Distance-Dependent Modifiable Threshold for Action Potential Back-Propagation in Hippocampal Dendrites

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Bernard, C. and D. Johnston. Distance-dependent modifiable threshold for action potential back-propagation in hippocampal dendrites. J Neurophysiol 90: 1807–1816, 2003; 10.1152/jn.00286.2003. In hippocampal CA1 pyramidal neurons, action potentials generated in the axon back-propagate in a decremental fashion into the dendritic tree where they affect synaptic integration and synaptic plasticity. The amplitude of back-propagating action potentials (b-APs) is controlled by various biological factors, including membrane potential (Vm). We report that, at any dendritic location (x), the transition from weak (small-amplitude b-APs) to strong (large-amplitude b-APs) back-propagation occurs when Vm crosses a threshold potential, θx. When Vm > θx, back-propagation is strong (mostly active). Conversely, when Vm < θx, back-propagation is weak (mostly passive). θx varies linearly with the distance (x) from the soma. Close to the soma, θs ≪ resting membrane potential (RMP) and a strong hyperpolarization of the membrane is necessary to switch back-propagation from strong to weak. In the distal dendrites, θx ≫ RMP and a strong depolarization is necessary to switch back-propagation from weak to strong. At ~260 µm from the soma, θ260 ≈ RMP, suggesting that in this dendritic region back-propagation starts to switch from strong to weak. θx depends on the availability or state of Na+ and K+ channels. Partial blockade or phosphorylation of K+ channels decreases θx and thereby increases the portion of the dendritic tree experiencing strong back-propagation. Partial blockade or inactivation of Na+ channels has the opposite effect. We conclude that θx is a parameter that captures the onset of the transition from weak to strong back-propagation. Its modification may alter dendritic function under physiological and pathological conditions by changing how far large action potentials back-propagate in the dendritic tree.

I N T R O D U C T I O N

In most mammalian central neurons, action potentials are generated in the axon region and then back-propagate into the dendritic tree (Colbert and Johnston 1996; Johnston et al. 2000; Magee et al. 1998; Spruston et al. 1995; Stuart et al. 1997). Back-propagation can be fully regenerative, decremental, or passive according to the geometrical features of the dendritic tree and the ratio between Na+ and K+ channels at any dendritic location—a property that appears to be neuron type-dependent (Hoffman et al. 1997; Martina et al. 2000; Stuart and Hausser 1994, 2001; Vetter et al. 2001). In CA1 pyramidal neurons, the amplitude of back-propagating action potentials (b-APs) decreases with the distance from the soma due to the increase in density of K+ channels and despite a relatively uniform density of Na+ channels (Hoffman et al. 1997; Johnston et al. 2000; Magee et al. 1998; Yuan et al. 2002). The amplitude of a b-AP at a given dendritic location is not constant either in vitro or in vivo (Quirk et al. 2001). Many factors controlling back-propagation have been described in detail (Johnston et al. 1999)—among them is the local membrane potential. Small-amplitude b-APs in the distal dendrites can be boosted by precisely timed excitatory postsynaptic potentials or appropriate membrane depolarization via A-type K+ channel inactivation and/or Na+ channel activation (Magee and Johnston 1997; Stuart and Hausser 2001). This amplification may allow the opening of voltage-gated channels and the removal of the Mg2+ block of NMDA receptors, hence modifying subsequent synaptic integration and providing an associative signal for synaptic plasticity (Magee and Johnston 1997; Watanabe et al. 2002). Conversely, the amplitude of b-APs can be decreased after membrane hyperpolarization (Tsubokawa and Ross 1996) or during repetitive firing of action potentials (Colbert et al. 1997). Back-propagation is thus context-specific: it depends on the availability or state (i.e., open, closed, inactivated) of Na+ and K+ channels, and the recent history of membrane potential will modify the availability (states) of these channels.

The relationship between the state of the membrane potential and the amplitude of b-APs has not been fully explored. We have investigated this issue in CA1 pyramidal neuron dendrites. We have identified two states of back-propagation, weak (small-amplitude b-AP) and strong (large-amplitude b-AP), according to the membrane potential at any given dendritic location. The transition threshold between the two states can be modified by changing the availability of Na+/K+ channels. The possibility to switch from weak to strong or from strong to weak back-propagation provides an efficient means of modifying information processing in the dendrites.

