How cesium dialysis affects the passive properties of pyramidal neurons: implications for voltage clamp studies of persistent sodium current

Ilya A Fleidervish\(^1\) and Lior Libman

Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, PO Box 12, Rehovot 76100, Israel
E-mail: fleider@agri.huji.ac.il

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**Abstract.** In order to accurately understand and model neuronal integration in the brain, we must know the biophysical properties of channels that are located far from the soma, in the axonal and dendritic membranes of central nerve cells. Reliable electrophysiological measurements in these regions are difficult to obtain, because the processes are too tiny to directly study with an electrode. One common strategy is to record with a somatic electrode that contains Cs\(^+\), to dialyze the intracellular space with this K\(^+\) channel blocker, and thereby to render the neuron electrotonically compact. Does this work? Here, we combine the experimental and modeling techniques to determine the extent to which a whole-cell voltage clamp, established with a Cs\(^+\)-containing pipette in the soma of a cortical pyramidal cell, attains adequate voltage control of distal neuronal processes. We focus on the low-voltage-activated, slowly inactivating ‘persistent’ Na\(^+\) current (\(I_{\text{NaP}}\)), that plays a crucial role in determining neuronal excitability and synaptic integration.

\(^1\) Author to whom any correspondence should be addressed.
1. Introduction

Somas, dendrites and axons of pyramidal neurons contain a non-homogeneously distributed mixture of molecularly distinct voltage-gated Na\(^+\) channels (Migliore and Shepherd 2002). Fast-inactivating, transient current through these channels plays a central role in initiation and propagation of action potentials (Hodgkin and Huxley 1952; Stuart and Sakmann 1994). In addition, there is a more slowly inactivating, tetrodotoxin (TTX)-sensitive, persistent Na\(^+\) current (\(I_{\text{NaP}}\)) (Chandler and Meves 1966; Crill 1996; Gutnick and Crill 1995), whose functional importance relates to dynamic control of the ‘gain’ (Crill 1996; Vervaeke et al 2006) of the neuronal input–output relationship. \(I_{\text{NaP}}\) operates in the subthreshold voltage range, where other large, voltage-gated conductances are not active (Crill 1996). It is thus ideally poised to influence both the synaptic integration properties of a neuron and the character of its spike output. A precise understanding of the role of \(I_{\text{NaP}}\) requires knowledge of the distribution and the biophysical properties of the ion channels that generate it.

The usual technical approach to study \(I_{\text{NaP}}\) in intact neurons is by whole cell voltage clamp, applying slowly rising or declining voltage ramps via a somatic patch pipette (Alzheimer et al 1993; Astman et al 2006; Fleidervish et al 1996; French et al 1990). The resultant instantaneous current–voltage curve reflects \(I_{\text{NaP}}\) activation since, if the ramp’s rising rate is slow enough, the transient Na\(^+\) current will be entirely inactivated. However, because the elaborate dendritic and axonal arbors which are preserved in the slice preparation may be sources of \(I_{\text{NaP}}\), recordings from a single electrode voltage clamp at the soma may be subject to serious error (Muller and Lux 1993; White et al 1995). A common strategy for improving the quality of voltage control is dialyzing cells with Cs\(^+\) ions, which block K\(^+\) channels and thereby enhance membrane resistivity (Coetzee et al 1999; Spruston et al 1993). Theoretical analysis (Spruston et al 1993) of the attenuation of voltage signals in a neuron dialyzed with Cs\(^+\) predicted satisfactory control (~98%) of near-dc (but not transient) voltage at a distance of up to 1000 \(\mu\text{m}\) from the somatic voltage-clamping pipette. This prediction only holds if membrane resistivity is uniformly high all along the processes. Cs\(^+\) diffusion into the dendrites and axon from a point source in the soma, however, is a slow process. One of the aims of the present study was to elucidate to what extent and how quickly optimal voltage clamp conditions are actually achieved in the case of focal dialysis of a pyramidal neuron with Cs\(^+\) ions via the somatic pipette.

