyramidal neuron recorded in neocortical layer V. Voltage dependency of $I_{\text{NaP}}$ activation had similar properties in layers II/III (C) and V (D).
The average amplitude of the current peak was variable in layer V but significantly larger than that in layer II/III neurons ($P < 0.001$). The cell capacitance was also significantly larger in layer V ($P < 0.02$), and the current density assessed using the cell capacitance as an approximate measure of the membrane surface was also variable but significantly higher in layer V than in layer II/III ($P < 0.003$). This difference was not attributable to differences in age-dependent $I_{\text{NaP}}$ maturation (Alzheimer et al. 1993a) because the ages of rats from which we recorded layer V (15.6 ± 0.2 days) and II/III (15.8 ± 0.3 days) neurons were similar.

Voltage dependency of steady-state $I_{\text{NaP}}$ inactivation

We estimated the voltage dependency of $I_{\text{NaP}}$ inactivation by delivering voltage ramp commands at the end of a 10-s depolarizing pulse at voltages ranging from -90 to -10 mV (see Fig. 2C, inset). To avoid the accumulation of slow inactivation, inactivating pulses were delivered every 45 s. In layer V pyramidal neurons, substantial inactivation began with prepulse potentials that were more positive than those needed to inactivate $I_{\text{NaP}}$ in layer II/III. The current traces shown in Fig. 2, A and B exemplify the different voltage dependencies of inactivation in representative layer II/III and layer V pyramidal neurons. In the layer II/III neuron, the peak amplitude of the current evoked by a ramp stimulus delivered at the end of a 10-s conditioning prepulse to a membrane potential of -50 mV decreased by 25.9% (Fig. 2A). On the contrary, in the layer V neurons, the current obtained after a 10-s conditioning prepulse at -50 mV was substantially unaffected, almost overlapping the current trace obtained after a conditioning prepulse to -90 mV (Fig. 2B).

Boltzmann fitting of the mean normalized current amplitudes confirmed a significant difference in voltage dependencies of inactivation between the neurons of the two layers. In fact, the voltage dependency of inactivation was significantly shifted to the right (about 4.5 mV) in layer V (Fig. 2C; Table 1), and the slope factor reflected the significantly steeper inactivation curve (Table 1). On average, after a 10-s prepulse to -60 mV the inactivation was negligible or absent in layer V (4.9 ± 2.0%), but already substantial in layer II/III pyramidal neurons (15.6 ± 2.0% of the maximal current peak; $P < 0.002$).

In all of the pyramidal neurons of both layers V and II/III, a fraction of $I_{\text{NaP}}$ remained after prepulses to -10 mV membrane potential, which was on average slightly larger in layer V (33.4 ± 2.3% of the maximum unconditioned current peak amplitude) than in layer II/III (27.9 ± 1.1%), but the difference was not statistically significant. The $I_{\text{NaP}}$ amplitude also did not further decrease in the case of conditioning potentials to 0 mV (10 neurons, data not shown).

**Kinetics of $I_{\text{NaP}}$ development of inactivation and recovery from inactivation**

The time dependency of inactivation was evaluated by applying depolarizing prepulses to -10 mV of increasing duration (from 0 to 40 s) and eliciting $I_{\text{NaP}}$ by means of a standard voltage ramp (see stimulus protocol in Fig. 3A). In both layer II/III and layer V, the time dependencies of slow $I_{\text{NaP}}$ inactivation were fitted by biexponential functions in the form $y = y_0 + A_1 \exp[-(x-x_0)t_1] + A_2 \exp[-(x-x_0)t_2]$.

