Site Independence of EPSP Time Course Is Mediated by Dendritic $I_{h}$ in Neocortical Pyramidal Neurons

STEPHEN R. WILLIAMS AND GREG J. STUART
Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT 0200, Australia

Williams, Stephen R. and Greg J. Stuart. Site independence of EPSP time course is mediated by dendritic $I_{h}$ in neocortical pyramidal neurons. J. Neurophysiol. 83: 3177–3182, 2000. Neocortical layer 5 pyramidal neurons possess long apical dendrites that receive a significant portion of the neurons excitatory synaptic input. Passive neuronal models indicate that the time course of excitatory postsynaptic potentials (EPSPs) generated in the apical dendrite will be prolonged as they propagate toward the soma. EPSP propagation may, however, be influenced by the recruitment of dendritic voltage-activated channels. Here we investigate the properties and distribution of $I_{h}$ channels in the axon, soma, and apical dendrites of neocortical layer 5 pyramidal neurons, and their effect on EPSP time course. We find a linear increase (9 pA/100 μm) in the density of dendritic $I_{h}$ channels with distance from soma. This nonuniform distribution of $I_{h}$ channels generates site independence of EPSP time course, such that the half-width at the soma of distally generated EPSPs (up to 435 μm from soma) was similar to somatically generated EPSPs. As a corollary, a normalization of temporal summation of EPSPs was observed. The site independence of somatic EPSP time course was found to collapse after pharmacological blockade of $I_{h}$ channels, revealing pronounced temporal summation of distally generated EPSPs, which could be further enhanced by TTX-sensitive sodium channels. These data indicate that an increasing density of apical dendritic $I_{h}$ channels mitigates the influence of cable filtering on somatic EPSP time course and temporal summation in neocortical layer 5 pyramidal neurons.

INTRODUCTION

The integration of synaptic potentials to form an output signal, the action potential, is the most fundamental operation neurons perform. As excitatory synaptic contacts are made predominantly on the dendrites of central neurons, excitatory postsynaptic potentials (EPSPs) must propagate to the soma and axon to influence action potential initiation (Stuart et al. 1997b). The propagation of synaptic potentials from dendrites to the soma is controlled by both the passive (Rall 1977; Spruston et al. 1994) and active membrane properties of the dendritic tree (Yuste and Tank 1996). The influence of dendritic filtering on EPSP amplitude and time course will be particularly apparent in neurons with large dendritic trees, such as layer 5 neocortical pyramidal neurons, where excitatory synaptic contacts made on apical dendrites can be up to 1 mm from the soma. Indeed, modeling studies have indicated that EPSPs generated in the apical tuft of uniformly passive layer 5 neocortical pyramidal neurons may be attenuated in amplitude by >100 times at the soma, and possess a time course that greatly outlasts the potential at source (Cauller and Connors 1994; Cauller and Connors 1994). Experimentally, however, little relationship between somatic EPSP time course and the presumed site of generation has been observed in both neocortical layer 5 and hippocampal CA1 pyramidal neurons (Andreasen and Lambert 1998; Nicoll et al. 1993), suggesting either that distal synaptic events have different time courses at source (Cauller and Connors 1994) or that their propagation to the soma is influenced by nonuniform membrane properties and/or voltage-activated channels.

Simulations predict that the degree of voltage attenuation from the soma along the main apical dendrite of layer 5 pyramidal neurons is best described if a higher membrane conductance and higher density of hyperpolarization-activated mixed cation channels ($I_{h}$) are present in distal apical dendrites (Stuart and Spruston 1998). These factors would be expected to influence the time course of EPSPs and suggest that nonuniform membrane properties and $I_{h}$ channel distributions may act to normalize EPSP time course at the soma. In support of this notion, it has recently been shown that the density of $I_{h}$ channels increases with distance from the soma in the apical dendrites of CA1 pyramidal neurons (Magee 1998), where they act to normalize temporal summation of somatic EPSPs (Magee 1999).

Here we directly investigate the distribution and properties of $I_{h}$ channels in the apical dendrites of neocortical layer 5 pyramidal neurons and their effect on the relationship between the site of EPSP generation and somatic EPSP time course.

