

Slow Recovery from Inactivation of Na⁺ Channels Underlies the Activity-Dependent Attenuation of Dendritic Action Potentials in Hippocampal CA1 Pyramidal Neurons

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Na⁺ action potentials propagate into the dendrites of pyramidal neurons driving an influx of Ca²⁺ that seems to be important for associative synaptic plasticity. During repetitive (10–50 Hz) firing, dendritic action potentials display a marked and prolonged voltage-dependent decrease in amplitude. Such a decrease is not apparent in somatic action potentials. We investigated the mechanisms of the different activity dependence of somatic and dendritic action potentials in CA1 pyramidal neurons of adult rats using whole-cell and cell-attached patch-clamp methods. There were three main findings. First, dendritic Na⁺ currents decreased in amplitude when repeatedly activated by

brief (2 msec) depolarizations. Recovery was slow and voltage-dependent. Second, Na⁺ currents decreased much less in somatic than in dendritic patches. Third, although K⁺ currents remained constant during trains, K⁺ currents were necessary for dendritic action potential amplitude to decrease in whole-cell experiments. These results suggest that regional differences in Na⁺ and K⁺ channels determine the differences in the activity dependence of somatic and dendritic action potential amplitudes.

Key words: potassium channels; modulation; whole-cell; cell-attached; electrophysiology; rat

Pyramidal neurons often initiate action potentials in the axosomatic region (Turner et al., 1991; Stuart and Sakmann, 1994; Spruston et al., 1995; Colbert and Johnston, 1996b). Once the action potential fires, it propagates not only along the axon but also “backward” throughout much of the dendritic arbor (Turner et al., 1991; Spruston et al., 1995). These backpropagating dendritic action potentials are primarily mediated by Na⁺ ions but drive an influx of Ca²⁺ ions that may provide a sufficient postsynaptic signal for associative synaptic plasticity (Jaffe et al., 1992; Markram and Tsodyks, 1996; Magee and Johnston, 1997). Unlike somatic action potentials, which remain relatively constant in amplitude, dendritic action potentials display an activity-dependent decrease in amplitude that is voltage-dependent and slow in its recovery (Callaway and Ross, 1995; Spruston et al., 1995; Tsubokawa and Ross, 1996a). These decreases in amplitude are accompanied by reduced Ca²⁺ influx (Callaway and Ross, 1995; Spruston et al., 1995) and are, therefore, likely to determine some of the time- and activity-dependent properties of synaptic plasticity. The mechanism of the decrease in dendritic action potential amplitude, however, is not known. A number of candidate mechanisms have been considered in the theoretical literature (Migliore, 1996).

The present study was aimed at identifying properties of voltage-gated ion channels that might underlie the activity-dependent decrease in dendritic action potential amplitude. We hypothesized that repetitive activation of voltage-gated channels, as would occur during a train of dendritic action potentials, might

alter channel properties. Using cell-attached patch-clamp techniques, we observed isolated currents in patches from the soma and dendrites of hippocampal pyramidal neurons under conditions of repetitive activation. Dendritic K⁺ currents showed no activity-dependent change in amplitude during trains of brief depolarizations. Dendritic Na⁺ currents, however, displayed a striking decrease in amplitude during such trains. The recovery from this attenuation was slow and voltage-dependent. Somatic Na⁺ currents also decreased during trains but to a much lesser degree. These results represent the first electrophysiological evidence of regional differences in somatic and dendritic Na⁺ channel properties in pyramidal neurons (cf. Stuart and Sakmann, 1994; Magee and Johnston, 1995).

The finding that somatic Na⁺ currents decrease during trains, although to a lesser degree than dendritic Na⁺ currents, seemed inconsistent with the constant somatic action potential amplitude reported previously (Callaway and Ross, 1995; Spruston et al., 1995; Tsubokawa and Ross, 1996a). Thus, we tested whether factors other than the decrease in Na⁺ current might contribute to the decrease in dendritic action potential amplitude. We found that the relationship between Na⁺ current and action potential amplitude became stronger as the Na⁺/K⁺ permeability ratio decreased. Thus, in the dendrites, in which the density of A-type K⁺ channels is high compared with the density in the soma (Hoffman et al., 1997), the effect of losing Na⁺ current during a train is more pronounced. Thus, regional differences in both density and electrophysiological properties of voltage-gated channels provide the basis for the different activity dependence of somatic and dendritic action potential amplitude.

Portions of this work have appeared in abstract form (Colbert and Johnston, 1996a).

MATERIALS AND METHODS

Preparation and solutions. The present study used 4–10-week-old male Sprague Dawley rats. Animals were anesthetized with a lethal dose of a

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combination of ketamine, xylazine, and acepromazine. Once deeply anesthetized, they were perfused through the heart with cold, modified artificial CSF (ACSF) containing (in mM): 110 choline-Cl, 2.5 KCl, 1.2 NaH_2PO_4 , 25 NaHCO_3 , 0.5 CaCl_2 , 7.0 MgCl_2 , and 20 dextrose. After removal of the brain, 400- μm -thick slices were cut using a Vibratome (Lancer), incubated submerged in a holding chamber for 30 min at 32°C, and stored submerged at room temperature.

During the slicing procedure, the slices were maintained in the same ACSF used for the perfusion. The external recording solution contained (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 25 NaHCO_3 , 2.0 CaCl_2 , 1.0 MgCl_2 , and 25 dextrose. Slices were maintained in a submerged holding chamber in normal external recording solution. All external solutions were bubbled continuously with 95% O_2 /5% CO_2 . The internal pipette solution used for whole-cell recordings contained (in mM): 120 potassium gluconate, 20 KCl, 10 HEPES, 0.4 EGTA, 4.0 NaCl, 4.0 Mg_2ATP , 0.3 Mg_2GTP , and 14 phosphocreatine. pH was adjusted to 7.25 with KOH. The pipette solution for cell-attached patch recordings of Na^+ currents contained (in mM): 120 NaCl, 30 tetraethylammonium (TEA) chloride, 10 HEPES, 2 CaCl_2 , 3 KCl, 1 MgCl_2 , and 5 4-aminopyridine (4-AP). pH was adjusted to 7.4 with NaOH. For recording K^+ currents, the pipette contained (in mM): 125 NaCl, 10 HEPES, 2 CaCl_2 , 1 MgCl_2 , and 2.5 KCl, plus 1 μM tetrodotoxin (TTX). pH was adjusted to 7.4 with NaOH.