**Table 1. $I_{\text{NaP}}$ activation and inactivation parameters in layer II/III and V pyramidal neurons**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Layer II/III</th>
<th>Layer V</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{NaP}}$ peak amplitude (pA)</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td>Cell capacitance (pF)</td>
<td>76.4 ± 3.2</td>
<td>87.4 ± 3.1*</td>
</tr>
<tr>
<td>Current density (pA/pF)</td>
<td>4.0 ± 0.3</td>
<td>5.9 ± 0.4**</td>
</tr>
<tr>
<td>$V_{\text{th}},$ mV</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>$k$</td>
<td>43.1 ± 0.3</td>
<td>44.2 ± 0.6</td>
</tr>
<tr>
<td>$V_{\text{th}},$ mV</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>$k$</td>
<td>-42.3 ± 1.1</td>
<td>-46.8 ± 1.6*</td>
</tr>
<tr>
<td>$\tau_1,$ ms</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>$\tau_2,$ ms</td>
<td>543.1 ± 411.3</td>
<td>7249.1 ± 615.2 (P = 0.065)</td>
</tr>
<tr>
<td>$\tau_3,$ ms</td>
<td>537.5 ± 104.9</td>
<td>417.6 ± 71.2 (P = 0.420)</td>
</tr>
<tr>
<td>$\tau_4,$ ms</td>
<td>4987.2 ± 600.7</td>
<td>4421.2 ± 259.4 (P = 0.477)</td>
</tr>
</tbody>
</table>

Values are means ± SE. *$P < 0.05$; **$P < 0.01$.
The faster time constant measured in layer V varied widely from 158.0 to 1133.8 ms. On average (425.9 ± 80.5 ms) it was significantly longer than in layer II/III (145.8 ± 18.2 ms; range 63–157; P < 0.003). The second time constant was variable but on average of about 5,000 ms, without any significant differences between the two layers. The curve of development of \( I_{\text{NaP}} \) inactivation reached a plateau with prepulses lasting >25 s. In fact, a small current remained in all of the neurons even after the longest inactivation prepulses (corresponding to 13–26% of the unconditioned current peak), without any difference between the two layers.

Figure 3B magnifies the divergence of the time course of the early component of slow inactivation in layers II/III and V by showing the two curves displayed in Fig. 3A on a linear scale and for prepulses ranging from 1 to 2,000 ms. The inset shows, with a box chart plot, the more variable values of the early time constant in layer V with respect to layer II/III. Figure 3C shows the current traces obtained after 200-ms, 1-s, and 40-s conditioning prepulses to −10 mV in two representative layer V and layer II/III pyramidal neurons. As a consequence of the slower development of inactivation, in the layer V neuron the \( I_{\text{NaP}} \) evoked after the 200-ms conditioning prepulse was only slightly lower than the unconditioned current amplitude (mean 16.2 ± 2.5%; \( n = 11 \)), whereas it was clearly reduced in the layer II/III neuron (mean 27.2 ± 4.1%; \( n = 6 \); \( P < 0.03 \)).

To verify the absence of unblocked currents possibly contaminating the traces, we evaluated the time-dependent inactivation properties in three layer V neurons in the presence of TTX. As shown in Fig. 3D, the time course of \( I_{\text{NaP}} \) inactivation measured on the original and TTX-subtracted traces completely overlapped, thus ruling out any effect of contaminating currents.

To evaluate the contribution of \( I_{\text{NaP}} \) to the depolarized plateau after APs observed when Ca\(^{2+} \) and K\(^+ \) currents are blocked (Franceschetti et al. 1995; Stafstrom et al. 1985), we did current-clamp experiments with the solutions used for the voltage-clamp recordings of \( I_{\text{NaP}} \) in nine neurons: three layer II/III and six layer V pyramidal neurons. In all layer II/III neurons, the depolarizing plateau did not exceed 100 ms, whereas extremely long depolarizations lasting >1 s consistently followed the AP evoked in three of six layer V neurons. The remaining three layer V pyramidal neurons showed depolarized plateaus lasting from 22 to 220 ms. We analyzed the decay of these long depolarizing plateaus, as shown in Fig. 4A for a representative layer V pyramidal neuron, and compared it with the time course of development of inactivation of the \( I_{\text{NaP}} \) recorded in the same neuron (Fig. 4B). Both decays could be fitted using a biexponential function with a similar fast time constant (\( \tau_1 \) values of 552.4 and 490.4 ms, respectively). The slow time constant of the voltage plateau decay was shorter (\( \tau_2 = 4,252.8 \) ms) than that of development of \( I_{\text{NaP}} \) inactivation (9,155.6 ms), probably because of the abrupt end (about 7 s from its onset) of the AP shoulder. We did not investigate the mechanism of the abrupt end of the depolarizing plateau, which is frequently observed in cells recorded under similar experimental conditions and may have arisen from residual unblocked voltage-dependent hyperpolarizing currents or K\(^+ \) currents activated by persistent Na\(^+ \) entry into the cell.