METHODS

Wistar rats (3–6 wk old) were anesthetized by inhalation of halothane, decapitated, and 300 μm-thick coronal or parasagittal neocortical brain slices prepared, according to institutional guidelines. Slices were perfused with oxygenated Ringer solution of the following composition (in mM): 125 NaCl, 25 NaHCO$_3$, 3 KCl, 1.25 NaH$_2$PO$_4$, 2 CaCl$_2$, 1 MgCl$_2$, and 25 glucose. Current-clamp recordings were made at 34–35°C, and cell-attached recordings were made at either room temperature (20–24°C) or 34–35°C. Simultaneous somatic (pipette resistance, 2–5 MΩ) and dendritic (8–12 MΩ) patch-clamp recordings were made from visually identified large layer 5 pyramidal neurons using two identical current-clamp amplifiers (Dagan) as previously described (Williams and Stuart 1999). Somatic, dendritic, and axonal cell-attached recordings (pipette resistance, 10–12 MΩ) were made using on-line leak subtraction (P/5) with a patch-clamp amplifier (Axon Instruments). No differences in the degree or time of negative pressure applied to the back of pipettes was required to form high resistance (3–10 GΩ) seals at somatic, dendritic, or axonal sites, suggesting that similar membrane areas were sampled. For whole cell recordings a patch electrodes were filled with (in mM) 135 K-gluconate,
properties and distribution of $I_h$ channels

Cell-attached recordings were made from the axon, soma, and apical dendrite of layer 5 pyramidal neurons ($n = 72$) with pipettes filled with a solution designed to block voltage-activated potassium and sodium currents. Voltage steps of $-100$ mV made from a holding potential set $20$ mV positive to resting membrane potential (RMP) revealed the presence of a slowly activating, noninactivating inward current (Fig. 1A; RMP determined from current-clamp recordings was found to be on average $-75.8 \pm 0.5$ mV, mean $\pm$ SE). The amplitude of this current was variable from patch to patch, but showed a clear location dependence (Fig. 1B). Recordings made from axonal and somatic sites revealed little or no slow inward current, whereas in patches from apical dendrites the magnitude of this slow inward current increased as recordings were made more distally from the soma (slope of linear regression $9 \mu A/100 \mu m$; Fig. 1B).

Current-voltage relationships indicated that this slow inward current first activated at potentials close to $-70$ mV and increased in magnitude with potential negativity (Fig. 1C). Tail current analysis revealed that the slow inward current was maximally activated at approximately $-110$ mV, and that activation could be well fit with a single Boltzmann function with values of $V_{1/2}$ of $-92$ mV and a steepness coefficient of $6.2$ at room temperature ($n = 29$), and $V_{1/2}$ of $-91$ mV and a steepness coefficient of $6.1$ at $34-35^\circ C$ ($n = 6$), indicating that

![Fig. 1](image)

$\text{FIG. 1.}$ Distribution and properties of $I_h$ channels in cell-attached patches. $A$: slow inward currents evoked in response to voltage steps from $-52$ to $-152$ mV (bottom trace) in cell-attached patches obtained from the axon, soma, and apical dendrite (the indicated locations from the same neuron). Each current trace is an average (30 trials; only 1 in 5 data points are shown for clarity) and was fit with single exponential function (smooth lines). $B$: pooled data (72 patches) of the average amplitude of slow inward currents measured at steady-state (as indicated in $A$) during voltage steps from $-52$ to $-152$ mV, indicate a poverty of $I_h$ channels in axonal or somatic patches, but an increase in $I_h$ channel density with distance along apical dendrites. All data obtained at $20-24^\circ C$. The line is an unconstrained linear regression with a slope of $9 \mu A/100 \mu m$. $C$: family of currents evoked in response to a series of voltage steps (bottom traces). Each current trace is an average of 5 trials and has been fit with a single exponential (smooth lines). $D$: activation curve for $I_h$ channels obtained by tail current measurement (as indicated in $C$). Each point is the mean $\pm$ SE, and data were fit with a single Boltzmann function (solid line, $20-24^\circ C, n = 29$). Dashed line represents the Boltzmann function obtained at $34-35^\circ C$ ($n = 6$). Arrow indicates the whole cell recorded resting membrane potential ($RMP$). $E$: bath application of ZD7288 (100 $\mu M$) reduces the amplitude of slow inward currents, revealing the presence of a very slow current, the time course of which was well fit with a biexponential function (smooth line). $F$: time constants of activation ($\bullet$) and deactivation ($\circ$) at different test potentials. Each point is the mean $\pm$ SE and was fit with a single exponential for recordings made at $20-24^\circ C$ and $34-35^\circ C$. 