Recording techniques. Recordings were made from somata and dendrites of hippocampal CA1 pyramidal neurons. Neurons were visualized using infrared-illuminated, differential interference contrast (DIC) optics (Axioskop; Zeiss) according to standard techniques (Stuart et al., 1993). Whole-cell patch recordings were made using microelectrode amplifiers (Axoclamp 2A, Axon Instruments; IX2-700 Dagan Instruments) in bridge mode. Cell-attached patch recordings were made using a patch-clamp amplifier with a capacitive headstage (Axopatch 200A or 1D; Axon Instruments). Pipettes (3–5 M Ω for whole-cell and 7–12 M Ω for cell-attached) were made from borosilicate glass and pulled using a P-87 Flaming-Brown pipette puller (Sutter Instruments). Except as noted, cell-attached patch recordings were made submerged at room temperature (~25°C). Membrane potentials were determined by rupturing the patch after the channel data were recorded. Whole-cell recordings were made submerged at ~35°C. Whole-cell series resistance was 6–20 M Ω for somatic recordings and 15–50 M Ω for dendritic recordings. Whole-cell recordings were low-pass filtered at 3 kHz (6 dB/octave) and digitized at 10 kHz. Cell-attached patch recordings were filtered at 2 kHz (eight-pole Bessel filter) and sampled at 10 kHz. Data were digitized at 16-bit resolution (ADC488/16; IOTech) and stored by computer for off-line analysis (Next Computer). Antidromic action potentials were stimulated by constant current pulses (Neurolog; Digitimer Ltd. or WPI Instruments) through tungsten electrodes (AM Systems) placed in the alveus. In some experiments, trains of backpropagating action potentials were evoked by brief depolarizing current pulses through the somatic electrode. Significance was determined by *t* test with *p* < 0.05 considered significant. Data are reported as mean \pm SEM.

RESULTS

Dendritic action potential amplitudes decrease during trains

Simultaneous whole-cell recordings from the soma (Fig. 1A) and dendrites (Fig. 1B) of CA1 pyramidal neurons demonstrated a number of differences between somatic and dendritic action potentials. First, single action potentials were larger in the soma (88 ± 1.4 mV; *n* = 8) than in the apical dendrites at ~200 μm from the soma (50 ± 4.5 mV; *n* = 8). Second, evoked at rates of 5–50 Hz, action potentials maintained a relatively constant amplitude in the soma (V_m , Fig. 1A) but decreased with successive action potentials in the dendrites (V_m , Fig. 1B). The amplitude ratio of the 10th to the first action potential was $97 \pm 1\%$ (*n* = 8) in the soma and $59 \pm 2\%$ in the dendrites. The maximum rate of rise of the somatic action potential was more sensitive than its amplitude, revealing that the somatic action potential changed throughout the train (dV_m/dt , Fig. 1A,C). The dV_m/dt ratio of the 10th to the first action potential was $86 \pm 2\%$. In contrast, the rate of rise of the dendritic action potential (dV_m/dt , Fig. 1B) decreased similarly to its amplitude (Fig. 1C). The dV_m/dt ratio of the 10th to the first dendritic action potential was $50 \pm 2\%$. Thus,

action potentials displayed quite different activity dependencies in the soma and dendrites of CA1 pyramidal neurons.

Na^+ channel current decreases during trains of simulated action potentials

To identify the underlying changes in voltage-gated currents that determine the frequency dependence of action potentials during trains, we took the approach of observing pharmacologically isolated currents in cell-attached patches of somatic and dendritic membranes. Patches typically had 7–20 channels, estimated from peak currents and unitary current amplitudes (Magee and Johnston, 1995; Hoffman et al., 1997). We simulated trains of action potentials in the cell-attached patches by trains (20 or 50 Hz) of brief depolarizations (2 msec; Fig. 2A). Ensemble averages were constructed from 20–40 individual sweeps. The change in the amplitudes of the ensemble currents was quantified as the relative amplitude of the current evoked by the 10th step in the train compared with the amplitude of the ensemble current evoked by the first step.

The first channel type that we explored was the Na^+ channel. As described in our previous studies (Magee and Johnston, 1995; Colbert and Johnston, 1996b), we isolated Na^+ currents by including 4-AP and TEA in the patch pipette. Because of the fast kinetics of Na^+ channels, the channels activated and substantially inactivated during the 2 msec command potential (Fig. 2B). From a holding potential equal to the resting potential of the cell (approximately -67 mV), ensemble Na^+ currents decreased in amplitude during trains. Consistent with the change in dV_m/dt from the whole-cell recordings (compare Fig. 1), the decrease was modest in somatic patches but quite striking in dendritic patches (Soma and Dendrite, Fig. 2A,B). This difference was apparent at both 20 and 50 Hz frequencies. In the dendrites, the ratio of the 10th current amplitude to the first was $38 \pm 3\%$ (*n* = 16) during the 50 Hz train and $37 \pm 3\%$ (*n* = 11) during the 20 Hz train (Fig. 2C). Na^+ currents evoked in somatic patches decreased to $63 \pm 5\%$ (*n* = 11) at 50 Hz and $73 \pm 4\%$ (*n* = 12) at 20 Hz.

To determine whether the decrease in Na^+ current during trains was dependent on holding potential, we held the patch at 20–30 mV hyperpolarized to its rest potential. With the hyperpolarized holding potential, the Na^+ current decreased significantly less than when held at rest (Fig. 2C). In the soma the current decreased by the 10th step to $85 \pm 3\%$ (*n* = 4) at 50 Hz and to $84 \pm 3\%$ (*n* = 10) at 20 Hz. In the dendrites the current decreased to $49 \pm 6\%$ (*n* = 7) at 50 Hz and to $47 \pm 4\%$ (*n* = 5) at 20 Hz. Thus, the decrease in both somatic and dendritic Na^+ channels was voltage-dependent. The difference in the magnitude of the decrease in the soma and dendrites, however, could not be explained by differences in resting potential. Even when held at 20 mV hyperpolarized to rest, the dendritic Na^+ currents decreased more than the somatic Na^+ currents held at rest.

