1 Title: Long-Term Inactivation of Sodium Channels as a Mechanism of Adaptation in CA1

2 Pyramidal Neurons

3 Abbreviated Title: Mechanisms of Adaptation in CA1 Pyramidal Cells

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Abstract

The hippocampus is involved in memory and spatial navigation. Many CA1 pyramidal cells function as place cells, increasing their firing rate when a specific place field is traversed. The dependence of CA1 place cell firing on position within the place field is asymmetric. We investigated the source of this asymmetry by injecting triangular depolarizing current ramps to approximate the spatially-tuned, temporally-diffuse depolarizing synaptic input received by these neurons while traversing a place field. Ramps were applied to rat CA1 pyramidal neurons *in vitro* (slice electrophysiology) and *in silico* (multi-compartmental NEURON model). Under control conditions, CA1 neurons fired more action potentials at higher frequencies on the up-ramp versus the down-ramp. This effect was more pronounced for dendritic compared to somatic ramps. We incorporated a five-state Markov scheme for Nav1.6 channels into our model and calibrated the spatial dependence of long-term inactivation according to the literature; this spatial dependence was sufficient to explain the difference in dendritic versus somatic ramps. Long-term inactivation reduced the firing frequency by decreasing open-state occupancy, and reduced spike amplitude during trains by decreasing occupancy in closed states, which comprise the available pool. PKC activators like phorbol ester phorbol-dibutyrate (PDBu) are known to reduce Nav long-term inactivation. PDBu application removed spike amplitude attenuation during spike trains *in vitro*, more visibly in dendrites, consistent with decreased Nav long-term inactivation. Moreover, PDBu greatly reduced adaptation, consistent with our hypothesized mechanism. Our synergistic experimental/computational approach shows that long-term inactivation of Nav1.6 is the primary mechanism of adaptation in CA1 pyramidal cells.

Significance statement (118 words)

The hippocampus plays an important role in certain types of memory, in part through context-specific firing of “place cells” that were first identified in rodents as cells that are particularly active when an animal is in a specific location in an environment, called the place field of that neuron. In this in vitro/in silico study, we found that long-term inactivation of sodium channels causes adaptation in the firing rate that could potentially skew the firing of CA1 hippocampal pyramidal neurons earlier within a place field. A computational model of the sodium channel revealed differential regulation of spike frequency and amplitude by long-term inactivation, which may be a general mechanism for spike frequency adaptation in the central nervous system.

Introduction

Area CA1 of the hippocampus is thought to play a key role in learning and memory, specifically episodic memories (Burgess et al., 2002), sequential order (Fortin et al., 2002; Hoang and Kesner, 2008) and trace conditioning (Shors, 2004; Hunsaker et al., 2006). A type of episodic memory involving the sequential order of places along a linear track has been well studied in rodents (O'Keefe and Nadel, 1978). Many CA1 pyramidal cells function as place cells (O'Keefe and Dostrovsky, 1971); they increase their firing rate as they traverse a place field. These fields are specific to a given environment (Colgin et al., 2008), and result from the activation of a particular subset of synaptic inputs by aspects of the place field in that environment. The firing rate of individual place cells is generally symmetric around a peak in the center of the place field, as recorded in head-restrained mice navigating a virtual spatial environment on a spherical treadmill (Harvey et al., 2009). However, intracellular recordings from the same mice revealed that traversing the place field evoked a depolarizing ramp of synaptic excitation that was not symmetric, but instead peaked after three quarters of the field had been traversed, such that the peaks of the firing rate and the underlying depolarization do not coincide. One explanation for this asymmetry is provided by the response of CA1 neurons to a temporally symmetric current ramp in anesthetized mice (Harris et al., 2002). The firing rate adapts such that the neuron fires substantially less on the down-ramp compared to the up-ramp. Such adaptation can explain how the firing rate peaks before the synaptic depolarizing ramp peaks. Although Harvey et al. (2009) observed a symmetric dependence of the firing rate on distance through the place field, this dependence becomes skewed in a predictive direction as the center of mass moves earlier in the place field as the environment becomes more familiar (Mehta et al., 2000), likely through synaptic plasticity mechanisms. Since adaptation is likely to contribute to the skew of the firing rate, here we investigate intrinsic mechanisms that determine firing rate adaptation in CA1 pyramidal cells.

Adaptation can result from accumulation of an outward current or a decrement in an inward current active in the inter-spike interval. The Ca^{2+} -activated small-conductance K^+ current (I_{SK})

(Stocker et al., 1999; Pedarzani et al., 2005) and the muscarinic M-type K^+ current (I_M) (Otto et al., 2006; Gu et al., 2008) are often implicated in firing rate adaptation in CA1 pyramidal neurons. Voltage-gated Na^+ channels in CA1 pyramidal neurons have previously been shown to exhibit long-term inactivation that increases with distance from the soma along the apical dendrite (Colbert et al., 1997; Jung et al., 1997), and the resulting decrease in the available pool of sodium channels has been suggested as a mechanism for frequency adaptation in the same neurons (Fernandez and White, 2010).

We systematically tested these possible mechanisms of adaptation using a combination of *in vitro* slice electrophysiology and computational modeling. We applied symmetric current ramps to the soma or apical dendrite of CA1 pyramidal cells to simulate the depolarizing synaptic input these cells receive *in vivo* while crossing a place field. Selective blockers of I_{SK} and I_M did not decrease adaptation. To investigate the putative contribution of long-term inactivation of Na^+ channels to adaptation in CA1 pyramidal neurons, we calibrated a Markov model of a Na^+ channel with a second inactivated state that recovered more slowly than the first, according to the spatially-dependent long-term inactivation shown in Mickus et al. (1999).