M E T H O D S

Recordings were performed from CA1 pyramidal cell dendrites (Yuan et al. 2002) in 350-µm-thick transverse hippocampal slices from male Sprague Dawley (5- to 7-wk old) according to local regulations. During recordings, slices were perfused with an oxygenated bath solution containing (in mM) 125 NaCl, 2.5 KCl, 2 CaCl2, 2 MgCl2, 1.25 NaH2PO4, 25 NaHCO3, and 10 dextrose, kept at 32–33°C (this particular temperature was chosen to be consistent with another study in progress). 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-ben-

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zo[f]quinazoline-7-sulfonamide (NBQX) (1 μM), 2-aminophosphonovaleic acid (APV, 100 μM), and bicuculline (10 μM)/picrotoxin (10 μM) were added to the perfusing solution to block AMPA, N-methyl-d-aspartate (NMDA) and GABA_A receptors, respectively. U0126 and phorbol diacetate (PDA) were dissolved in DMSO to 10 mM. In some experiments, TTX (10 μM) was applied near the recording microelectrode (5 μm) using a puffer pipette (1-2 μm tip).

Whole cell current-clamp recordings (Yuan et al. 2002) were performed using an Axoclamp 2A amplifier in bridge mode (Axon Instruments, Foster City, CA) with pipettes filled with a solution containing (in mM) 120 KMeSO_4, 20 KCl, 0.2 EGTA, 2 MgCl_2, 10 HEPES, 4 Na_2ATP, 0.3 Tris GTP, 14 phosphocreatine; 0.4% biocytin and KOH were added to adjust to pH 7.3. Stimulation and data acquisition were controlled by Igor software (Igor Wavemetrics). Signals were digitized with an ITC-18 interface (Instrutech), at a sampling rate of 5–20 kHz. Access resistance as estimated from the bridge balance was 33.9–20 kHz. Acquisition were controlled by Igor software (Igor Wavemetrics).

In the absence of any drug, the amplitude of the back-propagating action potential (b-AP) decreased with the distance from the soma at resting membrane potential (Fig. 1A), consistent with previous studies (Golding et al. 2001; Hoffman et al. 1997; Spruston et al. 1995; Yuan et al. 2002). The decrease in amplitude of b-APs was correlated to the decrease of the maximal rate of rise of the b-APs (Fig. 1B and C). The half-width of the b-APs increased with the distance from the soma (Fig. 1D).

Current pulses were injected into the dendrites in a step-wise manner to achieve various membrane potentials (\(V_m\)) from hyperpolarized (−120 to −90 mV) to depolarized (subthreshold to action potential initiation) levels. b-APs were evoked 300 ms after the start of current injection to allow membrane potential to reach steady state. Figure 2 illustrates the results obtained with such a protocol (dendrite recorded 260 μm from the soma). The amplitude of b-APs remained small and roughly constant over a wide range of hyperpolarized potentials (Fig. 2, A and B). As the membrane was further depolarized, there was a rapid increase of the relative amplitude of b-APs until it decreased because the membrane potential at the peak of the b-AP reached an asymptotic value (Fig. 2C). The maximum rate of rise of the b-APs followed the same type of curve (not shown). The half-width of the b-APs was large at hyperpolarized levels and decreased as \(V_m\) became more positive till it reached a minimum (Fig. 2D). The half-width then increased as the membrane was further depolarized. The broadening of b-APs at depolarized levels might reflect the activation of voltage-gated Ca^{2+} channels.

We define \(\theta\) as the membrane potential where the second derivative of the b-AP amplitude (Amp) with respect to \(V_m\) is maximum, i.e., \(\theta = \max[\frac{d^2\text{Amp}(V_m)}{dV_m^2}]\) (Fig. 2E). \(\theta\) represents the onset of the transition between small- and large-amplitude (weak and strong) back-propagation. In the example shown in Fig. 2, the resting membrane potential (RMP) of the dendrite was −68 mV and \(\theta\) was −58 mV. In this dendrite, at 260 μm from the soma, b-APs had a small amplitude because RMP was more negative than \(\theta\) (RMP < \(\theta\)). For boosting or amplification to occur, \(V_m\) had to reach \(\theta\) = −58 mV.

Rapid changes in membrane potential occur in vivo, in particular during oscillations (Buzsáki 2002). Sinusoidal currents were injected in the dendrites at various frequencies, and b-APs were appropriately timed to mimic physiologically relevant conditions (Stuart and Hausser 2001). b-APs displayed a transition from weak to strong (or from strong to weak) back-
The half-width of the b-AP decreased with membrane depolarization as more and large amplitude b-APs (V_m > \theta_e) occurred on reaching the peak (trough) of the oscillation. Arrowhead indicates stimulus artifact. Small arrow: foot of b-AP. B: the transition between small- and large-amplitude back-propagation occurred on a very fast time scale.