Materials and methods: All membrane potentials have been corrected for an experimentally determined liquid junction potential of $-10$ mV. For cell-attached recordings, patch electrodes contained $120$ mM KCl, $20$ mM tetraethylammonium chloride, $5$ mM 4-aminopyridine, $2$ mM CaCl, $1$ mM MgCl, $1$ mM BaCl, $10$ mM HEPES, and $1$ $\mu M$ TTX (pH 7.2 adjusted with KOH), and all patch potentials were corrected for an experimentally determined liquid junction potential of $-3$ mV. Voltage and current signals were filtered at $10-30$ kHz for whole cell recordings, or $2-5$ kHz for cell-attached recordings, and acquired at $20-100$ kHz using an ITC-16 interface (Instrutech) controlled by an Apple PowerPC. Activation curves were fit with a single Boltzmann equation of the following form: $y = 1/[1 + e^{(V_{1/2} - V)/k}]$, where $V_{1/2}$ is the voltage of half-maximal activation and $k$ is a constant. The time course of current activation and deactivation were fit with single or biexponential functions. Numerical values are given in the text as means $\pm$ SE, unless stated otherwise. Statistical analysis was performed with Student’s $t$-test ($\alpha = 0.05$).
$\approx 7^{-8}\%$ of maximal $I_h$ current is activated at the whole cell resting membrane potential (Fig. 1D). The activation properties of the slow inward current were found to be similar at all dendritic recording locations. The time course of current activation increased with membrane negativity and could be well fit with single exponential functions for all voltages examined (Fig. 1C). The activation time constant was $274 \pm 47$ ms at $-82$ mV and decreased exponentially to $55 \pm 6$ ms at $-152$ mV when measured at room temperature ($n = 19$) and were accelerated to $103 \pm 20$ ms at $-82$ mV and $18 \pm 4$ ms at $-152$ mV at $34^{-35}^\circ C$ ($n = 6$; Fig. 1F). Deactivation after maximal activation at $-152$ mV was also well described with a single exponential function with values that decreased exponentially from $275 \pm 55$ ms at $-62$ mV to $91 \pm 22$ ms at $-22$ mV at room temperature ($n = 7$) and $30 \pm 4$ ms at $-62$ mV and $10 \pm 1$ ms at $-22$ mV at $34^{-35}^\circ C$ ($n = 4$; Fig. 1F). The average reversal potential of the slow inward current, extrapolated from tail currents following maximal activation, was $-7.94 \pm 0.77$ mV ($n = 6$).

These voltage-dependent properties are similar to those described for $I_h$ currents during whole cell recording in layer 5 neocortical pyramidal neurons (Solomon et al. 1993; Solomon and Nerbonne 1993; Spain et al. 1987). Consistent with this, bath application of the bradycardiac agent ZD7288 (100 $\mu$M), which blocks $I_h$ at an intracellular site in cardiac cells and central neurons (BoSmith et al. 1993; Harris and Constanti 1995; Williams et al. 1997) caused a $74.5 \pm 2.2\%$ ($n = 6$) reduction (measured just before test step termination) in the slow inward current activated by voltage steps to $-152$ mV (Fig. 1E). Block by ZD7288 appeared to decrease with time during maximally activating voltage steps, leading to the appearance of a very slow inward current that was well fit with a double exponential function (Fig. 1E). The appearance of a very slow inward current in the presence of ZD7288 has been previously observed in whole cell recordings from other central neurons and presumably reflects a voltage-dependent relief of ZD7288 blockade (BoSmith et al. 1993; Harris and Constanti 1995; Williams et al. 1997). In summary, we have observed a predominant apical dendritic distribution of a conductance that by analogy with the properties of whole cell currents reflects the activation of the mixed cationic current $I_h$.

Independence of EPSP time course on dendritic location

Simulated EPSPs (sEPSPs) generated by current injection through somatic and dendritic whole cell recording pipettes (exponential rise 0.3 ms, decay 3 ms) (Stuart and Sakmann 1995) were used to explore the location dependence of EPSP time course (Fig. 2A). The time course of sEPSPs generated by apical dendritic current injection recorded at source and after propagation to the soma were distinct and demonstrated a characteristic crossing during their decaying phase (Fig. 2A) (see Stuart and Spruston 1998). The half-width of somatically and dendritically generated sEPSPs recorded at the soma were similar regardless of the site of sEPSPs generation ($n = 38$; Fig. 2, B and C). Regression analysis revealed that, as sEPSPs were generated more distally, the half-width of sEPSPs recorded at the soma increased by just 0.4 ms/100 $\mu$m (Fig. 2C). The 20–80% rise time of sEPSPs at the soma, however, increased as the site of generation was more distal (Fig. 2D).
These data indicate an independence of somatic sEPSPs duration with the site of EPSP generation, and a dissociation between rise and decay kinetics that would not be apparent in a uniformly passive system (Rall 1977). The independence of somatic sEPSPs duration on the site of sEPSP generation collapsed in the presence of the $I_h$ channel blocker ZD7288 (50 μM; $n = 20$). In ZD7288 the half-width of somatic sEPSPs generated by dendritic current injection outlasted sEPSPs generated by somatic current injection, and increased as sEPSPs were generated more distally (Fig. 2, B and C). Furthermore, the crossover between dendritic sEPSPs recorded at source and after propagation to the soma was abolished (the ratio of somatic to dendritic sEPSP amplitude at 25 ms was $1.32 \pm 0.08$ in control and $1.01 \pm 0.02$ in ZD7288; $P < 0.05; n = 20$). The rise time of propagated dendritic sEPSPs was largely unaffected by ZD7288 (Fig. 2D).