Spruston et al. (1995) reported that the recovery of action potential amplitude in the dendrites after a train of action potentials was slow and voltage-dependent. If the loss of Na^+ current during trains was responsible for the loss of action potential amplitude in the dendrites, then the recovery of the Na^+ current amplitude after a train should be slow as well. To test this hypothesis, we observed the recovery of Na^+ current amplitude after a 20 Hz train of brief depolarizations. After giving a train, we waited a variable length of time (100, 400, 800, or 1600 msec) and then gave a final brief test depolarization (Fig. 3A). The minimum duration before a new train was given was 5 sec. To quantify the time course of recovery, we first normalized the

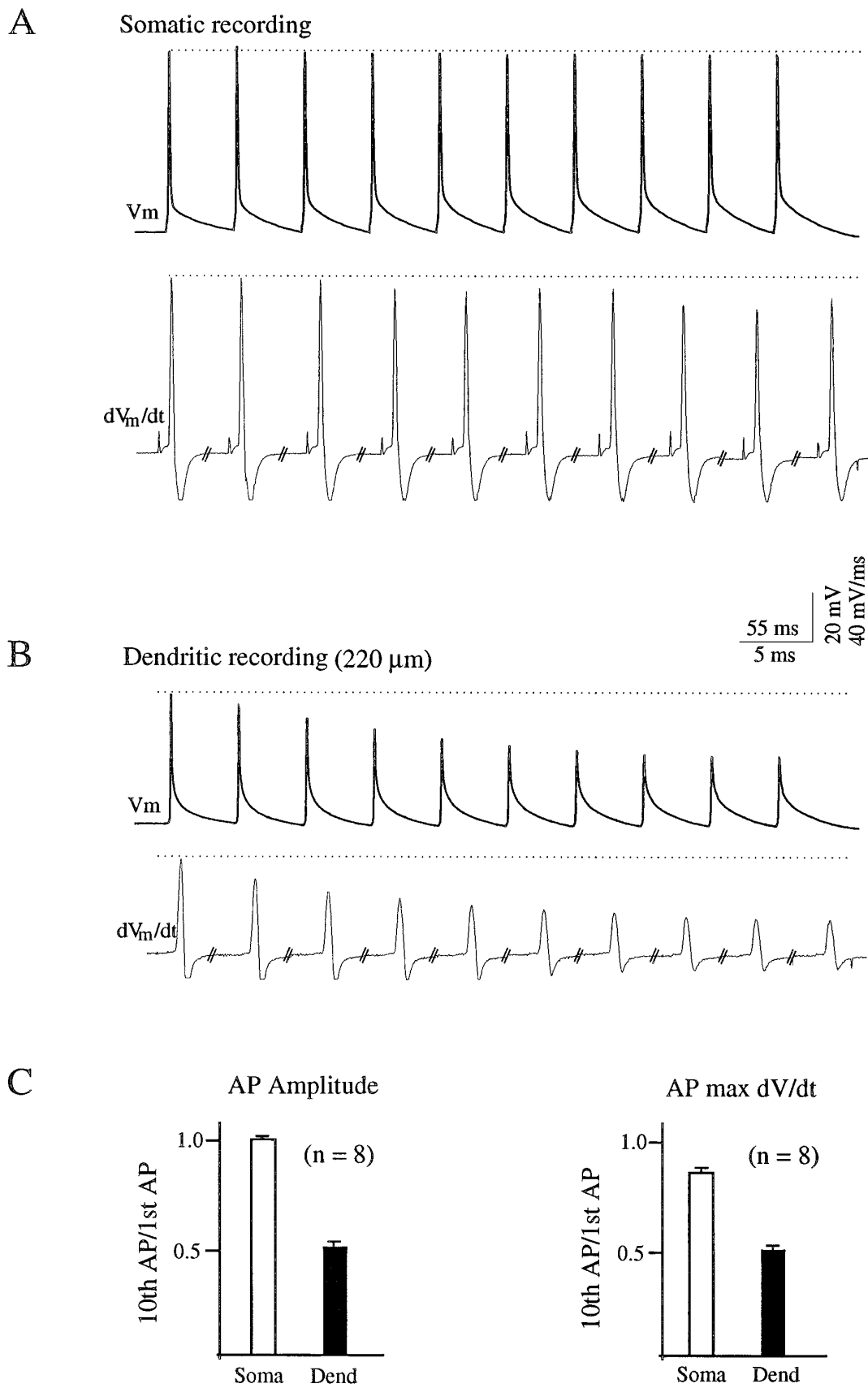


Figure 1. Dendritic action potential amplitude and rate of rise both decrease during repetitive activity. *A*, Membrane potential V_m and its temporal derivative dV_m/dt during a 20 Hz train of action potentials recorded from a CA1 pyramidal soma. Action potential amplitude does not decrease although maximum rate of rise decreases slightly during the train (*dashed line*, dV_m/dt of first spike). *B*, Membrane potential V_m (Figure legend continues)