From strong to weak back-propagation

A transition threshold was found in 62 of the 65 recorded dendrites. In three cases (distal recordings >300 \mu m), the transition could not be identified because action potentials were triggered by the current injection before the occurrence of the b-AP. The value of \theta varied linearly along the dendritic tree, increasing with distance (x) from the soma (Fig. 3A), from hyperpolarized values (with respect to RMP) for distances close to the soma to depolarized values in the distal part of the dendritic tree. By subtracting \theta_x from RMP at each recording site, we found two main regions of back-propagation along the dendritic tree (Fig. 3B). Below the zero line, RMP was more positive than \theta_x and b-APs had a large amplitude (strong back-propagation). Membrane hyperpolarization could switch back-propagation from strong to weak, provided that V_m < \theta_x. Above the zero line, RMP was more negative than \theta_x and b-APs had a small amplitude (weak back-propagation). Membrane depolarization switched back-propagation from weak to strong, provided that V_m > \theta_x. Therefore the critical factor that determined whether strong or weak back-propagation occurred was where V_m was with respect to \theta_x at any dendritic location (x). The intersection between the zero line and the regression line of \theta_x gave the average distance from the soma in the propagation similar to that obtained with constant dendritic current injection (n = 50, Fig. 2F), showing that transitions can occur on a very fast time scale.

FIG. 3. From strong to weak back-propagation in dendritic trees. A: the threshold for amplification increased as a function of the distance from the soma. The blue line represents the average RMP in the dendrite of the 65 recordings. The red line is the linear least-square best fit of the dataset. B: difference between the transition threshold and RMP at each recording site as a function of the distance from the soma. Above (below) the blue line, where RMP = \theta_x b-APs had a small (large) amplitude. The intersect between the blue line and the linear least-square best fit of the dataset gives the distance in the dendritic tree where, on average, RMP = \theta_x i.e., 263 \mu m from the soma. C: we propose the following model: <263 \mu m, b-APs have a large amplitude because RMP > \theta_x (red portion of the dendritic tree). In the intermediate zone around 263 \mu m, the amplitude of the b-AP decreases sharply (light blue). The extent of the light blue zone (extent of the transition zone) is arbitrary as it varied from cell to cell. At distances more than 263 \mu m, b-APs have a small amplitude because RMP < \theta_x (dark blue portion of the dendritic tree). D: the maximum depolarization reached at the peak of the b-AP was roughly constant along the dendritic tree when the membrane was held at -35 mV (●) or at -100 mV (○). The dynamic range of the membrane depolarization achieved by b-APs was constant along the portion of the dendritic tree tested with these two holding potentials as limits.
dendritic tree where $RMP = \theta_s$. We found a value of $x = 263 \pm 6 \mu m$.

$\theta_s$ captures the onset of the transition between weak and strong back-propagation. We define the length of the transition zone as the interval between $\theta_s$ and the membrane potential corresponding to the end of the rising phase of Amp$(V_m)$. In Fig. 2B, $\theta_s = -58 \text{ mV}$, the end of the rising phase was at $-49 \text{ mV}$, which gave a length for the transition zone of 9 $\text{ mV}$. There was no obvious relationship between the length of the transition zone and the distance from the soma, although the general trend was an increase of the length of the transition zone with the distance from the soma (not shown). In Fig. 8C, the recording site was at 280 $\mu m$, and the transition length was 4 $\text{ mV}$ [Amp$(V_m)$ was more like a step function], shorter than the 9 $\text{ mV}$ measured 260 $\mu m$ from the soma in Fig. 2.

We propose the following scheme (Fig. 3C). When an action potential is generated in the axon, it back-propagates into the dendritic tree. Its amplitude decreases with the distance from the soma as the density of A-type $K^+$ channels increases. Between the soma and 263 $\mu m$, the b-AP amplitude remains large because $RMP \gg \theta_s$ before reaching 263 $\mu m$, there is a sudden acceleration of the decrease of amplitude (falling phase of Amp$(V_m)$). At distances more than 263 $\mu m$, the b-AP has a small amplitude because $RMP < \theta_s$, and its amplitude still decreases with distance from the soma. Consistent with this scheme, most recordings performed at distances <263 and >263 $\mu m$ displayed large and small b-APs, respectively (Fig. 3B).

The amplitude of b-APs is a critical parameter to consider for $Ca^{2+}$ signaling in dendrites (Magee et al. 1998). We thus determined the dynamic range of membrane potentials generated by b-APs along the dendritic tree. For a measured (or extrapolated) $V_m$ of $-100 \text{ mV}$, b-APs had a small amplitude because $V_m \ll \theta_s$ (weak back-propagation). The amplitude of the b-APs was remarkably constant at this potential at all recording sites ($21 \pm 1 \text{ mV}, n = 59$). For a holding potential of $-100 \text{ mV}$, the membrane potential reached at the peak by b-APs was thus $-79 \pm 1 \text{ mV}, n = 59$, Fig. 3D. The amplitude of the b-APs for a holding potential of $-35 \text{ mV}$ was also remarkably constant along the dendritic tree ($30 \pm 1 \text{ mV}, n = 59$). At this holding potential, $V_m \gg \theta_s$ (strong back-propagation). For a holding potential of $-35 \text{ mV}$, the membrane potential reached at the peak by b-APs was thus $-5 \pm 1 \text{ mV}, n = 59$, Fig. 3D. If we consider $-100$ and $-35 \text{ mV}$ as lower and upper limits for membrane potential fluctuations, respectively, the membrane potential reached at the peak by b-APs can evolve between location-independent lower ($-79 \text{ mV}$) and upper ($-5 \text{ mV}$) boundaries with a wide dynamic range ($-75 \pm 2 \text{ mV}, n = 59$).