Experimentally, we observed that adaptation was more pronounced in the dendrites compared to the soma. The model demonstrated that the increase in occupancy in the long-term inactivated state of sodium channels along the apical dendrite was sufficient to account for this difference. The application of a PKC activator, known to decrease occupancy in the long-term inactivated state, significantly attenuated adaptation, as predicted by the model.

Materials and Methods

Experimental methods

Slice preparation. All the procedures described were conducted according to protocols approved by the Louisiana State University Health Sciences Center-New Orleans Institutional

Animal Care and Use Committee, following guidelines on the responsible use of laboratory animals in research from the National Institutes of Health. 7 to 11-week-old male Sprague Dawley rats were deeply anesthetized via intraperitoneal injection of ketamine and xylazine (90 and 10 mg/kg, respectively). Once the toe-pinch and palpebral reflexes ceased, rats were transcardially perfused with ice-cold oxygenated cutting solution containing, in mM: NaHCO_3 28, KCl 2.5, NaH_2PO_4 1.25, MgCl_2 7, CaCl_2 0.5, dextrose 7, sucrose 234, L-ascorbic acid 1, sodium pyruvate 3, and decapitated. The brains were rapidly removed and a vibratome used to cut 400 μm -thick transverse hippocampal slices that were then transferred to a chamber filled with an oxygenated artificial cerebro-spinal fluid (ACSF) containing, in mM: NaCl 125, NaHCO_3 25, KCl 2.5, NaH_2PO_4 1.25, MgCl_2 1, CaCl_2 2, dextrose 25, ascorbate 1, sodium pyruvate 3. After the cutting procedure, slices were allowed to recover for one hour at 36°C, with an additional recovery period of at least one hour at room temperature.

Patch clamp electrophysiology. Individual slices were transferred to a submerged recording chamber, and superfused with ACSF. All experiments were performed at 34-36°C. CA1 pyramidal cells were identified via differential interference contrast-infrared video microscopy. Whole-cell current clamp recordings were made using Dagan BVC 700A amplifiers in the active “bridge” mode. Recording pipettes had a resistance of 1-3 M Ω (for somatic recordings) and 3-5 M Ω (for dendritic recordings) when filled with an internal solution that contained, in mM: potassium methanesulphonate 125, KCl 20, HEPES 10, EGTA 0.5, NaCl 4, Mg_2ATP 4, Tris_2GTP 0.3, phosphocreatine 14. Cells with resting membrane potentials depolarized beyond -60 mV at break-in were discarded. Series resistance was monitored throughout the recordings and was usually less than 20 M Ω ; recordings were discarded when series resistance reached 25 M Ω or 30 M Ω , for somatic and dendritic recordings, respectively.

NBQX (10 μM), DL-APV (50 μM), Gabazine (12.5 μM) and CGP55845 (1 μM) were applied in the external solution in all experiments to block glutamatergic and GABAergic

neurotransmission, respectively, in order to isolate the contribution of the intrinsic ion channels to the ramp responses. Apamin (100 nM), XE-991 (10 μ M), phorbol-2,13-dibutyrate (PDBu, 5 μ M), and *Androctonus mauretanicus mauretanicus* toxin 3 (AmmTx3, 300 nM) were added to ACSF as needed, from stock solutions made with water or DMSO; the concentration of DMSO in the final solution was $\leq$ 0.1%. Gabazine and CGP55845 were purchased from Abcam (Cambridge, MA), DL-APV and XE-991 were from HelloBio (Princeton, NJ), NBQX, apamin and AmmTx3 were from Alomone Labs (Jerusalem, Israel), and PDBu was from Sigma (St. Louis, MO).

In order to approximate the depolarizing input that place cells receive as an animal traverses the place field, symmetric ramp-shaped depolarizing current injections were applied via the recording electrode to CA1 pyramidal neurons at either the soma or the apical dendrite (150-250 μ m from soma). Two second ramps (1 second up, 1 second down) and ten second ramps (5 seconds up, 5 seconds down) were delivered to simulate different running speeds. Current amplitude was adjusted to evoke peak frequencies between 10 and 25 Hz that resemble place cell firing as recorded *in vivo* (Hargreaves et al., 2007; Resnik et al., 2012; Bittner et al., 2015).

Experimental design and statistical analyses

Experimental data were recorded and analyzed with Igor Pro software (WaveMetrics). Asymmetry of action potential firing with respect to the depolarizing ramp was quantified as a spike ratio: the number of action potentials fired on the up-ramp divided by the number fired on the down-ramp (Harris et al., 2002). Plots of instantaneous frequency versus current (f/I) use the current value at the midpoint of the interspike interval (ISI). For experiments with PDBu, changes in the rate of rise of the action potentials during a spike train were compared between conditions by comparing the time derivative of the membrane potential dV/dt (Colbert et al., 1997), to estimate the degree of attenuation of sodium conductance along the train. As most ramps evoked at least 7 action potentials, a ratio was obtained of the maximum dV/dt for the 7th over the 1st action potentials during the spike trains.

A typical patch clamp electrophysiology experiment with a difference in means of 1.3-2X and a standard deviation of 0.2-0.3 requires $n = 6-12$ for $p = 0.05$ and a power of 0.9 (Cohen, 1977); each experimental group in this study is made up of $n \geq 9$ recording sessions. The number of cells recorded for each experiment is indicated in the Results section. Recordings were from one cell per slice; in general, a maximum of three recordings were obtained for each animal.