The time course of \( I_{\text{NaP}} \) recovery from inactivation was determined using the protocol shown in Fig. 5A. The voltage protocols included a 20-s prepulse at −10 mV, followed by a recovery period at −80 mV varying from 1 ms to 40 s, and by the standard voltage ramp to elicit \( I_{\text{NaP}} \). As in the case of the development of slow inactivation, the time course of recovery from inactivation could be best fitted using biexponential functions (Table 1). However, we did not find any difference between the curves describing the recovery from inactivation in the two neocortical layers. Figure 5B shows the current traces obtained after 1 ms and after 1-, 10-, and 40-s-long recovery periods at −80 mV in two representative layer V and layer II/III neurons.
Time-dependent $I_{	ext{NaP}}$ inactivation in pyramidal neurons previously classified according to their firing characteristics

With the aim of directly assessing the relationship between $I_{	ext{NaP}}$ properties and different physiological firing characteristics, in some pyramidal neurons we recorded in sequence with a KGluconate-based solution, the physiological firing (in standard ACSF), the firing with perfusion of Ca$^{2+}$/H11001 and K$^{+}$/H11001 channel blockers and, switching to voltage clamp, the properties of $I_{	ext{NaP}}$.

In response to the injection of depolarizing pulses, all neurons recorded in layer II/III ($n = 5$) discharged with individual APs, each followed by postexcitatory hyperpolarization and showing a more or less robust firing frequency adaptation (Fig. 6A). Three of the seven neurons recorded in layer V showed a firing behavior similar to that of layer II/III neurons (Fig. 6B). Four pyramidal layer V neurons discharged with nonadapting individual APs, each followed by a prominent depolarized potential (Fig. 6C, arrow), that were preceded by an early burst in two neurons. In these neurons, the AP bursts also appeared as an all-or-none response to short depolarizing stimuli threshold. During the perfusion of Ca$^{2+}$ and K$^{+}$ channel blockers recurrent firing disappeared and a depolarized plateau after an early AP was evident. The depolarized plateau had a shorter duration in adapting regular-spiking pyramidal neurons of both layer II/III and layer V (Fig. 6A), but exceeded 700 ms in both intrinsically bursting and nonadapting layer V pyramidal neurons (Fig. 6C). In all of the neurons, the curve of the development of inactivation of $I_{	ext{NaP}}$ followed a biexponential decay. The values of the early time constant measured in layer II/III pyramidal neurons was similar to that assessed in neurons recorded directly in voltage-clamp configuration (between 115.0 and 268.3 ms, $n = 3$). In layer V neurons, the early time constant was substantially slower in intrinsically bursting and nonadapting neurons (between 662.6 and 1,256.3 ms; $n = 4$), whereas in adapting regular-spiking neurons it showed intermediate values (328.3–332.8 ms; $n = 2$).

**DISCUSSION**

Our results show that $I_{	ext{NaP}}$ current density and inactivation characteristics are uneven in neocortical layers II/III and V of rat sensorimotor cortex, and this may significantly contribute toward modulating the particular firing properties characterizing different neocortical pyramidal neurons.