In the distal part of the dendritic tree, b-APs may not open sufficient numbers of $Na^+$ channels to be actively propagated. To test this, we selected dendritic recordings for which RMP was less than $\theta_s$ (weak back-propagation) and for which the amplitude of the b-AP was between 20 and 30 $\text{ mV}$ at RMP. Local application of the $Na^+$ channel blocker TTX ($10 \mu M$) had no effect on the amplitude of b-APs ($n = 3$, Fig. 4A), consistent with a passive back-propagation. This lack of effect was not due to a failure of TTX ejection as moving the puff pipette closer to the soma on the same dendrite (200 $\mu m$) slightly reduced the amplitude of b-APs recorded more distally ($n = 3$, not shown). We also selected dendritic recordings for which RMP was over $\theta_s$ (strong back-propagation) and for which the amplitude of the b-AP was between 40 and 50 $\text{ mV}$. Local application of TTX decreased the amplitude of b-APs ($n = 3$, Fig. 4B), consistent with a back-propagation containing an active component. Interestingly, the basic features of b-APs evoked at $V_m < \theta_s$ at any dendritic location ($\lambda$) were identical to those measured at RMP at a distance >270 $\mu m$ from the soma; i.e., amplitude (20–30 $\text{ mV}$), maximum rate of rise (20–30 $\text{ mV/msec}$) and half-width (3–15 $\text{ ms}$). This suggests that at potentials less than $\theta_s$, back-propagation is mostly passive, and we propose that $\theta_s$ corresponds to the potential where the transition from a back-propagation containing an active component to a passive one occurs.

$\theta$ and $Na^+/K^+$ channel availability

The amplitude of b-APs appears to be controlled by the degree of activation/inactivation as well as by the density of $Na^+$ and $K^+$ channels (Pan and Colbert 2001). We investigated the consequences of slight modifications of Na/K channel availability on the transition threshold $\theta$.

Partial blockade of $Na^+$ channels with 10 nM TTX decreased the amplitude of b-APs to 61 $\pm 10\%$ of control at RMP ($n = 6, P < 0.02$) and shifted $\theta$ by 10 $\pm 4 \text{ mV}$ ($n = 6, P < 0.03$) toward depolarized values (Fig. 5A). The distance in the dendritic tree where RMP was close to the new threshold with 10 nM TTX was now 254 $\pm 30 \mu m$ from the soma.

Partial blockade of $K^+$ channels with 50 $\mu M$ 4-aminopyridine (4-AP) (Hoffman et al. 1997) produced a small but not
FIG. 5. Modification of $\theta$ after partial blockade of Na$^+$ and K$^+$ channels. A: application of a small dose of the Na$^+$ channel blocker TTX (10 nM) shifted the transition threshold to a more positive value. Example of a dendrite recorded 160 $\mu$m from the soma. Top: variation of b-AP amplitude as a function of membrane potential before (filled black circles) and after TTX (empty red circles). $\text{Amp}(V_{\text{con}})$, here a step-like function, and $\theta$ were shifted to the right. The amplitude of b-APs was smaller at all membrane potentials, including at RMP ($-60$ mV, arrowhead) because of the partial blockade of Na$^+$ channels. Inset: b-AP before (black) and after (red) TTX at RMP. Traces are superimposed, because the decrease in amplitude was small. Scale bar: 10 mV/10 ms. Bottom: the shift to the right of $\theta$ resulted in a shift toward the soma of the portion of the apical dendrite that had b-APs with a large amplitude, from 263 to 254 $\mu$m. B: application of the K$^+$ channel blocker 4-AP (50 $\mu$M) shifted the transition threshold to a more negative value. Example of a dendrite recorded 280 $\mu$m from the soma. Top: variation of b-AP amplitude as a function of membrane potential before (filled black circles) and after 4-AP (empty red circles). $\text{Amp}(V_{\text{con}})$, here a smooth sigmoid-like function, and $\theta$ were shifted to the left. In this example, the amplitude of b-APs was increased in the rising phase of the sigmoid-like function after 4-AP. The minimum and maximum amplitudes of b-APs were similar before and after 4-AP. Inset: b-AP before (black) and after (red) 4-AP at RMP ($-68$ mV). Traces superimpose, because b-AP amplitudes were identical at RMP ($-60$ mV, arrowhead) because of the partial blockade of Na$^+$ channels. Scale bar: 5 mV/10 ms. Bottom: the shift to the left of $\theta$ resulted in a shift toward the distal portion of the apical dendrite that sees b-APs with a large amplitude, from 263 to 328 $\mu$m. C: application of the K$^+$ channel blocker 4-AP (5 mM) shifted the transition threshold to a more negative value. Example of a dendrite recorded 290 $\mu$m from the soma. Top: variation of b-AP amplitude as a function of membrane potential before (filled black circles) and after 5 mM 4-AP (empty red circles). $\text{Amp}(V_{\text{con}})$, here a smooth sigmoid-like function, and $\theta$ were shifted to the left. The minimum and maximum amplitudes of b-APs were increased after 4-AP. Inset: b-AP before (black) and after (red) 4-AP at RMP ($-63$ mV). Scale bar: 20 mV/10 ms. Bottom: the shift to the left of $\theta$ resulted in a shift toward the distal portion of the apical dendrite that sees b-APs with a large amplitude, from 263 to 438 $\mu$m.