The application of ZD7288 hyperpolarized the somatic RMP (by $11.1 \pm 1.3$ mV; $n = 20$), indicating that $I_h$ is active at the RMP. To compensate for any effect this could have on somatic EPSP time course, depolarizing current injection was used to maintain somatic membrane potential in the presence of ZD7288. Furthermore, ZD7288 completely abolished the depolarizing sag apparent during hyperpolarizing voltage steps generated at somatic and dendritic recording locations ($n = 20$). The small ($<5$ mV) membrane potential gradient between somatic and dendritic (more depolarized) recording locations (see Stuart et al. 1997a) was also abolished by ZD7288 ($n = 20$), indicating that this gradient is generated by $I_h$ active at RMPs.

To explore whether these findings were valid for sEPSPs with different time courses, we generated sEPSPs by current injections with a range of exponential rise and decay time constants (rise: 0.2, 0.3, 0.5, 1.0, 2.0 ms; decay: 2, 3, 5, 10, 20 ms; the amplitude of these current injections were varied to maintain the same amplitude of sEPSPs at the soma). Under control conditions the ratio of somatic sEPSP half-width for sEPSPs generated at the soma and those generated at dendritic sites was close to unity for all but the fastest sEPSPs ($0.87 \pm 0.03, 0.94 \pm 0.01, 1.06 \pm 0.06, 1.07 \pm 0.03, 1.09 \pm 0.03, n = 6$). In the presence of ZD7288 this ratio decreased to less than one for all sEPSPs ($0.68 \pm 0.05, 0.79 \pm 0.04, 0.93 \pm 0.03, 0.93 \pm 0.01, 0.93 \pm 0.02, n = 6$), indicating that the effect of $I_h$ normalization of somatic sEPSP half-width is relatively independent of the time course of the excitatory postsynaptic current.

$I_h$ channels prevent temporal summation of dendritic EPSPs

In a uniformly passive system, distal EPSPs would be expected to show greater somatic temporal summation than proximal or somatic EPSPs because of their slower decay kinetics at the soma. The normalization of sEPSP half-width observed here, however, suggests that this may not occur in layer 5 pyramidal neurons. To test this, a train of five sEPSPs at 50 Hz...
were generated at somatic and apical dendritic sites, and the integral of the voltage response was measured at the soma after normalization of the amplitude of the first EPSP. Under control conditions we observed that sEPSPs did not appreciably summate when generated from any recording location (Fig. 3, A and B), with the integral of sEPSPs increasing only marginally as the site of sEPSP generation was more distal (3.8 \( \mu \text{V} \cdot \text{s/100 \mu m} \); Fig. 3B). Bath application of ZD7288 (50 \( \mu \text{M} \)) unmasked temporal summation of sEPSPs generated at both somatic and dendritic locations (Fig. 3A). In the presence of ZD7288, however, dendritically generated sEPSPs summed to a greater extent than somatically generated sEPSPs, an effect that was more pronounced for more distally generated sEPSPs (Fig. 3, A and B).

The temporal summation of sEPSPs unmasked by ZD7288 may lead to the recruitment of other membrane currents, such as the persistent sodium current, \( I_{\text{Nap}} \), which has been shown to effect the amplitude and time course of both single and bursts of EPSPs at depolarized membrane potentials in neocortical layer 5 pyramidal neurons (Stuart and Sakmann 1995; Williams and Stuart 1999). Although temporal summation at the soma was largely absent at RMP under control conditions (Fig. 3A), we observed that trains of dendritic sEPSPs generated at depolarized membrane potentials (on average 8.3 \( \pm 0.8 \) mV positive to RMP) showed significant temporal summation (Fig. 3C; increase in integral of 95 \( \pm 19\% \); \( n = 5 \)). This effect of somatic depolarization was enhanced in the presence of ZD7288 (Fig. 3C). Increased temporal summation of sEPSPs at depolarized potentials in ZD7288 was largely abolished by the addition of the sodium channel blocker TTX (1 \( \mu \text{M} \); \( n = 5 \); Fig. 3C). TTX also reduced temporal summation of sEPSPs at the RMP in ZD7288 (Fig. 3C; decrease in integral 17 \( \pm 7\% \); \( n = 5 \)), indicating that the increased temporal summation of sEPSPs in the presence of ZD7288 is in part due to the activation of \( I_{\text{Nap}} \).