The study by Spruston et al (1993) assumed that the leak conductance of the membrane, which remains after Cs\(^+\) blockade, has a reversal potential near resting potential. However, in addition to its desired action on membrane resistivity, Cs\(^+\) ions have also been shown to depolarize the membrane in various neuronal preparations (Adelman and Senft 1966; Araki...
et al 1962; Chandler and Meves 1965). In the perfused squid giant axon, the depolarization that ensues when Cs$^+$ is added to the perfusate is quite rapid (Adelman and Senft 1966). What are the dynamics of Cs$^+$-induced depolarization in central pyramidal neurons? Are the long cell processes isopotential? What are the practical implications of these complex changes in passive properties for $I_{NaP}$ voltage clamp experiments? The present investigation was designed to explore these questions using whole cell current clamp recording in rat cortical slices and compartmental computer simulations.

2. Methods

2.1. Patch-clamp recording

Experiments were performed in coronal slices of somatosensory cortex of P14–P30 Wistar rats, using procedures that are standard in our laboratory (Fleidervish et al 1996; Fleidervish et al 1998). Animals of either sex were deeply anesthetized with Nembutal (60 mg kg$^{-1}$), killed by decapitation, and their brains were rapidly removed and placed in cold (6 °C), oxygenated (95% O$_2$–5% CO$_2$) artificial cerebrospinal fluid (aCSF). Coronal slices (300 µm) from a region corresponding to the primary somatosensory cortex were cut on a vibratome (Series 1000, Pelco International, Redding, Canada) and placed in a holding chamber containing aCSF at room temperature; they were transferred to a recording chamber after more than 1 h of incubation. Whole-cell recordings from layer 5 neurons were made under IR-DIC microscopic control (Stuart et al 1993). Slices were held submerged in a chamber on the fixed stage of an Axioskop FS microscope (Carl Zeiss, Oberkochen, Germany). Current-clamp voltage recordings were obtained using an Axoclamp-2B amplifier in bridge mode. Patch pipettes were manufactured from thick-walled borosilicate glass capillaries (1.5 mm o.d., Hilgenberg, Germany) and had resistances of 2.5–3.5 MΩ. Command voltage protocols were generated and data were acquired on-line with a Digidata 1320A A/D interface. Data were low-pass filtered at 10 kHz (−3dB, single-pole Bessel filter) and digitized at 20 kHz. All recordings were made at 36 ± 1 °C.

Apparent cell capacitance and input resistance were estimated by applying small hyperpolarizing pulses and determining the time constant and the amplitude of the resulting voltage deflection.

The aCSF contained (mM): NaCl 124; KCl 3; CaCl$_2$ 2; MgSO$_4$ 2; NaH$_2$PO$_4$ 1.25; NaHCO$_3$ 26; glucose 10 (pH 7.3 at 37 °C when bubbled with a 95% O$_2$–5% CO$_2$ mixture). The pipette solution for whole-cell current clamp experiments contained (mM): CsCl 135; MgCl$_2$ 2; Heps (cesium salt) 10 (pH 7.25). All chemicals were obtained from Sigma.

2.2. Simulations

Numerical simulations were performed in the NEURON simulation environment (Hines and Carnevale 1997). The compartmental model of a layer 5 pyramidal neuron was based on a detailed morphological reconstruction available in the literature (Ascoli 2006; Mainen et al 1995; see http://NeuroMorpho.Org). The neuron contained 261 compartments; to enhance the spatial resolution, each of these compartments was subdivided into ten equal segments. Passive parameters were $R_i = 125$ Ộ cm, $C_m = 1$ μF cm$^{-2}$. Leak conductance ($g_L = 2.4–240$ μS cm$^{-2}$) reversed at 0 mV and was distributed uniformly. The channel density was $g_{NaP} = 0.24$ nS cm$^{-2}$, $g_{K(DR)} = 24–2400$ μS cm$^{-2}$. Equilibrium potential for Na$^+$ ions was set.
Figure 1. Effect of Cs\(^+\) dialysis on resting membrane potential. Resting membrane potential of six individual layer 5 neurons after establishment of whole cell configuration with Cs\(^+\)-containing pipette (time 0). Note that immediately following break-in membrane potential is negative; the neurons depolarize almost completely over 3–10 min of Cs\(^+\) dialysis.