A statistically significant increase of the average amplitude of b-APs (128 $\pm$ 16% of control at RMP; $n = 6, P = 0.15$) but significantly shifted $\theta$ by $7.8 \pm 3.3$ mV ($n = 6, P < 0.05$) toward hyperpolarized values (Fig. 5B). The distance in the dendritic tree where RMP was close to the new threshold with 50 $\mu$M 4AP was now 328 $\pm 49$ $\mu$m from the soma. Larger blockade of K$^+$ channels with 5 mM 4AP, in the presence of 0.2 mM Cd$^{2+}$ and 0.2 mM Ni$^{2+}$ (Hoffman et al. 1997), increased the amplitude of back-propagating action potentials to $237 \pm 33\%$ of control at RMP ($n = 11, P < 0.001$) and shifted the threshold by 14.8 $\pm 4.4$ mV ($n = 11, P < 0.001$) toward hyperpolarized values (Fig. 5C). The distance in the dendritic tree where RMP was close to the new threshold with 5 mM 4AP was now 438 $\pm 48$ $\mu$m from the soma. In conclusion, $\theta$ was modified by changing the number of Na$^+$ and K$^+$ channels available for activation. A decreased number of Na$^+$ channels limited the extent of the dendritic tree where strong back-propagation occurred. Conversely, a decreased number of K$^+$ channels led to large-amplitude b-APs invading farther into the dendritic tree.

$\theta$ is not $I_h$ sensitive

Hyperpolarization-activated current ($I_h$) is present at high density in CA1 pyramidal neuron dendrites. Similarly to A-type K$^+$ channels, the density of $I_h$ channels increases many-fold with the distance from the soma (Magee 1998). We therefore tested whether $I_h$ played a role in controlling $\theta$. Application of the $I_h$ antagonist ZD7288 (30 $\mu$M) slightly decreased the average amplitude of back-propagating action potentials at RMP by $7 \pm 3\%$ of control (Fig. 6), but this decrease was not statistically significant ($n = 5, P = 0.06$). The transition threshold was also not modified (Fig. 6, $-0.3 \pm 0.5$ mV, $n = 5, P = 0.3$). We conclude that $I_h$ does not affect the distance from the soma where the transition from strong to weak back-propagation occurred in the apical dendrites.

Effect of Na$^+$ channel inactivation on $\theta$

b-APs undergo activity-dependent attenuation of their amplitude (Callaway and Ross 1995; Colbert et al. 1997; Spruston et al. 1995). During repetitive firing, there is a change in the Na/K channel ratio in favor of K$^+$ channels, because Na$^+$ channel inactivation increases during the train while K$^+$ channels do not (Colbert et al. 1997; Pan and Colbert 2001). To study the consequences of Na$^+$ channel inactivation on $\theta$, a train of five b-APs was generated in the dendrites with a 50-ms interstimulus interval. The amplitude of b-APs decreased in an activity-dependent manner and was seen as soon as the second b-AP in the train (Fig. 7A), consistent with previous studies.
Membrane potential did not reach $-92.58\text{ mV}$ (9.1 difference between firing amplitude and membrane potential; the amplitude was also voltage-dependent (Fig. 7A) (1995). However, this activity-dependent decrease of b-AP amplitude was not reversed but the repolarization phase of the b-AP was increased. Scale bar: 5 mV/10 ms. Bottom: blocking $I_h$ did not change back-propagation in the dendritic tree.