Statistical analyses were performed in SPSS (IBM, RRID:SCR_002865), following the tutorials and software guide from Laerd Statistics (2015, Statistical tutorials and software guides, retrieved from <https://statistics.laerd.com/>). Where possible, comparisons were made in the same cell before and after pharmacological treatment; the summary plots for these data include individual points and means $\pm$ standard error of the mean. For these experiments, parametric analyses (paired samples t-test and repeated-measures ANOVA) were used to compare treatments in the same neurons, since data were normally distributed as determined by the Shapiro-Wilk test for normality. Significant differences revealed by repeated measures ANOVA were followed up by post-hoc analysis using Bonferroni-corrected pair-wise comparisons. In the comparison between somatic and dendritic experiments, the Mann Whitney U test for unpaired data was used, since the data were not normally distributed. In this case, the summary plot consists of a box and whisker plot, including individual data. Differences were considered to be statistically significant when $p < 0.05$.

Computational modeling

A multicompartmental CA1 pyramidal neuron model from our lab was used as a starting point (Combe et al., 2018). This model was based on the (Poirazi et al., 2003a) model, with subsequent changes made in Shah et al. (2008) and Bianchi et al. (2012). The multicompartmental model from Combe et al. (2018) has 144 compartments in a reconstructed morphology (Megías et al., 2001) (Figure 1A); each compartment can be represented with an equivalent circuit (Figure 1B). Currents carried over from previous models include the leak

conductance, an A-type K^+ current (with different parameters for proximal and distal dendrites), a hyperpolarization-activated mixed cationic h-current, and T-type, R-type and L-type Ca^{2+} currents. Consistent with previous models, a 7-fold gradient in the h-conductance (Magee, 1998) and a 6-fold gradient in the A-type potassium conductance (Hoffman et al., 1997) were implemented along the apical trunk. In addition, in order to match the known lower amplitudes of back propagating spikes in the dendrites compared to the soma (Gasparini and Migliore, 2015), our model has a lower sodium conductance density in the dendrites compared to the soma (75% of the somatic value). The delayed rectifier in the previously cited models was replaced by separate models of K_v1 and K_v2 currents known to be present in CA1 pyramidal cells (Kirizs et al., 2014; Liu and Bean, 2014; Morgan et al., 2019). The K_v1 model was taken from Zbili and Debanne (2020) and the K_v2 model from <https://senselab.med.yale.edu/ModelDB/showModel?model=184176>. An inward rectifying potassium channel I_{KIR} was also added, consistent with the literature (Chen and Johnston, 2005): $I_{KIR} = \bar{g}_{KIR} \frac{(V-E_K)}{1+\exp\left(\frac{V-E_K+8.2}{10}\right)}$. For the simulations in this work, I_M and I_{SK} were removed from the model for reasons explained in the text. In the previous model, intracellular calcium decayed with first order kinetics to baseline values. Calcium dynamics are not central to this study, but we incorporated the calcium balance module in Ashhad and Narayanan (2013) to make this model suitable for future extensions that will rely on calcium dynamics.

To better model long-term inactivation of $Na_v1.6$, the dominant somato-dendritic isoform in these cells (Lorincz and Nusser, 2010), the fast Hodgkin-Huxley-type Na^+ current was replaced in the apical dendrites and soma with a Markov model modified from Balbi et al. (2017). The original fast Hodgkin-Huxley-type Na^+ currents were retained in the axon and basal dendrites in the absence of any data regarding long-term inactivation in those regions. This Markov model has 5 states: 2 closed states (C1 and C2), an inactivated state (I1), a long-term inactivated state (I2) and an open state O (Figure 1D1). Current can only flow through the open state, and transitions between initial state i and final state j are governed by voltage-dependent equations in which a

Boltzman function is multiplied by a scale factor, R_{max} , specific to each transition rate, that gives the maximum transition rate. $R_{i \rightarrow j} = R_{max} / (1 + \exp(-(V - V_H) / V_S))$.

The O-I1 and I1-O transition rates are the sum of two scaled Boltzman functions; the parameters are reported in Table 1. To calibrate the Markov model, we simulated the experimental protocol described in Mickus et al. (1999). To replicate the cell-attached patch-clamp data therein, a simulated voltage-clamp was applied to a single-compartment iso-potential model containing only the Markov model of the Na^+ channel. The model was held at -65 mV, and a 20 Hz train of ten 2-ms square pulses depolarized to 50 mV from rest was applied. The percentage steady-state inactivation was calculated as the difference in current amplitude between the first pulse and the tenth pulse divided by the amplitude of the first pulse. The transition rate for entry from I1 to I2 was calibrated in different spatial compartments to reproduce the observed increase in percent steady state inactivation with distance from the soma along the apical dendritic trunk as shown in Mickus et al. (1999). The following monotonically increasing expression for the maximum rate for the I1 to I2 transition produced the steady-state inactivation shown in Figure 1D3, with x representing distance from the soma in μm : for $x < 49.4$, $R_{max} = 0.0299 + 0.0707(1 - e^{-x/126})$, for $49.4 < x \leq 124.1$, $R_{max} = 0.0091 + 0.1360(1 - e^{-x/126})$, and for $x > 124.1$, $-0.0933 + 0.3(1 - e^{-x/126})$. Compare Figure 1D2 containing the data (reproduced with permission from the original Elsevier source, Mickus et al. 1999) with simulations in Figure 1D3.