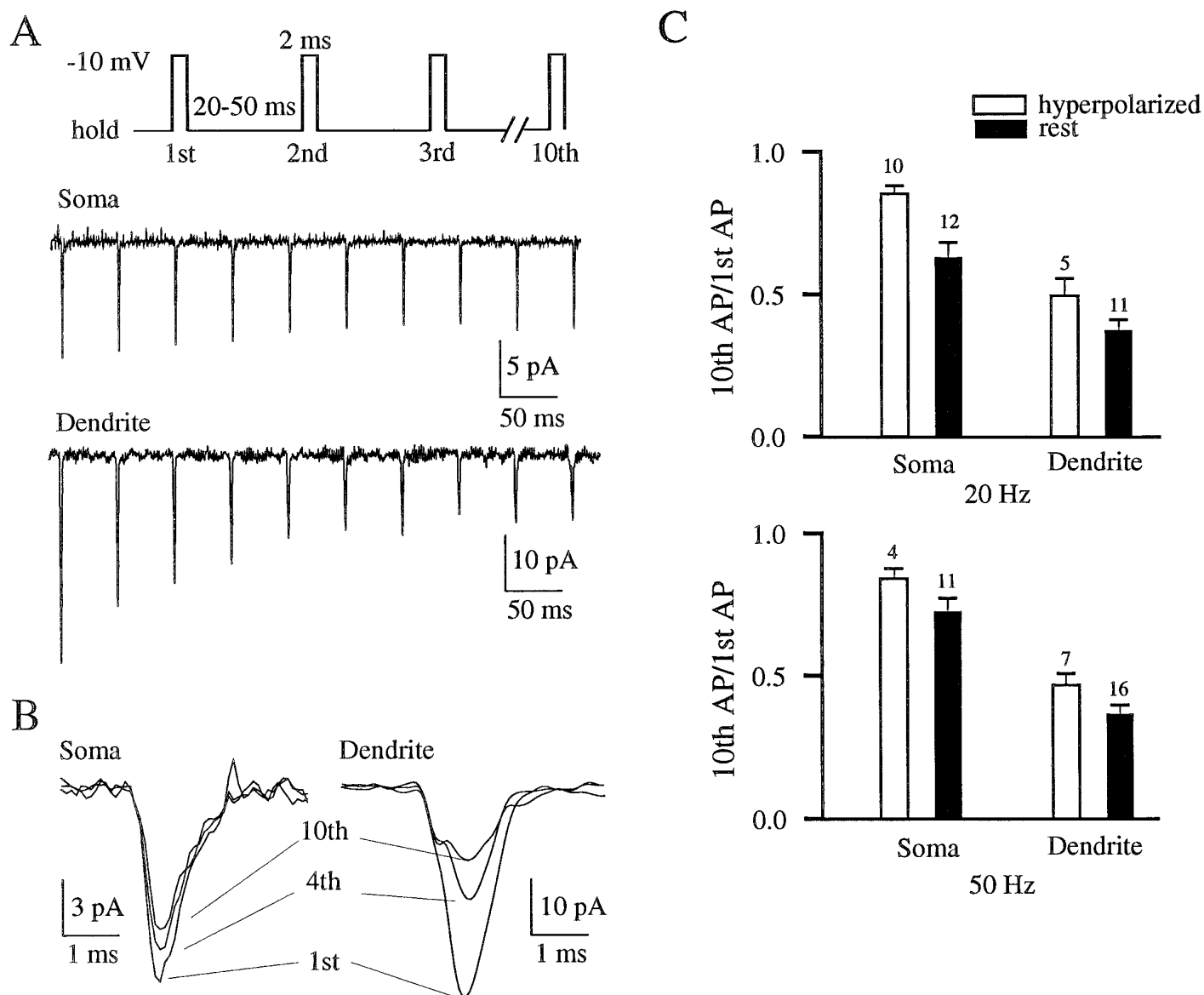


Figure 2. Repetitive activity decreases available Na^+ current. *A*, To simulate trains of action potentials, we gave trains of 10 depolarizing steps 2 msec in duration to cell-attached patches at a frequency of 20 or 50 Hz. Between depolarizations, the patch was held at either the resting potential of the neuron or 20 mV hyperpolarized to the resting potential of the neuron. 4-AP (5 mM) and TEA (30 mM) were included in the patch pipette to block K^+ channels. Soma waveform is a leak-subtracted ensemble average of currents evoked by trains applied to a somatic patch. The amplitude of the 10th evoked current is smaller than the amplitude of the first evoked current. Rest potential recorded after rupturing the patch was -63 mV. Dendrite waveform is a leak-subtracted ensemble average of currents evoked by trains applied to a dendritic patch. The amplitude of the 10th evoked current is greatly decreased compared with the first evoked current. Rest potential recorded after rupture of the patch was -67 mV. *B*, First, fourth, and 10th evoked currents from *A* at expanded (1 msec) time base. *C*, Group data. To quantify the decrease in current amplitudes during the train, we reported the amplitude of the current evoked by the 10th depolarizing step as a fraction of the amplitude of the current evoked by the first depolarizing step. *Top*, Group data for the 20 Hz train. *Bottom*, Group data for the 50 Hz train. Error bars indicate SEM, and the numbers above each bar indicate the numbers of patches. *Open bars* correspond to trains from a hyperpolarized holding potential. *Filled bars* correspond to trains from rest. Note that the decrease in Na^+ current was always greater in the dendrites. Note also that the decrease in both somatic and dendritic Na^+ channels was dependent on the holding potential.

and its temporal derivative dV_m/dt , during the same train of action potentials recorded simultaneously from the dendrite of the same CA1 pyramidal neuron used in *A*. In the dendritic recording, both action potential amplitude and maximum rate of rise drop significantly during the train (*dashed line*, maximum dV_m/dt of first spike). *C*, Group data comparing changes in somatic and dendritic action potential (AP) amplitudes and maximum rates of rise. Amount of change during the train is expressed by dividing the amplitude of the 10th action potential in the train by the amplitude of the first action potential (train frequency was 20–40 Hz). Note that derivative traces are on an expanded time scale compared with the voltage traces. *Hash marks* indicate where recordings between action potentials were removed to compress the trace. Error bars indicate SEM, and the numbers of recordings are shown in *parentheses*. All recordings were simultaneous somatic and dendritic recordings from the same neuron.

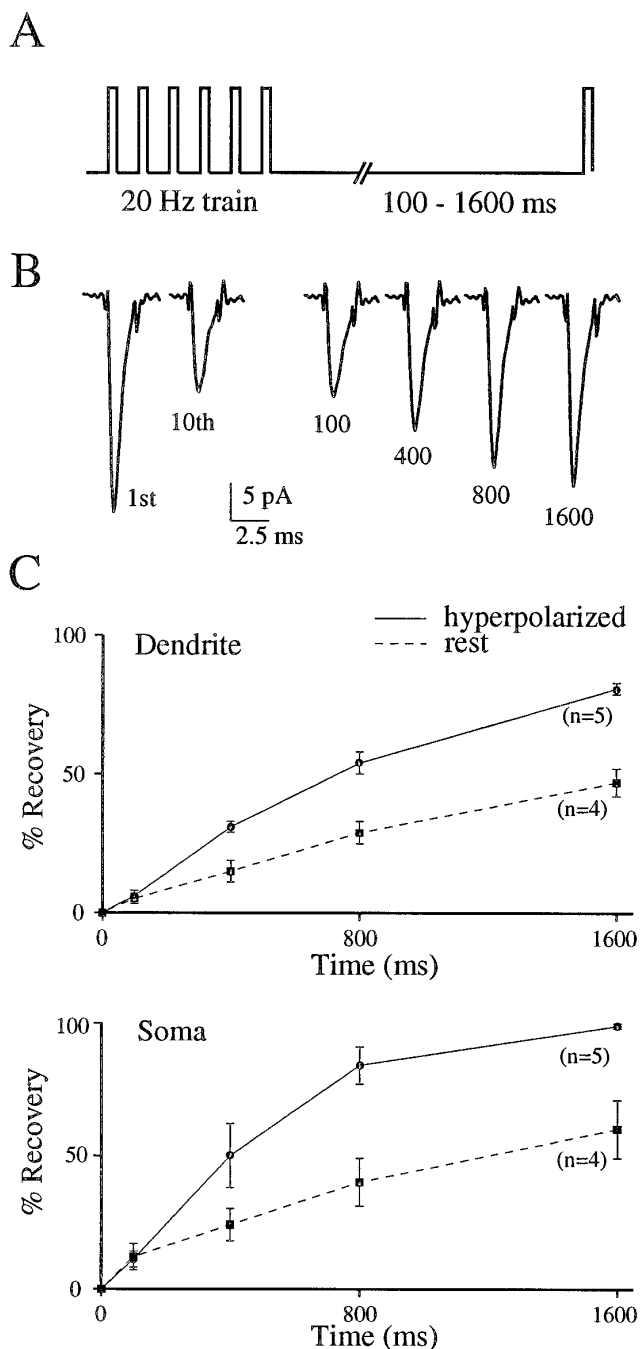


Figure 3. Recovery of Na^+ current after a train is slow and voltage-dependent. *A*, To measure the rate of recovery of available Na^+ current, we decreased Na^+ current to -10 mV by a 20 Hz train of 10 depolarizing steps each of 2 msec duration. Each train was followed by a single test depolarization after a wait of 100, 400, 800, or 1600 msec. *B*, Waveforms are leak-subtracted ensemble currents during the recovery paradigm. The first evoked current in the train represents the maximum (control) amplitude of the evoked current. At the end of the train, the 10th evoked current is decreased in amplitude. The amplitude of the current recovers as the duration of the waiting period (100–1600) is increased. *C*, Group data for recovery from holding potentials at rest and at 20–30 mV hyperpolarized to rest. *Top*, Dendritic patches. *Bottom*, Somatic patches. Values of the test currents are scaled between the amplitude of the current evoked by the 10th depolarizing step in the train (0% recovery) and the amplitude of the current evoked by the first depolarizing step (100% recovery). Note that the recovery of currents in both somatic and dendritic patches was slow and voltage-dependent. Error bars indicate SEM, and the numbers of patches are in parentheses.