**DISCUSSION**

The main findings of the present investigation are 1) \( I_{\text{h}} \) channels are located at a high density in the apical dendrites of neocortical layer 5 pyramidal neurons, 2) somatic EPSP time course is independent of the site of generation, as is temporal summation, and 3) blockade of \( I_{\text{h}} \) channels reveals site dependence of somatic EPSP time course and temporal summation.

Under control conditions dendritically generated sEPSPs recorded at source decayed faster than the propagated sEPSPs recorded at the soma, leading to a crossover of their decay phase. This behavior is not expected from a uniformly passive system and is a consequence of a higher membrane conductance (\( G_m \)) at dendritic sites (London et al. 1999; Stuart and Spruston 1998). In the present investigation we observed that crossing of source and propagated sEPSPs was abolished by blockade of \( I_{\text{h}} \) channels, indicating that \( I_{\text{h}} \) channels open at the RMP are responsible for increased \( G_m \) in apical dendrites and are not supplemented by other nonuniform conductances as previously suggested (Stuart and Spruston 1998). \( I_{\text{h}} \) does not, however, operate simply as a shunt conductance; the crossover of source and propagated sEPSPs will be further increased as a consequence of the voltage-dependent deactivation of \( I_{\text{h}} \) during EPSPs, leading to the generation of a net outward current that curtails their decay (Magee 1999; Nicoll et al. 1993). The properties of \( I_{\text{h}} \) observed in cell-attached patches indicates that \( I_{\text{h}} \) deactivation at physiological temperatures is sufficiently rapid to mediate this effect. At increasing distal dendritic locations these effects will be greater due to both the higher density of \( I_{\text{h}} \) channels and the increased local EPSP amplitude. The physiological consequence of this is site independence of EPSP time course at the soma. This effect was observed for a wide range of sEPSP kinetics but was less for sEPSPs with fast kinetics.

The pattern of local integration in dendrites will be effected by \( I_{\text{h}} \) channels. At distal dendritic sites the integration time window, in a uniformly passive system, is shorter than at the soma as a consequence of the rapid flow of current from source to neighboring dendritic sites (Koch et al. 1996). The high density of dendritic \( I_{\text{h}} \) channels, observed here, will further reduce the time window for dendritic integration, decreasing the likelihood that summation of distal synaptic inputs will reach threshold for activating dendritic regenerative events (Andreasen and Lambert 1998; Golding and Spruston 1998; Schiller et al. 1997; Schwinding and Crill 1997; Stuart et al. 1997a). This will have the effect of focusing EPSP integration at the level of the soma and axon.

The site independence of EPSP time course and temporal summation ensures that the temporal nature of synaptic integration at the site of action potential initiation in the axon of neocortical layer 5 pyramidal neurons will be similar for distal and proximal synaptic inputs. The nonuniform \( G_m \) produced by \( I_{\text{h}} \) and the activation/deactivation properties of \( I_{\text{h}} \) should ensure that temporal integration is normalized across a range of membrane potentials, EPSP amplitudes, and repetition frequencies (see also Magee 1999). Under control conditions, however, EPSPs summate more effectively when generated at somatic membrane potentials close to firing threshold, due to the activation of \( I_{\text{Nap}} \). Given that \( I_{\text{Nap}} \) has a predominant axosomatic location (Andreasen and Lambert 1999; Stuart and Sakmann 1995), and \( I_{\text{h}} \) an apical dendritic location, temporal summation will be increased by somatic depolarization following the activation of \( I_{\text{Nap}} \), and by dendritic depolarization through a reduction in \( I_{\text{h}} \) availability, whereas hyperpolarization of these different compartments will have the opposite effect. Temporal summation of EPSPs at the soma in layer 5 pyramidal neurons will therefore be determined by the spatial pattern of excitation and be inhibition as a consequence of nonuniform distributions of \( I_{\text{h}} \) and \( I_{\text{Nap}} \).

This work was supported by the Wellcome Trust.

Address for reprint requests: G. Stuart, Division of Neuroscience, John Curtin School of Medical Research, Mills Rd., Australian National University, Canberra, ACT 0200, Australia.

Received 16 December 1999; accepted in final form 4 February 2000.

**REFERENCES**