Diffusion of Cs\(^+\) was modeled as the exchange of Cs\(^+\) ions between the patch pipette and the soma and then between adjacent neuronal compartments (Hines and Carnevale 2000) assuming a diffusion coefficient of 0.9 \(\mu\)m\(^2\) ms\(^{-1}\) (Neil et al 1996). Blockade of K\(^+\) conductance by Cs\(^+\) ions was simulated as

\[
g_{K}^{DR}(\lbrack\text{Cs}^+\rbrack_i) = g_{K}^{DR}o / (1 + 10^{-\lbrack\lbrack\text{Cs}^+\rbrack_i - 2\rbrack0.3}),
\]

assuming that half-block is achieved at 2 mmol l\(^{-1}\) of Cs\(^+\) (Coetzee et al 1999).

3. Results

Figure 1 shows the resting membrane potential \((V_r)\) of six layer 5 pyramidal neurons recorded with Cs\(^+\)-filled pipettes in the presence of TTX (1 \(\mu\)M) and Cd\(^{2+}\) (200 \(\mu\)M). During the course of dialysis with Cs\(^+\), \(V_r\) depolarized from \(-55 \pm 6\) mV at the time of break-in to a steady state value of \(-12 \pm 9\) mV \((n = 6\) cells, mean \(\pm\) SD) by 3–12 min. The depolarization was accompanied by a parallel increase in apparent input resistance from 181 \pm 94 M\(\Omega\) to 1.4 \pm 0.4 G\(\Omega\) and membrane time constant from 14 \pm 4 to 42 \pm 6 ms \((n = 6)\). It is noteworthy that even at the time of break-in with the Cs\(^+\)-containing electrode, \(V_r\) was significantly more depolarized than we usually see in slices (Fleidervish et al 1998). This indicates that some depolarization commences at once, such that it is already apparent by the time of the first measurement, only a second or two after membrane rupture.
Figure 2. Compartmental simulation of Cs⁺ dialysis. (A) Reconstructed layer 5 pyramidal neuron (Ascoli 2006; Mainen et al 1995) (top) with the sketch of pipette-cell assembly (bottom). Cs⁺ concentration was kept constant in the most distant section of the pipette. [Cs⁺], was measured in the soma (black), in the axon (red, 195 µm from soma), in the basal dendrite (blue, 187 µm from soma) and in the distal apical dendrite (green, 601 µm from soma). (B) The time course of Cs⁺ diffusion as a function of time (left) and space over apical dendrite–soma (dashed line)–axon axes (right). (C) The effect of varying the series resistance on the time course of Cs⁺ diffusion as a function of time (left) and space (right).

For a small round cell, the time constant of equilibration of a substance between pipette and cell interior is proportional to the cell volume and to the pipette resistance and inversely proportional to the diffusion coefficient of this substance (Pusch and Neher 1988; Marty and Neher 1995). This analytical solution, however is not applicable to layer 5 pyramidal neurons because of their complex geometry. The rate of Cs⁺ ions diffusion was therefore determined in a compartmental model of a reconstructed neuron (figures 2(A) and (B)) (Ascoli 2006; Mainen et al 1995; see http://NeuroMorpho.Org). Simulated patch pipette was divided into ten 10 µm-long, frustum-shaped compartments (figure 2(A)); their geometry was adjusted to produce a desired value of series resistance (Rₛ), assuming an effective pipette solution resistivity of 240 Ω cm (Pusch and Neher 1988). Cs⁺ concentration was ‘clamped’ to 150 mmol l⁻¹ in the most distal compartment representing the pipette bulk (figure 2(A)). Following break-in (t = 0), Cs⁺ concentration in the rest of the pipette compartments, initially set equal to that in the bulk, dropped rapidly due to diffusion of Cs⁺ ions into the soma via the pipette tip.