There was no obvious correlation with the absence of the firing threshold for the last b-AP was found in 27% of the recordings compared to the soma (same as Fig. 2). Top: total blockade of $I_h$ with ZD 7288 (30 $\mu$M) did not modify the relationship between b-AP amplitude and membrane potential; $\theta$ was not modified. Inset: b-AP before (black) and after (red) ZD 7288 at RMP ($\sim$ 64 mV). The amplitude of the b-AP was not modified but the repolarization phase of the b-AP was increased. Scale bar: 5 mV/10 ms. Bottom: ZD 7288 (30 $\mu$M) changed the relationship between b-AP amplitude and membrane potential; $\theta$ was not modified. The amplitude of the b-AP was not modified but the repolarization phase of the b-AP was increased. Scale bar: 5 mV/10 ms. Bottom: blocking $I_h$ did not change back-propagation in the dendritic tree.

$I_h$ did not affect $\theta$. Example of a dendrite recorded 260 $\mu$m from the soma (same as Fig. 2). Top: total blockade of $I_h$ with ZD 7288 (30 $\mu$M) did not change the relationship between b-AP amplitude and membrane potential; $\theta$ was not modified. Inset: b-AP before (black) and after (red) ZD 7288 at RMP ($\sim$ 64 mV). The amplitude of the b-AP was not modified but the repolarization phase of the b-AP was increased. Scale bar: 5 mV/10 ms. Bottom: blocking $I_h$ did not change back-propagation in the dendritic tree.

Protein kinase A and protein kinase C (PKC) activations produce similar positive shifts in the activation curve of A-type $K^+$ channels via the MAPK pathway. As a result, fewer $K^+$ channels become available for opening, and the amplitude of b-APs is consequently increased (Hoffman and Johnston 1998, 1999; Yuan et al. 2002). Also, increases in PKC activity reduce slow $Na^+$ channel inactivation resulting in less b-AP attenuation during a train (Colbert and Johnston 1998) (Fig. 8A). Such phenomena could be physiologically important given that synaptic plasticity is accompanied by both transient and persistent increases in protein kinase activity (Adams and Sweatt 2002; Colbert and Johnston 1998; English and Sweatt 1996). We thus tested the consequences of PKC activation on the transition threshold $\theta$. PKC activation after application of 10 $\mu$M PDA increased the amplitude of b-APs to 157 ± 16% of control at RMP ($n = 6$, $P < 0.02$) and shifted the threshold by 17.8 ± 5.9 mV ($n = 6$, $P < 0.02$) to more negative values (Fig. 8A). The distance in the dendritic tree where RMP was close to the new threshold after PDA was now 425 ± 102 $\mu$m from the soma. Although PKC-dependent changes of $K^+$ and $Na^+$ activation curves should result in opposite shifts of $\theta$ (toward more negative and positive values, respectively), the effect on $K^+$ channels is predominant and PKC activation significantly increases the extent of the dendritic tree that sees large-amplitude b-APs.

**PKC activation favors strong back-propagation**

An endogenous phosphorylation of A-type $K^+$ channels has been reported in vitro (Watanabe et al. 2002; Yuan et al. 2002). Furthermore, certain properties of Na/K channels are modulated by phosphorylation in a distance-dependent manner (Gasparini and Magee 2002; Hoffman and Johnston 1998; Yuan et al. 2002), further suggesting that there may be a gradient of endogenous kinase activity along the dendrites. We thus tested the effect of the broad spectrum protein kinase inhibitor H7 on $\theta$. Bath application of H7 for 30 min resulted in a decrease of the amplitude of b-APs to 88 ± 4% of control at RMP ($n = 5$, $P < 0.03$), in keeping with the hypothesis of an endogenous phosphorylation of Na/K channels under physiological conditions. H7 also shifted $\theta$ by 4.7 ± 0.7 mV ($n = 5$, $P < 0.002$) toward depolarized values (Fig. 8B). The distance in the dendritic tree where RMP was close to the new threshold with H7 was now 243 ± 22 $\mu$m from the soma.

Protein kinase A and PKC can phosphorylate Kv4.2 channels via the extracellular regulated kinase (ERK) pathway (Yuan et al. 2002). Kv4.2 channels constitute the most abundant transient $K^+$ channels in CA1 pyramidal cells, in particular, in the dendrites where they directly control the membrane potential.
amplitude of b-APs. We have thus applied the MEK inhibitor U0126 to prevent the phosphorylation of Kv4.2 channels. Bath application of U0126 for 30 min resulted in a decrease of the amplitude of b-APs to 81 ± 3% of control at RMP (n = 10, P < 0.0001), in keeping with the endogenous phosphorylation of Kv4.2 channels under physiological conditions (Adams and Sweatt 2002; Yuan et al. 2002). U0126 also shifted θ by 5.1 ± 1.0 mV (n = 10, P < 0.0001) toward depolarized values (Fig. 8C). The distance in the dendritic tree where RMP was close to the new threshold after U0126 application was now reduced to 222 ± 31 μm from the soma.

Therefore the endogenous phosphorylation of Na⁺ and K⁺ channels extends the portion of the apical dendrite that sees large-amplitude b-APs.