	C1-C2	C2-C1	C2-O	O-C2	O-I1	I1-O	C2-I1	I1-C1	C1-I1	I1-I2	I2-I1
R_{max}	12	0.5	14	4	0.5 , 2.5	5e-4 , 25e-5	0.06	0.2	0.2	*	36e-5
V_H	-8	-50	0	-48	-42 , 10	-42 , 10	-65	-65	-65	-25	-50
V_S	-10	9	-6	9	12 , -12	12 , -12	-11	10	-11	-5	12

Table 1: Parameters for transition rates between Markov states. The asterisk indicates a distance-dependent parameter as described in the text. R_{max} is in units of ms^{-1} and V_H and V_S are in mV.

The passive properties of the model were adjusted consistently with the literature. To account for the additional membrane area due to the higher density of dendritic spines at distal

dendritic sites (Megías et al., 2001), a spine factor was incorporated into the model that multiplied the capacitance and divided the membrane resistance. This factor was 1.0 in the soma, the first 40 μm of the basal tree, and the first 100 μm of the apical trunk. In the apical tree, the factor stepped to 2.0 at 100 μm , then increased with a linear dependence up to 3.5 at the point where the apical dendrite branches into the tuft (394 μm in neuron pc2b). In the basal tree, the factor stepped to 3.5 at 40 μm from the soma. These values were based on spine densities reported by Megías et al. (2001). A sigmoidal decrease in membrane and axial resistance along the apical dendrites was implemented to further account for the decrease in input resistance along the apical dendrites (Magee et al., 1998; Poirazi et al., 2003b).

To replicate the experimental ramp protocol in the multicompartmental model, enough current was injected in the model to hold the membrane potential at -60 mV, and all transients were allowed to equilibrate before the application of the symmetric current ramp. These ramps, of two and ten second durations, as described above, were applied in the soma, and at 219 μm from the soma in the dendrites for the morphology in Figure 1A. The amplitude of the ramp was adjusted to reach similar peak frequencies as in the experiments, 10 to 12 Hz in the long ramps and 15 to 20 Hz in the short ramps. For the simulations, spike threshold was defined as the voltage at which the second temporal derivative of the membrane potential exceeded 20 mV/ms^2 .

Software accessibility

Model code is freely available and can be downloaded from ModelDB at: <http://modeldb.yale.edu/267140>, access code: adaptationmodel.

Results

In vivo patch clamp recordings in rats on a linear track revealed that the synaptic input received at the soma of CA1 pyramidal cells while traversing the neuron's place field resulted in

a hill-shaped depolarization above the baseline (Epsztein et al., 2011) that generally closely followed the shape of the cell's firing rate. Since there were no defined, repeatable shapes of this hill-shaped depolarization (Epsztein et al., 2011), in a first approximation to this spatially modulated synaptic input, we injected symmetric triangular-shaped ramps, two or ten seconds in total duration, either in the soma (Figure 2A), or in the apical trunk at ~200 μm from the soma (between 140 and 240 μm , Figure 2B). The different durations could roughly correspond to different running speeds. For the two second ramp in the soma (Figure 2A1), there are clearly fewer spikes on the down-ramp compared to the up-ramp, which is quantified in the summary data of the ratio of spikes on the up-ramp to spikes on the down-ramp in Figure 2C1. Moreover, the inset that plots the instantaneous frequency versus the current (f/I) clearly shows hysteresis; the frequency on the down-ramp is always slower than that on the up-ramp at the same average level of depolarizing current. Concomitantly, firing initiates at a lower current amplitude than it terminates. This type of f/I plot characterizes rate adaptation (Venugopal et al., 2015). The ten second somatic ramps (Figure 2A2) show a similar pattern. The dendritic response to a two second ramp (Figure 2B1) exhibits a much more pronounced decrease in spike height during the train as well as more pronounced spike rate adaptation than the somatic ones. A Mann-Whitney U test was run to compare the ratios of spikes on the up-ramp to spikes on the down-ramp between somatic and dendritic injections, since the data were not normally distributed. For the two second ramps, the distributions of the dendritic and somatic ratios were not similar, as assessed by visual inspection. The ratio for dendritic ramps (mean rank 42.00) was significantly higher than that for somatic ramps (mean rank 22.91), $U = 795$, $z = 4.129$, $p < 0.001$; Figure 2C1). As with the shorter ramps, there is a much more pronounced decline in spike height in the dendrites than in the soma during the ten second ramps; however, in this case a substantial recovery of spike amplitude occurs on the down-ramp (Figure 2B2). The frequency, however, does not recover on the down-ramp, indicating that these two aspects are regulated somewhat independently, as we later demonstrate. A Mann-Whitney U test was run to compare the ratios of

spikes on the up-ramp to spikes on the down-ramp between somatic and dendritic injections for ten second durations, since the data were not normally distributed. In this case, the distributions of the dendritic and somatic ratios were similar, as assessed by visual inspection. Median ratio was significantly higher for dendritic than somatic ramps (2.12 vs. 1.66, $U = 370$, $z = 3.019$, $p = 0.003$; Figure 2C2).