The lowest practically achievable series resistance in the patch-in-slice whole cell recordings is ∼3 MΩ (Alzheimer et al 1993; Astman et al 2006; Fleidervish et al 1996; French et al 1990). A model of whole cell somatic recording with pipette resistance set to 3 MΩ (figure 2(B)) predicts that [Cs⁺]ᵢ in the soma, basal dendritic tree and proximal axon will rise with a time constant of ∼2 min. In the model, Cs⁺ concentration in the distal part of the apical
dendrite began to rise with a lag of 30–60 s, and by the third minute after break-in, it reached values between 25 and 60 mmol l\(^{-1}\) (figure 2(B)). The rate of Cs\(^+\) dialysis was significantly slower when the pipette resistance was higher than 3 M\(\Omega\) (figure 2(C)). Thus, for pipettes with resistance of 10, 30 and 100 M\(\Omega\) the time constants of somatic [Cs\(^+\)]\(_i\) elevation were \(\sim 4\), \(\sim 18\) and \(\sim 55\) min, respectively. Within 3 min after break-in, somatic [Cs\(^+\)]\(_i\) reached values of 117, 60, 26 and 8 mmol l\(^{-1}\) for pipettes with \(R_s\) of 3, 10, 30 and 100 M\(\Omega\), respectively (figure 2(C), left). The spatial differences in Cs\(^+\) concentration at the third minute were greatest when the pipette resistance was low (figure 2(C), right). At the highest pipette resistance tested (100 M\(\Omega\), figure 2(C), right), the rise in [Cs\(^+\)]\(_i\) everywhere in the neuron was slow, but its time course was not different among different neuronal compartments. It is noteworthy that even at the lowest \(R_s\) tested (3 M\(\Omega\)), an almost complete equilibrium of Cs\(^+\) ions between a pyramidal neuron and a patch pipette was reached \(\sim 40\) min after the establishment of whole cell configuration.

We then determined to what extent and at what rate neuronal K\(^+\) channels in the various compartments are blocked by diffusing Cs\(^+\) ions, assuming that Cs\(^+\) blocks all K\(^+\) channels with a \(K_D\) of 2 mmol l\(^{-1}\) (Coetzee et al 1999). Figure 3(A), left shows that in recording with \(R_s\) of 3 M\(\Omega\) the K\(^+\) channels in the basal dendrites and in the axon were completely blocked within

**Figure 3.** Compartmental simulation of the effect of Cs\(^+\) dialysis on \(g_K\). (A) The time course of block of \(g_K\) as a function of time (left) and space (right) when series resistance is low (\(R_s = 3\) M\(\Omega\)). (B) The effect of varying the series resistance on the time course of [Cs\(^+\)]\(_i\) in the soma as a function of time (left) and space over apical dendrite–soma–axon axes (right) 3 min after the break-in.
15–20 s after break-in, while in the distal apical dendrite, the effect of the Cs⁺ started with a lag of 15–25 s. By 3 min, Cs⁺ redistribution was sufficient to block K⁺ conductance uniformly throughout the neuron’s membrane (figure 3(A), right). Figure 3(B), left shows the effect of variation of $R_s$ on the rate of Cs⁺ block of the somatic K⁺ channels. The times to 90% blockade were 3.2, 6.7, 17 and 68 s for $R_s$ of 3, 10, 30 and 100 MΩ, respectively (figure 3(B), left). Within the 3 min after break-in, $g_K$ was almost completely blocked in all neuronal processes if $R_s$ was $<10$ MΩ (figure 3(B), right). At $R_s = 30$ MΩ, 20–25% of the K⁺ channels in the apical dendritic tuft were not blocked (figure 3(B), right). In simulation with 100 MΩ pipette, at third minute after the break-in the non-blocked K⁺ channels persisted in all the distal half of the apical dendritic tree (figure 3(B), right); the 90% blockade in the most distal portion of the apical tuft was achieved at ~7 min after the break-in.