In the neocortex, as in other structures, the basic characteristics of $I_{	ext{NaP}}$ are its low threshold of activation and its persistence. The low threshold of activation allows $I_{	ext{NaP}}$ to exert its depolarizing effect at potentials that are more negative than firing levels, thus amplifying low depolarizing inputs such as small excitatory postsynaptic potentials (Schwindt and Crill 1995). The persistence of the current over time allows $I_{	ext{NaP}}$ to maintain its contribution during long depolarizations, sustaining recurrent neuronal firing (Crill 1996; Do and Bean 2003; Stafstrom et al. 1985) or long plateau potentials in hyperexcitable neurons (Bikson et al. 2003; Mantegazza et al. 1998; Segal and Douglas 1997). However, $I_{	ext{NaP}}$ undergoes a slow inactivation process that may critically define its ultimate effect on membrane excitability (Fleidervish and Gutnick 1996; French...
Differential $I_{\text{NaP}}$ properties in pyramidal neurons of layer II/III and V of the sensorimotor cortex

On the basis of findings obtained in cortical neurons (Alzheimer et al. 1993b; Brown et al. 1994), most studies suggest that $I_{\text{NaP}}$ is generated by a fraction of Na$^+$ channels that escape the fast transition to a nonconductive state affecting most activated Na$^+$ channels. In agreement with this assumption, a small channel fraction remains capable of late openings in the “persistent” mode despite sustained membrane depolarizations. Our data indicate that the characteristics of $I_{\text{NaP}}$ activation in both layers II/III and V of rat sensorimotor cortex are similar to those previously described in dissociated cortical neurons (Alzheimer et al. 1993b; Brown et al. 1994; Franceschetti et al. 2000; Taverna et al. 1999), neocortical slices (Pleidervish and Gutnick 1996), hippocampus (French et al. 1990), and entorhinal cortex (Magistretti and Alonso 1999), by activating at potentials that are a few millivolts more positive than the spontaneous resting potential. This finding suggests that in all these brain structures $I_{\text{NaP}}$ plays a similar role in increasing subthreshold membrane excitability, thus facilitating neuronal AP generation. In our experiments, one significant difference between the neocortical layers was the amplitude of the current, which was larger in layer V than that in layer II/III. This difference could be attributed to a different cell size, although the current density calculated on the basis of the cell capacitance as an approximate measure of the membrane surface was also greater in layer V than that in layer II/III. Because of the different voltage dependencies of $I_{\text{NaP}}$ inactivation in the two layers, at the holding potential of $-70 \text{ mV}$ used to study the activation properties, $I_{\text{NaP}}$ is partially inactivated in layer II/III neurons (by $8.8 \pm 1.6\%$; Fig. 2C). Thus we also compared the data applying a correcting factor for the recordings made in layer II/III, but the difference maintained the statistic significance. The larger current amplitude in layer V may well account for the particular firing properties and threshold of excitability of the pyramidal neurons in this layer. In fact, in rat somatosensory cortex, a considerable percentage of layer V neurons typically generate high-frequency bursts of APs that arise from a prominent subthreshold depolarization and that are produced in an all-or-none fashion in response to very small membrane depolarization (Connors and Gutnick 1990).

Unlike activation, $I_{\text{NaP}}$ inactivation properties were significantly different in the pyramidal neurons of the two neocortical layers.
layers. Moreover, the parameters describing both the voltage and the time dependencies of inactivation were different from those previously reported in other neuronal populations. This undoubtedly partially depends on the applied stimulus protocols (i.e., the length of the conditioning pulses and imposed membrane depolarization), although some evidences suggest that different inactivation kinetics may differentiate the \( I_{NaP} \) recorded in different structures.

In the entorhinal cortex, \( I_{NaP} \) completely inactivates after a 15-s conditioning depolarization at a membrane potential of \(-10\) mV (Magistretti and Alonso 1999) and is half-inactivated at about \(-49\) mV. Our findings in neocortical neurons showed that \( I_{NaP} \) reached its inactivation midpoint at a more depolarized potential in layer V (about \(-42\) mV), thus suggesting that a significant current fraction remains available for sustaining neuronal excitability at rather depolarized membrane potentials. Moreover, a current fraction corresponding to about 25% of maximum \( I_{NaP} \) amplitude did not inactivate despite long conditioning prepulses to 0 mV. A similar fraction also remained at the end of the longest conditioning pulses we used when evaluating the development of slow inactivation (40 s at \(-10\) mV), which suggests that a fraction of \( I_{NaP} \) in neocortical neurons is a real “persistent current” and does not obviously inactivate regardless of the extent and duration of the conditioning depolarization.