amplitude of each test current in the train to the amplitude of the first current. We then scaled the amplitude of each test current (Fig. 3*B*) between the amplitude of the 10th current in the 20 Hz train (i.e., 0% recovery) and the amplitude of the first current in the 20 Hz train (i.e., 100% recovery). Finally, these normalized values were fit with single exponentials to determine an approximate time constant of recovery. Dendritic Na^+ currents recovered with a single-exponential time constant of 5.6 sec (Fig. 3*C*, *Dendrite*). Somatic Na^+ currents recovered with a single-exponential time constant of 4.1 sec (Soma, Fig. 3*C*). That the time constant of recovery for the Na^+ current was measured in seconds suggests that the decrease in Na^+ current was more consistent with reported slow inactivations of the Na^+ channel than with accumulation of fast inactivation (see Discussion).

To test for voltage dependence of recovery, we repeated the recovery paradigm using a holding potential 20–30 mV hyperpolarized from rest. At this holding potential, the rate of recovery increased in both somatic and dendritic patches (Fig. 3*C*). The single-exponential time constant of recovery was 0.79 sec for somatic patches and 2.2 sec for dendritic patches. As seen with the magnitude of the decrease in Na^+ current, the voltage dependence of recovery of Na^+ current seemed to differ between somatic and dendritic patches.

K^+ currents remain constant during trains

After identifying the activity dependence of Na^+ channels in cell-attached patches, we turned to the observation of K^+ channels. From a negative holding potential, a long step to +40 mV evoked an outward current with two major components: a fast transient A-type current and a smaller sustained delayed-rectifier-type (DR-type) component (Fig. 4*A*). We have characterized these currents previously and found them to be the dominant outward currents in dendritic patches from CA1 pyramidal cells (Hoffman et al., 1997).

As described for the Na^+ channels, we tested the activity dependence of the K^+ channels by simulating trains of action potentials with trains of brief depolarizations of dendritic cell-attached patches. Trains of 8–10 brief depolarizations from rest to +40 mV resulted in essentially no change in the evoked current between the first and the last depolarization (0.98 ± 0.02). To provide a better comparison with the Na^+ current experiments and to simulate a dendritic action potential, we repeated the trains in some patches from rest to -10 mV (Fig. 4*B,C*). We also tried a number of different combinations of frequency and duration of the steps (up to 30 msec; data not shown). In all cases there was no change in the outward current during the train. The outward current deactivates very rapidly at the end of the 2 msec depolarization, and there does not seem to be any residual current in the waveform between the depolarizing steps (Fig. 4*B,C*). These experiments additionally provide a useful technical control for the Na^+ channel experiments; there is no recording artifact that results in an apparent alteration of currents during a train.

Hyperpolarization between action potentials reduces the decrease in action potential amplitude during trains

The results of the cell-attached patch experiments suggested that trains of action potentials in CA1 pyramidal neurons lead to a slowly recovering, voltage-dependent decrease in available Na^+ current but no long-lasting change in K^+ currents. To test the role of this decrease in Na^+ current on action potential amplitude

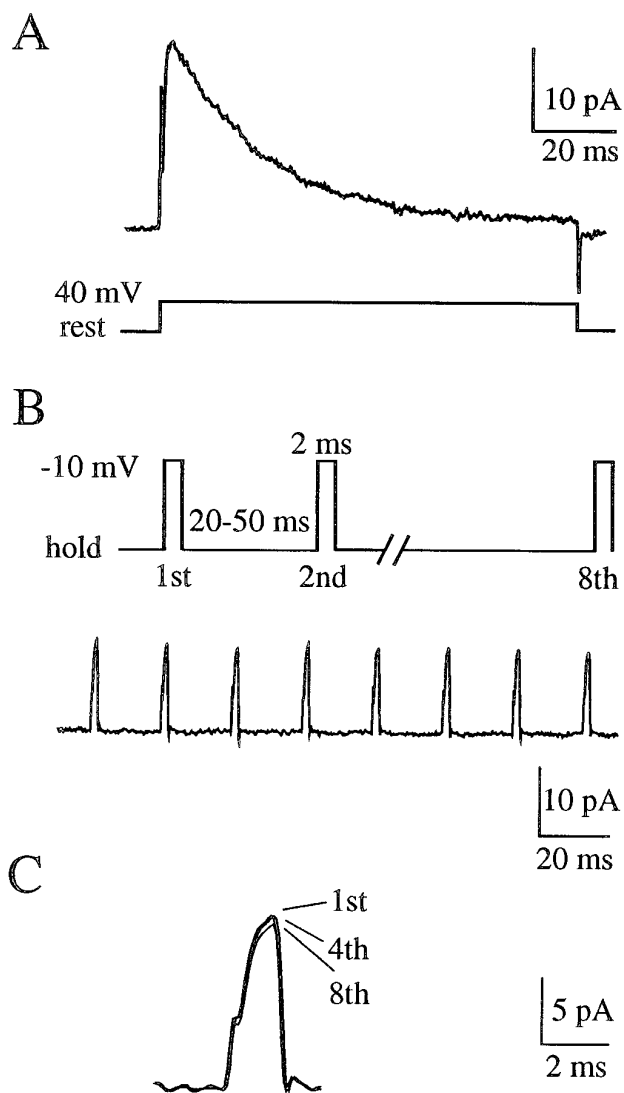


Figure 4. Repetitive activity does not alter available K^+ current. *A*, Waveform is a leak-subtracted ensemble average of K^+ current in a cell-attached dendritic patch ($\sim 150 \mu\text{m}$) evoked by a step to a depolarized command potential for 100 msec. The waveform demonstrates the early fast transient A-type current and a sustained DR-type current typical of dendritic patches. *B*, *Top*, To test whether repetitive activity alters available K^+ channels, we applied trains of brief depolarizations to a patch as described in *A* from a holding potential near rest to a potential of -10 mV . *Bottom*, Waveform is a leak-subtracted ensemble current during the train. Note that the currents rapidly inactivate at the end of each step and that there is no current between steps. *C*, First, fourth, and eighth evoked currents from *B* at expanded (2 msec) time base. Note in *B* and *C* that there is no alteration of current amplitude throughout the train. Note also that to account for a decrease in dendritic action potential amplitude during a train, the K^+ currents would be expected to increase in amplitude throughout the train.