In actual neurons, many different intrinsic and synaptic conductances combine to determine $V_i$. For simplicity, however, our simulations set $V_i$ by assuming a non-specific leakage conductance ($g_L = 0$ mS) and a partially activated K⁺ conductance ($g_K = -80$ mS). Figure 4(A), left shows the effect of Cs⁺ diffusion on $V_i$ in an electrotonically compact neuron ($g_L = 2.4 \mu S \text{ cm}^{-2}$ and $g_K = 24 \mu S \text{ cm}^{-2}$) with passive membrane characteristics similar to those experimentally determined for pyramidal cells in brain slices and $R_s = 3$ MΩ. The time course of the change in membrane potential was complex, reflecting the complicated time course of Cs⁺ diffusion, Cs⁺ interaction with K⁺ channels, and electrotonic interaction between the parts of the neuron with different degrees of K⁺ channel blockade. It is noteworthy (figure 4(A), right) that the neuron was nearly isopotential at any given stage in the development of the full Cs⁺ effect, such that the differences in $V_m$ in different regions of the neuron never exceeded 10 mV. Development of depolarization was significantly slower when $R_s$ was higher (figure 4(B), left). Thus, at $R_s = 100$ MΩ the neuron became fully depolarized within ~7 min after the break-in. The ‘compact’ neuron, however, remained practically isopotential at all stages of the Cs⁺ dialysis at all $R_s$ tested (figure 4(B), right). In a more ‘leaky’ neuron (figure 4(C), left, $g_L = 240 \mu S \text{ cm}^{-2}$ and $g_K = 2400 \mu S \text{ cm}^{-2}$), depolarization of soma was significantly more rapid than in the ‘compact’ neuron (figure 4(B), left). In this case, regional variations in $V_m$ were marked during the course of the development of depolarization to 0 mV (figure 4(B), right), at which time the neuron again became isopotential.