**DISCUSSION**

We describe a distance-dependent threshold θ, that captures the onset of the transition from weak to strong or from strong to weak back-propagation in the main apical dendrite of CA1 pyramidal neurons. The transition from strong (mainly active) to weak (mainly passive) back-propagation occurs ~260 μm from the soma at RMP. The transition from strong (weak) to weak (strong) back-propagation can also take place anywhere along the dendritic tree following appropriately timed changes in membrane potential Vm with the direction of the transition depending on the difference between Vm and θ, reflects the availability of ionic channels at any dendritic location, in particular Na⁺/K⁺ channels, and can be dynamically modified. A change in
Na+/K+ channels availability changes \( \theta_x \), in particular the dendritic region where \( \theta_x = \text{RMP} \), and thus the degree of penetration of the dendritic tree by large b-APs. This allows us to propose a general framework for back-propagation.

**Back-propagation in dendrites**

The state of the membrane potential and back-propagating action potentials are in constant interaction. On the one hand, the amplitude of a b-AP at any dendritic location depends on the availability of ionic channels at this location, such as the density of Na\(^+\) and K\(^+\) channels, their activation state (open, inactivated, closed), and their phosphorylation level. On the other hand, a b-AP activates voltage-gated Na\(^+\), K\(^+\), and Ca\(^{2+}\) channels. The state of the membrane is changed thereafter as some of these channels may remain inactivated, or as Ca\(^{2+}\) entry may in turn activate Ca\(^{2+}\)-dependent channels and trigger various intracellular second messengers. Therefore b-APs can modify subsequent events including later b-APs, synaptic inputs, and synaptic plasticity. The manner in which a b-AP modifies the state of the membrane at a given dendritic location depends on several parameters that characterize the b-AP, including its rise time, decay phase, and amplitude. We have focused on the amplitude of the b-AP because of the direct relationship between the potential reached by the membrane at the peak of the b-AP and the opening of the various voltage-gated channels. The availability of voltage-gated channels in dendrites depends on their density, their state of activation/inactivation, and their voltage ranges for activation/inactivation. Opening, inactivation, de-inactivation, and closing of ionic channels are all voltage-dependent processes. A change in membrane potential will thus modify the respective ratio of open/inactivated/closed channels (Pan and Colbert 2001). Previous studies have reported that small-amplitude b-APs can be boosted when appropriately timed with membrane depolarization and that the amplitude of large b-APs could be decreased after membrane hyperpolarization (Magee and Johnston 1997; Stuart and Hausser 2001; Tsubokawa and Ross 1996). Here, we have characterized the membrane potential dependence of b-AP amplitude. We report that if the membrane potential at a given dendritic location is lower than \( \theta_x \), the amplitude of the b-AP is small and roughly constant across the range of potentials more negative than \( \theta_x \). We suggest that these b-APs correspond to a mostly passive back-propagation because the Na\(^+\) channel blocker TTX does not affect their amplitude. In contrast, the amplitude of the b-AP becomes larger as the difference between membrane potential and \( \theta_x \) increases. We suggest that when \( V_m \) becomes larger than \( \theta_x \), the active component of back-propagation becomes predominant be-
cause the amplitude of the b-AP becomes TTX-sensitive. The value of $\theta_e$ determines at which membrane potential the amplification of b-APs starts to occur in the distal part of the dendritic tree when b-APs and excitatory postsynaptic potentials (EPSPs) or membrane oscillations are appropriately timed (Johnston et al. 2000; Magee and Johnston 1997; Stuart and Hauser 2001).

A transition threshold was found in 62 of 65 dendritic recordings. Pyramidal neurons with split dendrites at a distance $>260 \mu m$ from the soma had a transition threshold regardless of which dendritic branch was recorded (the recording site was identified morphologically). In three instances ($>300 \mu m$ from the soma), membrane depolarization evoked an action potential before the arrival of the b-AP, and the transition threshold could not be measured. These recordings were not the most distal ones from our database.

We have found that the rising phase of the curve giving the amplitude of b-APs as a function of $V_m$ (duration of the transition zone) was variable even for dendritic recordings performed at similar distances from the soma, from step-like to smooth sigmoid-like functions. The parameters controlling the duration of the transition remain to be investigated. Small variations of membrane potential around $\theta_e$ can produce a jitter of b-AP amplitude that depends on the length of the transition zone.

Theta is a meta-parameter that takes into account the state of ionic channels. Because voltage-gated channels interact with each other, it is very difficult to establish the exact contribution of one type of channel to a b-AP. Not surprisingly, because back-propagation is primarily controlled by $Na^+$ and $K^+$ channels, a modification of their availability modifies $\theta_e$. This was demonstrated using a variety of approaches including a decrease in the number of channels (via their partial blockade), their inactivation (via repetitive stimulation), and a change in their activation curve (via phosphorylation-dephosphorylation). It is tempting to correlate the linear increase of $\theta_e$ with the linear increase of $A$ type of $K^+$ channels as a function of the distance from the soma (Hoffman et al. 1997). However, because the distribution and properties of the various types of $Ca^{2+}$ channels also vary along the dendritic tree (Magee et al. 1998), the contribution of these channels to $\theta_e$ remains to be investigated.