Since the SK and M-type potassium currents are likely candidates to contribute to rate adaptation, we applied pharmacological blockers of each current separately and then together, and recorded responses in the apical dendrite where adaptation is most prominent (Figure 3). We have previously shown that the SK current prevents CA1 neurons from following fast (70-100 Hz) trains of Schaffer collateral stimulation but not 40 Hz stimulation (Combe et al., 2018). In this set of experiments with current ramp injections, the SK current blocker apamin (100 nM) did not occlude firing rate adaptation (Figure 3A) and the spike ratios were not significantly different (3.70 ± 0.67 under control conditions and 3.38 ± 0.62 in the presence of apamin, $t(9) = -1.411$; $p = 0.192$, $n = 10$, Figure 3D). The M-type K^+ channel blocker XE-991 (10 μ M) caused the cells to fire faster at the onset of spiking (Figure 3B), but did not significantly affect the spike ratio (3.75 ± 0.74 under control conditions and 3.84 ± 0.72 in the presence of XE-991, $t(8) = 0.434$, $p = 0.676$, $n = 9$; Figure 3E). When both blockers were applied simultaneously, they still failed to occlude spike rate adaptation (Figure 3C); in fact, they appeared to enhance adaptation. Even with a lower amplitude current ramp, the firing frequency on the up-ramp was consistently high in the presence of apamin and XE-991, and cells tended to fire more action potentials on the up-ramp than they did in control. Thus, the spike ratio increased from 2.97 ± 0.28 in control conditions to 3.85 ± 0.47 in the presence of apamin and XE-991, $t(10) = 2.349$, $p = 0.041$, $n = 11$; Figure 3F). The voltage waveforms are different when both K^+ currents are blocked, in that the after-hyperpolarizations (AHPs) are shallower (see inset in Figure 3F). I_{SK} and I_M govern different parts of the AHP (Gu et al., 2005). When only one of them is blocked, the other appears to compensate to some degree.

When both are blocked, unconstrained spiking on the up-ramp significantly increases the spike ratio.

The observations that adaptation is more pronounced in the dendritic than in the somatic ramps, and that dendritic ramps show a prominent decrease in spike height along the train made us hypothesize that the two features could be related. The decrease in spike height during the train in the dendrites is known to be due to long-term inactivation of Na^+ channels (also called slow, cumulative inactivation, see Colbert et al. (1997) and Jung et al. (1997); in order to add this feature to our model, we replaced the fast Hodgkin-Huxley-type Na^+ current in the apical dendrites and soma with a Markov model modified from Balbi et al. (2017, see Methods). The previous studies in CA1 neurons often refer to slow, cumulative inactivation (Jung et al., 1997; Mickus et al., 1999). We and others (Dover et al., 2010; Navarro et al., 2020) call this process long-term inactivation instead, in order to differentiate it from a separate process called slow inactivation, that requires seconds to develop (Fleidervish and Gutnick, 1996; Ulbricht, 2005). In contrast, the long-term inactivated state is entered rapidly (within milliseconds) when fibroblast growth factor homologous factors (FHF) bind to cytoplasmic domains of voltage-gated sodium channels (Goldfarb, 2012), but the recovery is slow, resulting in the longevity of the occupancy in the long-term inactivated state. We therefore simulated the typical ramp responses recorded experimentally under control conditions in this modified multicompartmental model containing the Markov model for the Na^+ channel; we also omitted the M-type and SK currents, since the above results indicated these currents did not substantially promote firing rate adaptation during the triangular ramp protocol.

Figure 4A shows two and ten second simulated somatic ramps. The ratio of spikes on the up-ramp to spikes on the down-ramp is ~ 2 for both ramps, and rate adapting f/I curves are also observed (insets in Figures 4A1 and 4A2), making both the voltage traces and the f/I plots qualitatively similar to those experimentally observed in Figure 2A. The state diagram plots

(Figures 4A3 and A4) show the fractional occupancy for each state of the Markov model for the Na^+ channels, i.e. the fraction of channels that occupy a given state, as a function of time; a color diagram of the transitions is shown as an inset at the right of Figure 4A4. By definition, the sum of the fractional occupancy in all states is equal to 1. The color code at the right of Figure 4A3 orders the states in order of increasing availability to open. In general, channels must progress from the fast-inactivated state (I1, orange) to the first closed state (C1, dark blue), then to the second closed state (C2, cyan) before opening (O, green). The least available state is the long-term inactivated (I2, magenta), since channels in this deeply inactivated state constitute a slow pool that is not readily available. Transitions from I1 to the open state are allowed, but at an extremely low rate (hence the red x indicating that this rate is very small in the inset in Figure 4A4), and transitions from C1 to the open state are not allowed (see Table 1). Therefore, in general, channels must progress from I1 (orange) to C1 (dark blue) then to C2 (cyan) before opening (O, green). The occupancy in the open state during the interspike interval (ISI) is too small to be observed when plotted on the same scale as the other states. For the two second ramp (Figure 4A3), occupancy in I2 accumulates during the spike train. Occupancy in I1 shows a pronounced increase during each spike, but is diminished by the end of the spike train, because a large fraction of channels become sequestered in I2.

Figure 4B shows two and ten second simulated dendritic ramps. The ratio of spikes on the up-ramp to spikes on the down-ramp is ~ 3 for the short ramps and still ~ 2 for the longer ramp, and rate adapting f/I curves were again observed (insets in Figures 4B1 and 4B2). The voltage traces and f/I curves are qualitatively similar to the experimental observations in Figure 2B. For short ramps, more adaptation is observed in the dendrites than in the soma. Notably, the simulations also capture the substantially steeper decrease in spike amplitude in the dendrites compared to the soma, consistent with the experimental observations in Figure 2. In addition, for longer ramps, the simulations capture the partial recovery of spike height on the down-ramp, while

the frequency keeps decreasing (compare Figure 4B2 to Figure 2B). One important contrast between the state diagram plots for somatic and dendritic current ramps is that for somatic ramps occupancy in I1 is always higher than in I2, whereas during dendritic ramps the I2 occupancy ends up exceeding that of I1. This is due to the spatial dependence of the parameter R_{max} that determines the occupancy in the I2 state (see Methods and Figure 1D3), and largely accounts for the differential effects of somatic versus dendritic current injection. Thus, the more prominent effects on both spike frequency and amplitude reduction in the dendrites are due to the greater build-up of occupancy in the I2 state in the dendrites as compared to the soma (compare magenta traces in Figures 4A and B).