In a compact neuron, holding the soma at −70 mV, whether by injecting hyperpolarizing current (not shown) or by voltage clamp, effectively established the desired membrane potential throughout the neuron, including the most distal parts of the dendritic tree (figures 5(A) and (C)). In a ‘leaky’ neuron, on the other hand, the attempt to hold $V_m$ at −70 mV was hampered by a significant voltage clamp error (figures 5(B) and (C)), which was significant even at the soma because of the finite series resistance of the recording pipette (in this case, 3 MΩ). Thus, the presence of the large leak current caused somatic $V_m$ to deviate from the pipette potential by ~20 mV in the depolarizing direction (figures 5(B) and (C)). Voltage clamp error increased further as a function of the distance from the soma, such that the actual $V_m$ of the tips of the distal apical dendrite was only about −10 mV. Figure 5(D) shows voltage clamp error in soma, axon, basal and apical dendrites measured while the magnitude of $g_L$ varied in range 2.4–240 $\mu S \text{ cm}^{-2}$. It is noteworthy that after complete block of K⁺ channels with Cs⁺, the satisfactory voltage clamp of soma and processes could only be achieved if residual $g_L < 10 \mu S \text{ cm}^{-2}$. The ‘compact’ neuron, which falls in this range of $g_L$, was clamped well at all tested $R_s$ (figure 5(E)) providing that Cs⁺-induced depolarization was complete. By contrast, attempts to control voltage of the ‘leaky’ neuron failed even at lowest $R_s$ (figure 5(F)).
Figure 4. Compartmental simulation of the effect of Cs$^+$ dialysis on membrane potential. (A) In a ‘compact’ neuron ($g_L = 2.4 \, \mu S \, \text{cm}^{-2}$, initial $g_{K(DR)} = 24 \, \mu S \, \text{cm}^{-2}$), the membrane depolarizes uniformly throughout soma, dendrites and axon. $V_m$ reaches its steady value of about 0 mV in about 3 min after the break-in. Color codes as in figure 2. (B) In a ‘compact’ neuron, an increase in series resistance delays depolarization in all compartments. (C) In a ‘leaky’ neuron ($g_L = 240 \, \mu S \, \text{cm}^{-2}$, initial $g_{K(DR)} = 2400 \, \mu S \, \text{cm}^{-2}$), soma, proximal axon and dendrites depolarize faster than in the ‘compact’ cell, creating non-isopotentiality along the apical dendrite. Note that the non-isopotentiality persists longer when Cs$^+$ diffusion is slowed by high series resistance.
Figure 5. $V_m$ in current-clamped and voltage-clamped Cs⁺-dialyzed pyramidal neuron. (A) Distribution of voltage (coded by color) in a compartmental model of a ‘compact’ neuron. Left, current clamp, immediately after establishment of whole cell configuration (control); middle, current clamp, after 3 min of Cs⁺ dialysis; right, voltage clamp, holding potential of $-70$ mV applied via a somatic patch pipette. Note that dc somatic command propagates throughout the dendrites and the axon almost isopotentially. (B) In a voltage clamped ‘leaky’ neuron even soma and proximal processes a clamped poorly. Note that application of holding potential of $-70$ mV via a somatic pipette results in a significant voltage gradient along the apical and basal dendrites. (C) $V_m$ as a function of distance over apical dendrite–soma (dashed line)–axon axes in a voltage clamped ‘compact’ and ‘leaky’ neurons. (D) Voltage clamp error as a function of neuronal leak conductance (range of 2.4–240 $\mu$S cm⁻²). (E) Voltage clamp error in a ‘compact’ neuron as a function of series resistance. (F) Voltage clamp error in a ‘leaky’ neuron as a function of series resistance.
The spatial non-isopotentiality of the voltage clamped, Cs⁺-dialyzed ‘leaky’ neuron, with the poorly clamped portions of the cell being held at very depolarizing potentials, must have a significant influence on the availability of Na⁺ channels. This is because both transient and persistent Na⁺ conductances in central neurons are subject to a slow inactivation process, whose voltage- and time-dependence we previously described in detail (Fleidervish and Gutnick 1996; Fleidervish et al 1996) (figure 4(D)). The steady-state slow inactivation ($s_\infty$) curve is such that in the ‘compact’ neuron, voltage clamping the soma to $-70$ mV almost completely rescued $I_{NaP}$ from slow inactivation throughout all the neuronal compartments (figure 6(A), left). By contrast, the poor voltage clamp in the ‘leaky’ cell resulted in partial activation and partial inactivation of $I_{NaP}$ in the soma and in the proximal neuronal processes even at the level of the holding potential (figure 6(A)). Unclamped, depolarized distal neuronal compartments almost entirely failed to generate $I_{NaP}$ because most of the Na⁺ channels there were inactivated (figure 6(A), left).

In the compact neuron, an uniform, near-perfect clamp was maintained throughout the course of somatically applied, slow, depolarizing voltage ramps (figure 6(B)) (providing that $g_{NaP}$ was not exceedingly high). In stark contrast, voltage clamp in the ‘leaky’ neuron was significantly distorted by space clamp error (figure 6(C)). The model predicts that among the distortions in recorded $I_{NaP}$ caused by increased leak conductance, the most significant ones include a very abrupt current onset, a leftward shift in activation and a decrease in amplitude (figure 6(C)).

4. Discussion

The main conclusion from these data is that within minutes after break-in with a Cs⁺-containing pipette, the passive characteristics of an intact pyramidal neuron change such that its processes can be readily controlled by a somatic voltage clamp electrode. This is only true, however, if series resistance is low, input resistance is high and the voltage command is extremely slow. Cs⁺-dialyzed cells depolarize to nearly 0 mV (figure 1). This means that unless an extrinsic experimental manipulation hyperpolarizes the membrane, all Na⁺ channels, including those responsible for $I_{NaP}$, will undergo slow inactivation and availability will fall to less than 30% of normal. Negative current injection or imposition of a hyperpolarizing voltage clamp serves to reprime the inactivated Na⁺ channels. In a poorly clamped neuronal process, the ability to generate $I_{NaP}$ would be significantly diminished, but not completely blocked.