during trains, we gave transient hyperpolarizing current injections, via the dendritic pipette, between each individual action potential in a train. Such membrane hyperpolarization should reduce Na^+ channel inactivation during the train, allowing action potential amplitude and maximum rate of rise to be maintained. Hyperpolarizations of 20–25 mV (Fig. 5*A*, arrow *S*) produced a graded effect on the attenuation of action potential amplitude, whereas even larger hyperpolarizations of 35–45 mV (Fig. 5*A*, arrow *L*) completely eliminated the decrease in action potential

amplitude ($3 \pm 2\%$ decrease; $n = 5$) and rate of rise ($11 \pm 7\%$ decrease; $n = 5$) that normally occurred during trains (Fig. 5*A*, arrow *C*). We repeated a similar paradigm at the same temperature as the whole-cell experiments using the cell-attached patches. Forty msec, 40 mV hyperpolarizing steps were given during the period between test commands to -10 mV in a train as described in Figure 2*A*. Without the hyperpolarizing steps, the attenuation was $55 \pm 3\%$ ($n = 3$). With the hyperpolarizing steps, the attenuation was reduced to $20 \pm 1\%$ ($n = 3$). The agreement between the channel data and the maximum rate of rise is consistent with the notion that the Na^+ channels underlie the activity-dependent decrease in action potential amplitude.

We have found previously that in CA1 pyramidal neurons the dendritic A-type K^+ channel density increases linearly with distance from the soma to reach a value fivefold higher than that found in the soma (Hoffman et al., 1997). Thus, we wanted to investigate the role of the relatively high density of dendritic A-type K^+ channels in the decrease of action potential amplitude during trains. The elevated A-channel density determines that dendritic membrane has a Na^+/K^+ permeability ratio that is significantly lower than that of somatic membrane. As a result, similar reductions in Na^+ current could potentially result in greater attenuation of action potential amplitude in the dendrites. We bath applied 4-AP (3–8 mM) to reduce the density of A-type K^+ channels, diminishing the somatodendritic gradient of Na^+/K^+ permeability. Cd^{2+} at $200 \mu\text{M}$ was also included to reduce Ca^{2+} spikes. After application of 4-AP, the decrease in action potential amplitude was reduced from $41 \pm 3\%$ to $15 \pm 2\%$ ($n = 5$; Fig. 6*A,B*), whereas the decrease in maximum rate of rise remained unchanged at $50 \pm 3\%$ ($n = 5$) for control versus $51 \pm 4\%$ ($n = 5$) with 4-AP application. These data suggest that without the high density of dendritic K^+ current, the decrease in Na^+ current during a train of spikes [which was unchanged by 4-AP application (see dV_m/dt plot in Fig. 4*B*)] is less effective in reducing action potential amplitude. Thus, along with the Na^+ channel inactivation observed in the dendrites, the elevated dendritic A-channel density enhances the decrease in action potential amplitude during trains.

To test this idea further, we lowered the relatively high Na^+/K^+ permeability ratio of the somatic membrane by applying a low concentration of TTX (100 nM) to the bath (Fig. 6*C,D*). TTX increased the drop-off in somatic action potential amplitude during a train from $4 \pm 1\%$ to $18 \pm 3\%$ ($n = 5$). Furthermore, there was a greater decrease in the maximum dV_m/dt ; $21 \pm 2\%$ ($n = 5$) for control versus $33 \pm 4\%$ ($n = 5$) with TTX application. Thus, lowering the Na^+ channel density of somatic membrane allowed the $\sim 20\%$ decrease in Na^+ current occurring during a train to have a more pronounced effect on somatic action potential amplitude. However, even in the presence of TTX, the decrease in somatic action potential amplitude was never as great as that recorded in the dendritic regions under control conditions (21% in the soma vs 41% in the dendrites).

DISCUSSION

In the present study, we have identified properties of ion channels that taken together predict the different activity dependence of action potential amplitude in the soma and apical dendrites of CA1 pyramidal neurons. First, repetitive activation of Na^+ channels leads to a loss of available Na^+ current that has a slow and voltage-dependent rate of recovery. The magnitude of this loss of current seems to be considerably greater in the dendrites than in the soma. Second, the degree to which action potential amplitude