During the short ramps, occupancy in I2 accumulates until the end of the spike train; in contrast, during the longer ramps, occupancy in I2 peaks and then declines before spiking ceases (compare Figure 4B3 and 4B4). For these longer ramps, as the frequency slows on the down-ramp, longer interspike intervals provide more time for long-term inactivation to be removed. For sufficiently long intervals, more long-term inactivation is removed during the ISI than is added during the preceding spike, allowing occupancy in I2 to decrease. As occupancy in I2 decreases, occupancy in I1 increases, shifting a fraction of channels from the slow pool to the available pool. The spike height, which decreases during the up-ramp because long-term inactivation is accumulating, recovers to some degree on the down-ramp, as occupancy in the I2 state starts to decrease. However, the frequency does not recover; in fact, it slows down even more as the spike height recovers. Our working hypothesis was that occupancy in the I2 state is responsible for both the decrease in spike height and the decrease in frequency; therefore, it was not immediately evident how these two quantities could be differentially regulated by occupancy in the I2 state.

An explanation for these counterintuitive findings comes from the observation that, as I2 occupancy decreases and I1 occupancy increases during the long ramps, occupancy in C1 increases as well (see dark blue trace in Figure 4B4). This suggests that the closed states, as

part of the readily-available pool, may play a role in the differential recovery of spike height and frequency. We looked more closely at the occupancy in various states during the longer ramps, using spike height as an additional measurement, and compared the differences between somatic and dendritic ramps by superimposing occupancy in the various states for the same levels of injected currents in the up- and down-ramps (Figure 5). These plots show that occupancy in each of the states follows a history-dependent (hysteretic) trajectory. If there were no hysteresis, occupancy on the up- and down-ramps at the same level of applied current would be identical. Figure 5A shows that the occupancy in the immediately available pool, consisting of channels in the C1 and C2 closed states (Baranauskas and Martina, 2006), is generally lower during the down-ramp than the up-ramp at comparable values of injected current. The hysteresis is more pronounced for the dendritic injections (Figure 5A2); moreover, the occupancy in the readily available states is, in general, lower in the dendrite than at the soma. At spike threshold, only the channels in the available pool (C1+C2 states) can be regeneratively recruited for the action potential upstroke. Therefore, in Figure 5B we plotted the spike height versus occupancy in the C1+C2 pool at spike threshold. The height was measured from threshold to peak in order to exclude contributions of the AHP to spike amplitude. Occupancy in the C1+C2 pool accurately predicted spike height, independent of whether injected current was increasing (up-ramp, filled circles) or decreasing (down-ramp, open circles), both for the soma (Figure 5B1) and for the dendrites (Figure 5B2). However, the dependence of the spike height on C1+C2 occupancy was much steeper in the dendrites than in the soma suggesting that a smaller change in C1+C2 occupancy can yield a larger difference in spike height during the spike train. Together with the larger hysteresis in occupancy states in the dendrites, this largely explains the greater decrement in spike height in the dendrites.

Given the dependence of dendritic spike height on the available pool in C1+C2, it is clear that the spike height in Figure 4B2 recovers during the down-ramp because C1+C2 is increasing

on the down-ramp (Figure 5A2), largely due to increases in C1 (dark blue trace in Figure 4B2), which is generally in equilibrium with I1. Recall that the decrease in I1 (and consequently C1+C2) is driven by the cumulative increase of occupancy in the slow pool (I2), as shown in Figure 4. We therefore looked at the occupancy in I2 for the superimposed up- and down-ramps. Again, the hysteresis is more prominent in the dendrites (Figure 5C2) than the soma (Figure 5C1). The differential regulation of the spike height and frequency by occupancy in the I2 state occurs in part because the frequency does not depend directly upon occupancy in C1+C2, which increases even as the frequency has yet to recover. Instead, the frequency depends on the very small fractional occupancy in the open state during the interspike interval (Figure 5D, with values during spikes removed). Occupancy in the open state during the interspike interval is generally lower for both soma (Figure 5D1) and dendrite (Figure 5D2) on the down-ramp compared to the up-ramp at comparable values of injected current, but again the effect is more prominent in the dendrite. Occupancy in the open state during successive interspike intervals also increases (but only slightly) on the down-ramp for the same reason the available pool increases, namely the decrease in occupancy in I2. However, the frequency, unlike the spike amplitude, does not depend solely on the occupancy in the states of the Markov model, but also on the injected current. Therefore the frequency does not recover because occupancy in the open state, although it increases during the down-ramp, is still below the values on the up-ramp, so the small increase is not sufficient to compensate for the constantly decreasing level of injected current on the down-ramp. Moreover, spiking ceases at higher values of injected current on the down-ramp than those that were required to initiate spiking on the up-ramp. A Markov model that differentiates between a fast and a slow pool as well as differentiating between a readily-available pool and the fast-inactivated pool easily accounts for this differential regulation of spike frequency and spike height, whereas we were never able to obtain these results with Hodgkin-Huxley type models. Our model describes well both frequency and height changes; the differential regulation of these two quantities initially appears counterintuitive and would be difficult to tease out experimentally.