The diffusional exchange of Cs⁺ ions between a pyramidal neuron and a patch pipette, as expected, is significantly slower than that in small round cells (Marty and Neher 1995; Pusch and Neher 1988). Thus, when $R_e$ is within the range of 3–10 MΩ, it takes from 40 min to several hours to pipette and cell Cs⁺ concentrations to equilibrate. In our simulations, the effect of Cs⁺ on neuronal membrane potential and on input resistance was significantly more rapid since we postulated that Cs⁺ ions block all K⁺ channels with low $K_D$ of 2 mmol l⁻¹. K⁺ conductance in actual pyramidal neurons is formed by tens of molecularly and pharmacologically distinctive types of K⁺ channels (Coetzee et al 1999). The sensitivity of the individual K⁺ channels to the Cs⁺ block varies over a fairly wide range (Coetzee et al 1999). This, along with the presence of various Cs⁺-insensitive ionic conductances (e.g. TRP, Cl-channels) which were not blocked in these experiments, might explain discrepancies between the predicted (figure 4) and actual (figure 1) time course of Cs⁺-induced depolarization.

Voltage clamp errors associated with high leak conductance, in addition to causing distortions to $I_{NaP}$, could potentially affect measurements of other currents in Cs⁺-dialyzed
pyramidal cells. For example, the technique of loading the post-synaptic neurons with Cs\(^+\) ions is widely used in long-term plasticity studies (cf Bolshakov et al 1997; Huang et al 2005). The prolonged recording times and high resistance pipettes that are common in such studies probably leads to a gradual shift in neuronal passive parameters which might significantly distort the amplitude and shape of PSCs as recorded in the soma. Moreover, even moderate depolarization of poorly controlled neuronal processes might cause persistent activation of those synaptic channels which are normally closed at \(V_{\text{rest}}\) (e.g. NMDARs). Indeed, depolarization of poorly

Figure 6. Quality of voltage clamp in ‘compact’ and ‘leaky’ neurons. (A) \(s_\infty\) (left) and \(m_\infty\) (right) as a function of distance over apical dendrite–soma (dashed line)–axon axes in voltage clamped ‘compact’ and ‘leaky’ neurons. Note that in the poorly clamped portion of the dendrite the persistent Na\(^+\) conductance is partially activated and partially suppressed by slow inactivation. (B) Slow voltage ramps from \(-70\) to \(0\) mV delivered via 3 M\(\Omega\) somatic pipette propagate isopotentially throughout the axonal and dendritic trees of a ‘compact’ neuron dialyzed with Cs\(^+\). Color codes as in figure 2. (C) Poor space clamp, significant series resistance error and distortion of the persistent sodium current in a model of ‘leaky’ neuron. Note that during the slow voltage ramps, at \(V_m < 0\), the processes are more depolarized than the soma; this results in partial activation and inactivation of \(I_{\text{NaP}}\) even at the level of holding potential.
controlled dendrites could affect virtually all voltage-sensitive currents (e.g. hyperpolarization-activated cation current, $I(h)$, TRP currents) by inducing partial activation or partial inactivation.

In a previous study, we showed that in layer 5 pyramidal neurons, the Na$^+$ channels responsible for $I_{NaP}$ generation are primarily located in the proximal axon (Astman et al 2006). Our evidence was based, in part, on whole-cell voltage clamp recordings of $I_{NaP}$ elicited by very slow voltage ramps in neurons in which Cs$^+$ had been replaced K$^+$. In these experiments, direct TTX application to the proximal axon consistently blocked $I_{NaP}$ while TTX application on the dendrites did not. Results of the present study show that the puzzling lack of $I_{NaP}$ in the proximal dendrites (Astman et al 2006) does not reflect inadequate voltage control but rather represents a biophysical feature of the dendritic Na$^+$ channels.

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