Courses of time-dependent slow \( I_{NaP} \) inactivation and recovery from inactivation were fitted by biexponential functions in all of the neurons, thus further indicating that \( I_{NaP} \) inactivation occurs as complex transitions in sensorimotor cortex. Our inactivating protocol included more data points and inactivating prepulses with very long durations; this probably accounts for the difference between our results and those previously obtained in sensorimotor cortex (Fleidervish and Gutnick 1996) that showed a time-dependent decay well fitted by a simple exponential function. However, development of \( I_{NaP} \) slow inactivation is also well fitted by a single exponential function in entorhinal cortex (Magistretti and Alonso 1999), despite the use of a stimulus protocol that was quite similar to that used in our experiments. The presence in entorhinal cortex of a peculiar Na\(^+\) channel subtype that opens in the persistent mode only (Magistretti and Alonso 2002) may explain the different inactivating behavior of entorhinal neurons with respect to the sensorimotor ones. In neocortical layers, the expression of channel subunit isoforms is uneven (Gong et al. 1999; Whitaker et al. 2000) and the level of persistent current that they generate may be modulated (Mantegazza et al. 2005); this may account for the differences that we observed in slow inactivation in layers II/III and V, without the need to hypothesize the presence of novel and specific channel subtypes opening in persistent mode only.

Our experiments were designed to characterize \( I_{NaP} \) inactivation and compare its time course in different neocortical layers, but we did not investigate the similar process affecting \( I_{NaT} \). However, it is well known that \( I_{NaT} \) also undergoes slow inactivation processes similar to those we studied for \( I_{NaP} \) (see Goldin 2003 for a review). Slow inactivation probably arises from conformational channel changes (mainly involving the channel pore), which substantially differ from those of fast inactivation and are likely to involve Na\(^+\) channels regardless of their “transient” or “persistent” opening. Slow Na\(^+\) channel inactivation modulates firing properties and oscillatory activities and seems to occur with different kinetics depending on channel location (i.e., cell soma versus dendrites) or molecular characteristics of channel subunits. Interestingly, abnormalities in slow Na\(^+\) channel inactivation in skeletal muscle and cardiac Na\(^+\) channels account for genetically determined human diseases, including hyperkalemic periodic paralysis (Bendahhou et al. 2002; Hayward et al. 1999) and Brugada syndrome (Veldkamp et al. 2000). Moreover, defective sodium channel slow inactivation may contribute to the inherited neuronal dysfunctions leading to epileptic disorders (Lossin et al. 2002; Spampanato et al. 2001). These observations underline the importance of slow inactivation processes and their specific kinetic properties in the neocortex, which may contribute to the specific ability of neocortical subpopulations to sustain neuronal synchronization and epileptogenesis (Chagnac-Amitai and Connors 1989).

In our experiments, we made whole cell recordings from the cell soma and we cannot exclude the possibility of a special contribution of dendritic Na\(^+\) channels to the \( I_{NaP} \) recorded in layer V. Moreover, neurons in this layer are morphologically and electrotonically more complex than those in layer II/III, and this in theory may bias the measurements. However, the properties of the activation and of the recovery from inactivation of \( I_{NaP} \) in the two layers are almost identical. Thus the differences observed in the other characteristics can be considered as specific features of \( I_{NaP} \) in different layers that can have considerable effects on the excitable properties of the superficial versus deep neocortical layers.

**Physiological significance of the different properties of \( I_{NaP} \) in layers II/III and V**

Pyramidal neurons are the principal elements of neocortical circuitry because they are recipients of the input system, the source of local excitatory circuits, and the sole output of the neocortex. The fine modulation of their firing properties is therefore fundamental to elaborating inputs and generating appropriate outputs.