In order to show that asymmetry and spike rate adaptation in the f/I plots in the model are unequivocally due to occupancy in the long-term inactivated state (I₂), we ran simulations with entry into that state blocked (Figure 6A and B). These simulations confirm that firing rate adaptation in the model is indeed due to this mechanism; the f/I plots cease to show any hysteresis and become linear instead, both for somatic (Figure 6A) and dendritic current injections (Figure 6B). Similar results were obtained with two additional morphologies (c80761 from Ishizuka et al. (1995) and pc1a from Megías et al. (2001); see extended data in Figures 6-1 and 6-2, respectively), to verify that this mechanism could be generalized. Since the model strongly suggests that spike rate adaptation is due to long-term inactivation of the sodium channels, and since phosphorylation by protein kinase C (PKC) has been shown to reduce long-term inactivation of dendritic Na⁺ channels (Colbert and Johnston, 1998), we attempted to reduce occupancy in the long-term inactivated state in CA1 neurons *in vitro* by applying a PKC activator, the phorbol ester phorbol-di-butyrate (PDBu, Figure 6C and D). As predicted, both somatic and dendritic ramps exhibit far less rate adaptation in the presence of PDBu compared to control. PDBu (5 μM) decreased the spike ratio in the soma (Figure 6C and E) from 2.31 ± 0.22 in control conditions to 1.37 ± 0.09 , $t(10) = -5.071$, $p < 0.001$, $n = 11$). PDBu also prevented the reduction in spike amplitude during dendritic ramps, consistent with an effect exerted via reducing long-term inactivation of sodium channels (Colbert and Johnston, 1998). In addition, the spike ratio for the dendritic recordings decreased from 3.48 ± 0.46 to 1.64 ± 0.16 , $t(9) = -4.278$, $p = 0.002$, $n = 10$, Figure 6D and F).

Since there are other targets of phosphorylation by PKC, most notably the A-type potassium current carried by Kv4.2 channels, which is expressed at higher densities in the dendrites of CA1 neurons (Hoffman et al., 1997; Hoffman and Johnston, 1998), we performed an additional set of experiments to rule out a contribution of the A-type current to the occlusion of firing rate adaptation and asymmetry. Figure 7 shows that the specific Kv4 blocker *Androctonus*

mauretanicus mauretanicus toxin 3 (AmmTx3, 300 nM) by itself has little effect on spike frequency adaptation or spike height (compare Figure 7B to 7A). When added in the presence of AmmTx3, PDBu 5 μ M still substantially reduced spike rate adaptation (Figure 7C) as quantified by the spike ratio (Figure 7E) and prevented the decrease in spike amplitude during a train. The spike ratio was significantly different across the three conditions according to a one-way repeated measures ANOVA ($F(2,16) = 9.231$, $p = 0.002$). Bonferroni-corrected post-hoc pairwise comparisons found no significant changes between the spike ratios under control conditions (3.46 ± 0.57) and in the presence of AmmTx3 (3.79 ± 0.68 , $p = 0.877$), but a significant decrease in the presence of PDBu (1.86 ± 0.16), with $p = 0.039$ and 0.034 , respectively, for $n = 9$. Figure 7D shows the profile of the rate of rise (dV/dt) of the dendritic voltage traces. The maximum rate of rise corresponds to the early part of the action potential and is dependent on the available Na^+ conductance (Fleidervish et al., 1996; Colbert and Johnston, 1998); the decrease in these peaks mirrors the decrease in spike amplitude along the action potential train evoked by the ramp. The maximum rate of rise decreased significantly along the train due to the progressively decreased availability of Na^+ channels during the train under control conditions and in the presence of AmmTx3, but far less in the presence of PDBu (Figure 7F). A one-way repeated-measures ANOVA found that the ratio between the 7th and the 1st dV/dt was significantly different between the three conditions ($F(1.102,8.819) = 134.262$, $p < 0.001$, $n = 9$). Bonferroni-corrected pairwise comparisons found no differences between control conditions (0.49 ± 0.04) and in the presence of AmmTx3 (0.49 ± 0.04 , $p = 1.00$), but a significant increase in the presence of PDBu (0.83 ± 0.02 , $p < 0.001$), suggesting a significant activity-dependent decrease in the fast sodium current in control and AmmTx3, that is prevented by PKC activation with PDBu. On the other hand, AmmTx3 clearly exerted an effect by broadening the AP (Figure 7G) as expected, given the important contribution of I_A via Kv4.2 channels to spike width in these neurons (Kim et al., 2005). These results support

our model prediction that entry of Na_v channels into the long-term inactivated state promotes firing rate adaptation in response to symmetric depolarizing input.

Discussion

Summary

In this study, we have shown that 1) experimentally observed adaptation in response to depolarizing ramps is more prominent in the dendrites than the soma, 2) adaptation is not mediated by the M-type and SK K^+ currents that mediate adaptation in other conditions, 3) adaptation is likely mediated by long-term inactivation of sodium channels, and 4) a phenomenological five-state Markov model calibrated with a distance-dependent long-term inactivation can account for the differences in adaptation between soma and dendrite, as well as for the differential regulation of spike height and frequency by long-term inactivation.

Significance

Since the excitatory inputs that mediate spatially-tuned firing of place cells are likely received in the dendrites, the adaptation mediated by long-term inactivation of Na^+ channels that we observe in the dendritic ramps likely affects the position of the center of mass of place fields, with implications for spatial coding. This study was performed in the context of a sustained depolarization that is thought to underlie activation of ensembles of CA1 place cells. However, the sequential activation of place cells along a linear track likely generalizes to sequential activation encoding other types of episodic memory (Wood et al., 1999), meaning that these findings may be important for understanding episodic memory in general. Also, gain control (Chance et al., 2002), or modulation of the slope of the frequency-current input-output relationship of a neuron, is a general feature of neural processing (Salinas and Thier, 2000); the spike frequency adaptation modulated by Na_v long-term inactivation is a form of divisive gain control as

sustained depolarization, and the consequent adaptation, can decrease the slope of the steady-state frequency-current relationship (Fernandez and White, 2010).