The existence of a location-dependent transition threshold in the dendrites means that at a given distance $\theta_e = RMP$. We have found a value of $x = 260 \mu m$, suggesting that at this distance there is a transition from predominantly active to predominantly passive back-propagation at RMP. This cut-off distance is very close to the $280 \mu m$ value found in vivo above which b-APs have a small amplitude (Kamondi et al. 1998a). The dispersion of b-AP amplitudes between 200 and 260 $\mu m$ in Fig. 1 is consistent with the jitter of amplitude expected to occur in this dendritic region. The dispersion may reflect the fact that the state of ionic channel may be different from one cell to another at similar recording locations, e.g., different phosphorylation levels. Therefore in CA1 pyramidal cell dendrites, b-APs decrease in amplitude as they travel away from the soma under a combination of factors including dendritic morphology (Vetter et al. 2001), the increase of A-type $K^+$ channels (Hoffman et al. 1997), and the value of $\theta_e$ at any dendritic location (present study). The large variability of amplitudes recorded in distal dendrites previously reported (Golding et al. 2001; Johnston and Spruston 1992; Magee and Johnston 1995; Tsubokawa and Ross 1996) may stem from the difference between RMP and $\theta_e$ in each experimental condition.

**Functional consequences**

In vivo recordings indicate that membrane potential can fluctuate between $-80$ and $-40 \text{mV}$ (Kamondi et al. 1998a,b). Because most values of $\theta_e$ (90%) are bounded by these two potentials, switching between weak and strong back-propagation is physiologically relevant. A stronger depolarization is required as the distance with the soma increases for the transition to occur. This condition can be met by appropriately timed EPSPs and b-APs because the increase in amplitude of dendritic EPSPs with the distance from the soma (Magee and Cook 2000) could compensate for the decremental back-propagation. This would constitute another normalization process in the dendrites (Magee 1999; Magee and Cook 2000). We have also observed the switch from large- to small-amplitude b-APs in more proximal parts of the dendritic tree using membrane potential oscillation or hyperpolarization. The functional consequences for limiting the invasion of the dendritic tree by b-APs remain to be investigated.

Numerous hippocampal functions are associated with rhythmical oscillations at various frequencies (Freund and Buzsáki 1996) for which dendrites may play an important role (Kamondi et al. 1998b; Magee 2001). b-AP timing in the oscillation and the frequency of the latter will determine the degree of invasion of the dendritic tree because close to the trough or the peak of the oscillation, b-APs will have a small or a large amplitude, respectively.

Repetitive firing is also part of the cell repertoire found in vivo. The progressive inactivation of $Na^+$ channels makes each b-AP have its own $\theta_e$ value. This defines a window of membrane potential inside which only the first of two consecutive b-APs has a large amplitude. Outside this window, the amplitude of both b-APs is either small or large. This window may be important for temporal coding in dendrites (Williams and Stuart 2000).

The other important characteristic of $\theta_e$ is its plasticity. Many physiological factors can change the availability of ionic channels. This includes a change in their number (internalization/insertion) or of their activation/inactivation/de-inactivation curves via their numerous modulatory sites. For example, their phosphorylation or dephosphorylation, which can occur in many physiological (synchronous plasticity) and pathological (epilepsy and ischemia) conditions, will modify these curves and thus $\theta_e$. A change in $\theta_e$ is likely to alter information processing in the dendrites.

All these arguments emphasize the importance of understanding the dynamic character of back-propagation. Given the high rate of spontaneous excitatory activity received by pyramidal cells in vivo in certain conditions (Pare et al. 1997), large-amplitude b-APs may invade extensively the dendritic tree. Conversely, GABAergic activity-dependent oscillations (Buzsáki 2002) may limit this invasion (Tsubokawa and Ross 1996), although this will be dependent on the depolarizing or hyperpolarizing action of GABA in the dendrites (Gulledge and Stuart 2003). Finally, during high-frequency firing, the long recovery rate from inactivation of $Na^+$ channels (Colbert...
et al. 1997) is an efficient way to limit the invasion and thus the triggering of Ca-dependent modifications.

In conclusion, the existence of state-dependent and modifiable transition threshold endows the cell with the ability to dynamically fine tune the degree of invasion of the dendritic tree by b-APs and thus the amount of depolarization and Ca\(^{2+}\) entry. The transition threshold may be both a useful parameter for characterizing a dendritic tree as well as a critical parameter for dendritic information processing.

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**DISCLOSURES**

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