Pyramidal cells can generate a series of individual APs in all neocortical layers of mammals, whereas a large subset of layer V pyramidal cells discharge with a burst of APs, which can be followed by recurrent bursts or by individual APs with a prominent depolarized afterpotential. Moreover, an intermediate class of layer V regular-spiking neurons discharge in response to both threshold and suprathreshold depolarizations with individual APs each followed by a depolarized after potential (\( R_{SAP} \)) according to the definition of Tseng and Prince 1993). These two neuronal phenotypes share similar morphological characteristics that are already visible in young rats (Franceschetti et al. 1998; Kasper et al. 1994) and contribute with their rhythmic firing behavior to intracortical synchronization (Silva et al. 1991). In both these neurons, the extremely long (TTX-sensitive) plateau potential observed in the presence of K\(^+\) and Ca\(^{2+}\) channel blockers (Franceschetti et al. 1995; Staffstrom et al. 1985) is consistent with the especially high contribution of \( I_{NaP} \) in sustaining their firing behavior. Most layer II/III pyramidal neurons and a subclass of layer V pyramidal neurons conversely discharge with individual APs not followed by a depolarizing afterpotential, and show fire frequency adaptation.
Differences in $I_{NaP}$ amplitude and kinetics may dramatically influence the firing properties of these different neuronal subtypes, and the more heterogeneous neuronal population in layer V with respect to layer II/III may explain the more heterogeneous values of $I_{NaP}$ amplitude and inactivation kinetics that we measured in neurons of this layer. Accordingly, control experiments that we performed to evaluate the firing properties, before evaluating the time course of $I_{NaP}$ inactivation, confirmed the presence of a slower early inactivation time constant in layer V intrinsically bursting and nonadapting RS neurons, with respect to adapting RS neurons of layer V and layer II/III pyramidal neurons.

The APs forming a burst arise from depolarization envelopes that reach a plateau at a membrane potential of about $-50$ mV, roughly corresponding to the firing level (Franceschetti et al. 1998). On the basis of our evaluation of the voltage dependence of inactivation in layer V, $I_{NaP}$ amplitude is just slightly affected by membrane depolarizations at $-50$ mV, and this may suitably maintain the plateau potential from which the AP bursts arise. The voltage- and time-dependent properties of $I_{NaP}$ inactivation may also contribute to maintaining the burst recurrence or the rhythmic (nonadapting) recurrence of AP characterizing both intrinsically bursting and nonadapting regular-spiking neurons. Conversely, the same mechanism may play a subsidiary role in the adaptation of regular-spiking neurons, which have a quite high firing frequency at the onset of membrane depolarization that quickly declines because of the strong adapting effect of various voltage-dependent and Ca$^{2+}$-activated K$^+$ currents (Sah and Davies 2000).

The level and duration of membrane depolarization that we imposed during conditioning pulses (many seconds) used to evaluate the kinetics of slow inactivation of $I_{NaP}$ largely exceed the range of the real depolarizations expected to occur physiologically in neocortical neurons. However, large and long-lasting depolarizations do occur in the case of pathological events such as epileptic discharges that lead to extreme firing frequencies or long depolarizing plateaus. In this case, the whole range of $I_{Na}$ inactivation kinetics becomes important in determining the characteristics and duration of such “paroxysmal” events. $I_{NaP}$ plays an important role in sustaining interictal and ictal epileptic events in various experimental preparations (Bikson et al. 2003; Segal and Douglas 1997; Timofeev et al. 2002, 2004), and similar mechanisms may occur in genetically determined (Lossin et al. 2002, 2004), and similar mechanisms may occur in genetically determined (Lossin et al. 2002) or acquired epilepsies (Vreugdenhil et al. 2004). The extremely long Na$^+$-dependent depolarizing plateau generated by layer V neurons after Ca$^{2+}$ and K$^+$ current blockade, which we found in the present and previous experiments (Franceschetti et al. 1995, 2000; Taverna et al. 1999), may be an example of a high degree of Na$^+$-dependent hyperexcitability. The decay of these plateau potentials was best-fitted by a biexponential function, and exactly followed the kinetic properties of $I_{NaP}$ slow inactivation assessed in the same neurons. Therefore an especially Na$^+$-dependent excitability may account for the greater likelihood of epileptic discharges to originate from layer V neurons (Chagnac-Amitai and Connors 1989; Connors 1984; Hoffman et al. 1994).

**REFERENCES**