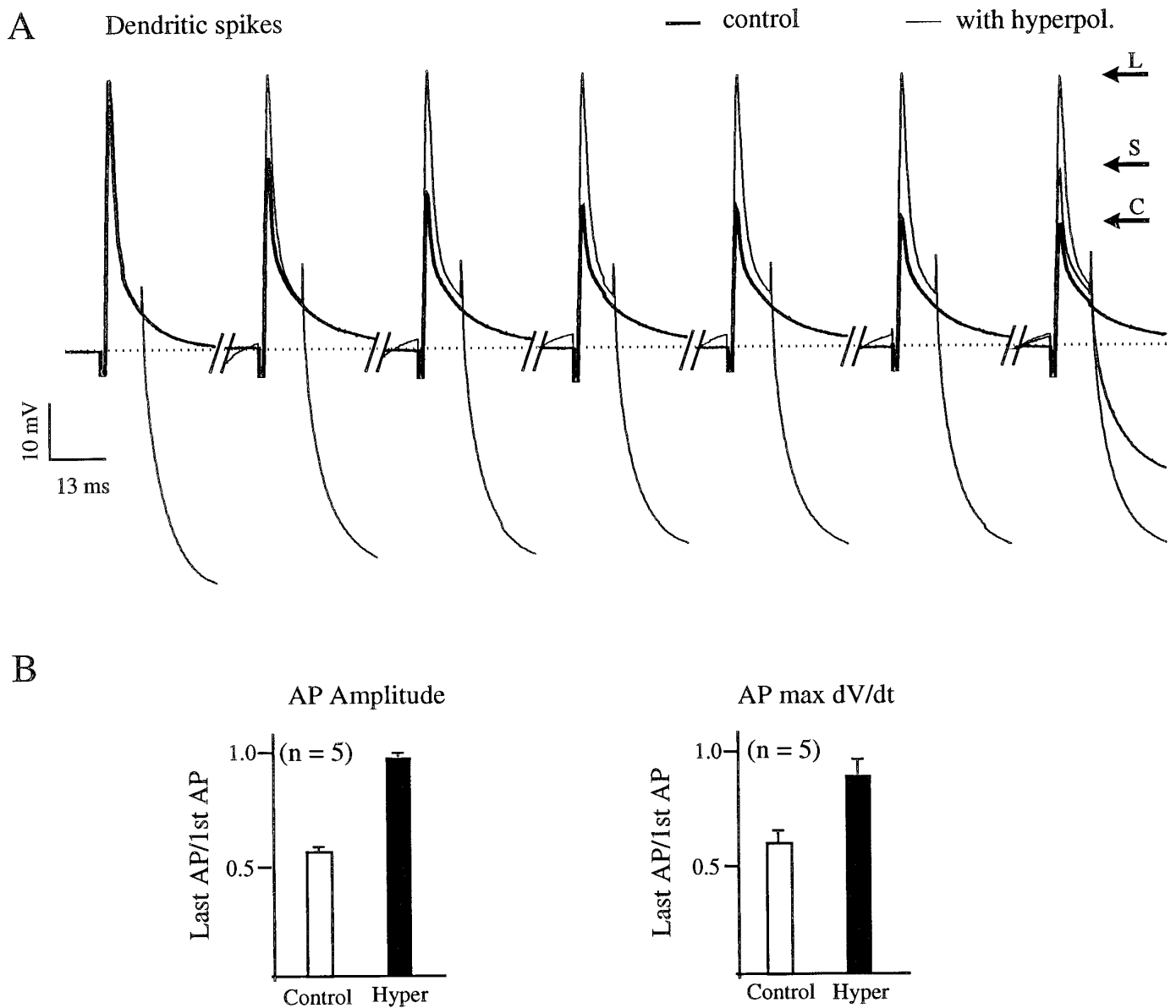


Figure 5. Membrane hyperpolarization modulates action potential amplitude decrease during trains. *A*, A train of action potentials (17 Hz) recorded from a CA1 dendrite ($\sim 200 \mu\text{m}$) with (light traces) and without (dark traces) the presence of membrane hyperpolarizations between the spikes. The largest hyperpolarizations (35–45 mV; 2.0 nA; 40 msec; arrow *L*) completely removed the decrease in action potential amplitude, whereas smaller hyperpolarizations (20–25 mV; 1.5 nA; 40 msec; arrow *S*) produced a graded effect on amplitude. The final action potential without interspersed hyperpolarizations is indicated by arrow *C*. *B*, Grouped data comparing changes in dendritic action potential amplitudes and maximum rates of rise with (open bars) and without (filled bars) ~ 40 mV hyperpolarizations between action potentials. Amount of change during the train is expressed by dividing the amplitude of the last action potential in the train (8th–10th) by the amplitude of the first action potential (train frequency was 20–40 Hz). Records were truncated between action potentials to compress the length of the trace. Error bars indicate SEM, and the numbers of recordings are shown in parentheses.

depends on the magnitude of Na^+ current is a function of the Na^+/K^+ permeability ratio. Therefore, the relatively high density of A-type K^+ channels and the relatively greater decrease in Na^+ current in the dendrites determine that backpropagation of action potentials into the dendritic arborization will be severely reduced during repetitive firing.

Slow Na^+ channel inactivation

Slowly accumulating inactivation and slow recovery from inactivation have been described in Na^+ channels from a number of preparations including skeletal muscle (Ruff et al., 1988), squid giant axon (Moore et al., 1964; Rudy, 1978), suprachias-

matic neurons (Huang, 1993), and most recently neocortical neurons (Fleidervish et al., 1996). Importantly, slow inactivation of Na^+ channels has traditionally been associated with long-sustained depolarizations. Because of this association, its role as a physiological or even pathophysiological process has been questioned (Cannon, 1996). In the present study, however, the slow inactivation state is rapidly entered (i.e., within a single train) and, thus, is likely to have a physiological role (see below).

Fleidervish et al. (1996) described previously a slow inactivation of Na^+ channels in somata of neocortical pyramidal neurons.

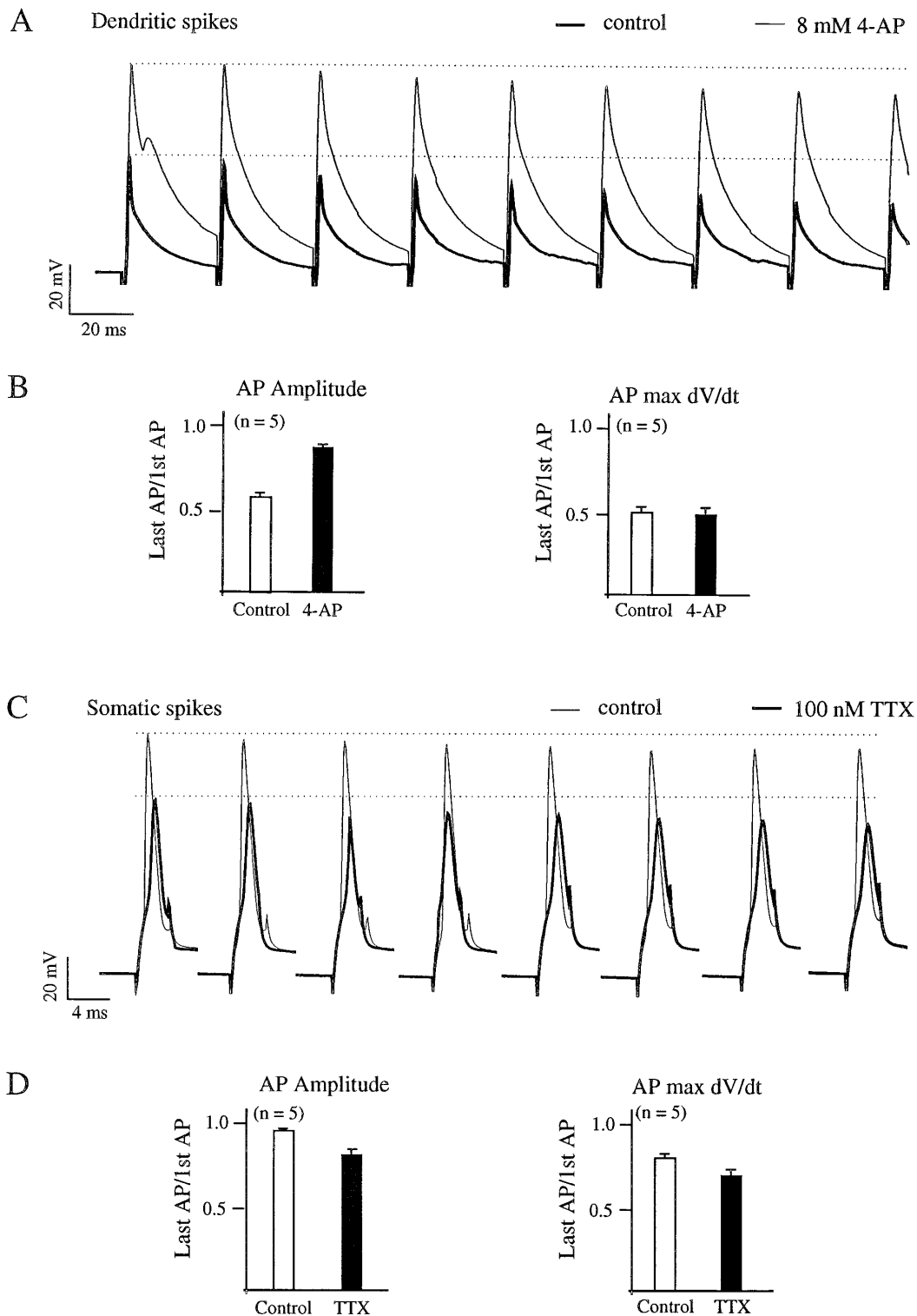


Figure 6. Manipulation of the Na^+/K^+ permeability ratio modulates action potential decrement during trains. *A*, A train of action potentials (33 Hz) recorded from a CA1 dendrite ($\sim 240 \mu\text{m}$) before (*dark traces*) and after (*light traces*) bath application of 8 mM 4-AP showing that high concentrations of 4-AP allowed action potential amplitude to remain fairly constant during repetitive stimulation. *B*, Grouped data showing that with a reduced K^+ permeability (*filled bars*) dendritic action potential amplitude remains fairly constant during a train, although the substantial decrease in maximum rate of rise remains unchanged (control, *open bars*). *C*, A train of action potentials (20 Hz) recorded from a CA1 soma before (*light traces*) and after (*dark traces*) bath application of 100 nM TTX showing that low concentrations of TTX increased the amount of action potential amplitude decrement during repetitive stimulation. *D*, Grouped data showing that with a reduced Na^+ permeability (*filled bars*) somatic action potential amplitude is substantially reduced during a train of repetitive activity, although the decrease in the maximum rate of rise is only slightly greater (control, *open bars*). Amount of change during the train is expressed by dividing the amplitude of the last action potential in the train (8th–10th) by the amplitude of the first action potential (train frequency was 20–40 Hz). Records from action potentials were truncated to compress the length of the *trace*. Error bars indicate SEM, and the numbers of recordings are shown in *parentheses*.

Using somatic recordings, they found that multiple long depolarizations (>500 msec) decreased somatic firing frequency. Using cell-attached patches on the soma, they found that a single depolarization to approximately -10 mV required 2 sec duration to inactivate 50% of the current. Recovery from this inactivation had a rate constant similar to that seen in the present study and, thus, may represent the same process. Fleidervish et al. suggested that such slow inactivation may explain the activity dependence of dendritic action potential amplitude (Callaway and Ross, 1995; Spruston et al., 1995). The present findings make this suggestion more plausible. First, we showed that a single train of brief depolarizations, rather than sustained depolarization, greatly decreases Na^+ current in dendritic patches. Second, we demonstrated that the relatively low K^+ channel density in the soma together with the more modest decrease in somatic Na^+ current predicts the modest activity dependence of somatic action potential amplitude. Importantly, this scheme reconciles the experimentally observed uniform density of Na^+ channels throughout the dendrites, soma, and initial segment (Magee and Johnston, 1995; Colbert and Johnston, 1996b) with the notion of different Na^+/K^+ permeability ratios in these regions. Traditionally, electrophysiological properties of pyramidal neurons consistent with a high Na^+/K^+ permeability ratio have been attributed to an extremely high density of Na^+ channels in the axon.

Differences in Na^+ inactivation between soma and dendrites

The observed differences in the attenuation of Na^+ current in the soma and dendrites imply regional differences in either the type of channels or their modulation. In previous electrophysiological studies of Na^+ channels in pyramidal neurons (Stuart and Sakmann, 1994; Magee and Johnston, 1995), no regional differences were noted in the steady-state or kinetic properties of activation or fast inactivation to suggest different channel types. Although there is evidence of segregation of Na^+ channel α -subunits within the neuron [i.e., a relatively high density of type I in the somatic region and a relatively high density of type II or IIA in fiber tracts (Westenbroek et al., 1989)], type IIA apparently accounts for $\sim 80\%$ of the total in all regions. Furthermore, because it is difficult to distinguish the presynaptic or postsynaptic location of the subunits in the dendritic fields, it is not clear whether the distribution of α -subunits can account for the regional differences in electrophysiological properties of the Na^+ channels reported here.

A number of modulations of the voltage dependence of activation and fast inactivation have been reported (reviewed in Catterall, 1992), including phosphorylation by protein kinase C (Renganathan et al., 1995; Cantrell et al., 1996) and by cAMP-dependent pathways (Li et al., 1992). Effects of these various pathways have been studied primarily in somata or in expression systems; thus differences in regional activity of the various modulators are not known. Regional differences in the activity of the various modulatory systems would provide an explanation for the differences in the magnitude of Na^+ current attenuation seen here without the need to invoke regional differences in Na^+ channel subtypes.

Functional consequences

The decrease in dendritic action potential amplitude represents a time- and activity-dependent process. It is important to note that the rates of action potential generation that decrease dendritic action potential amplitude include not only high frequency burst firing but also sustained firing that is not much above background

rates (Tsubokawa and Ross, 1997). Spikes that occur at 5–10 Hz will cause significant reductions in dendritic Na^+ current. Furthermore, these reductions will impact not only action potential amplitude but any process that depends on Na^+ current, such as boosting of EPSPs or setting thresholds for action potential initiation. Although we demonstrated that a significant block of A-type K^+ channels greatly minimized the effect of Na^+ current attenuation on spike amplitude, significant activity-dependent attenuation of dendritic spikes still occurs under less extreme conditions in which inactivation of A-type channels boosts the amplitude of the early spikes in the train (Andreassen and Lambert, 1995; Spruston et al., 1995).

A decrease in the influx of Ca^{2+} ions accompanies any decrease in dendritic action potential amplitude (Jaffe et al., 1992; Callaway and Ross, 1995; Spruston et al., 1995; Tsubokawa and Ross, 1996b). Therefore, any process that depends on the influx of Ca^{2+} will be affected by changes in dendritic action potential amplitude. Although the relationships between Ca^{2+} influx and processes such as long-term potentiation are not fully understood, the backpropagating dendritic action potential has been demonstrated to be an important postsynaptic signal for the induction of such processes (Magee and Johnston, 1997; Markram et al., 1997). Thus, it is probable that regulation of dendritic spike amplitude determines some of the activity dependence of the induction of various synaptic plasticities. The present result that both Na^+ and K^+ channels contribute to the expression of the decrease in dendritic action potential amplitude suggests two independent targets for modulation of dendritic action potential amplitude by second-messenger systems (Tsubokawa and Ross, 1997).

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