Previous work on long-term inactivation of Na_v channels in CA1 pyramidal cells

This work bridges the work by Fernandez and White (2010) and Venkatesan et al. (2014) using somatic recordings to suggest that long-term inactivation of sodium channels is responsible for frequency adaptation and the work by Colbert et al. (1997, 1998) showing that slow sodium channel recovery from inactivation is responsible for a decrease in amplitude during a train of dendritic back-propagating action potentials. Our modeling work unifies these phenomena with a common mechanistic explanation of how these two processes are differentially regulated.

Essential aspects of the phenomenological Markov model

Much more complicated Markov models of Na_v gating exist (Goldfarb, 2012; Navarro et al., 2020), but we attempted to use the simplest model possible to gain insight into long-term inactivation. We started with a published six-state model (Balbi et al., 2017) and removed one open state (Knowlton et al., 2021) leaving the five-state model as in Figure 1D1. Figure 8 recapitulates the main features of the model and how the various states/pools differentially regulate adaptation and spike height during the train. The first essential aspect of the model is that the slow pool (represented by a single state, I2, indicated in magenta in Figure 8) sequesters a fraction of the Na_v channels and renders them completely unavailable for hundreds of milliseconds. Channels are recruited into this state during a spike and recover slowly during the interspike interval (see Figure 4), allowing accumulation across multiple spikes. The choice to connect I2 to the fast pool via I1 rather than O, as some models do (Goldfarb, 2012; Navarro et al., 2020), is not fundamental but phenomenological and inherited from Balbi et al. (2017). The second essential aspect of the model is that the fast pool is not monolithic but separated into three conceptual pools: the fast-inactivated (I1) pool, the readily available (C1+C2) pool and the open pool. Therefore, including I2, a minimum of four pools of Na_v channels is required to explain our

results. Since the transition from C1 to C2 is fast, these closed states (indicated in shades of blue in Figure 8) are immediately available to be recruited into a regenerative, positive feedback loop that drives the spike upstroke, and as shown in Figure 5B, the occupancy in this pool uniquely determines spike height. The I1 fast-inactivated state constitutes its own pool (orange in Figure 8); it is not available immediately prior to a spike but can be quickly recruited into the available pool during the after-hyperpolarization and contribute to the following spike. Finally, the open pool (indicated in green in Figure 8) has up to 20% occupancy during spiking, but in the order of 0.01% during the interspike intervals, as shown in Figure 5D. The slow pool has more occupancy on the down-ramp than on the up-ramp at comparable values of injected current, due to the history of depolarization, therefore by a material balance the fast pool must have less occupancy on the down-ramp. We have shown that although hysteresis in I2 drives both decreases in spike height and in frequency, these aspects are differentially regulated by the available and open pools, respectively. As shown in Figure 4, the long-term inactivated state is entered quickly during a spike, and, if the subsequent interspike interval is too short to remove the amount of long-term inactivation induced by the spike, inactivation accumulates. However, for sufficiently long intervals, more long-term inactivation is removed during the ISI than is added during the preceding spike, allowing occupancy in the long-term inactivated state to decrease. As a consequence, occupancy in the readily available (C1+C2) pool increases, allowing spike height to recover somewhat on the down-ramp. In contrast to the complete dependence of spike height on the available pool, frequency depends not only upon the hysteresis in the open pool, but also on the injected current. Therefore, spike height can recover to some degree on the down-ramp while the frequency is still decreasing.

Contributions of SK and M-type potassium currents

Previous work has implicated the SK and M-type K^+ currents in spike frequency adaptation in CA1 pyramidal cells (Madison and Nicoll, 1984; Aiken et al., 1995; Peters et al., 2005; Otto et

al., 2006). Our previous work (Combe et al., 2018), showed that the SK current limited the spiking frequency of CA1 pyramidal cells in response to trains of Schaffer collateral stimulation. Action potentials failed on average for every other stimulus for > 50 Hz train in control, and blocking SK with apamin allowed the cells to follow the stimulus train more faithfully. However, at the firing frequencies (< 25 Hz) examined in this study, the SK K^+ current did not significantly contribute to adaptation (Figure 3A). In addition, blocking the M-type K^+ current with XE-991, allowed the initial spiking frequency to increase, but did not shift the spike ratio in our experiments (Figure 3B). Similarly, linopirdine, a specific M-type channel blocker, changed the time course of spike frequency adaptation in CA1 pyramidal cells, but not the degree of adaptation attained at steady state (Fernandez and White, 2010). In either case, if only one current was blocked, it appeared the other could compensate during the AHP, resulting in little effect on adaptation; on the other hand, combined SK and M-type K^+ current block increased adaptation, as visible from the increased spike ratio in the presence of apamin and XE-991 (Figure 3F). This counterintuitive effect may be due to a shallower after-hyperpolarization that 1) led to a general increase in excitability, allowing for a greater number of action potentials on the up-ramp and/or 2) removed less Na_v inactivation, and therefore ultimately decreased the fast pool.

Modulation

We have shown that adaptation can be modulated by phosphorylation state (Figure 6). Since phosphorylation states depend on the neuromodulatory milieu, adaptation may be plastic, which is relevant to spatial navigation; increasing adaptation moves the center of mass of the place field in a direction opposite the direction of motion, whereas decreasing adaptation moves it in the direction of motion. As stated above, there are also implications for gain control of the frequency-current relationship. Although we have modeled the transition rates between Markov states as parameters, in the context of neuromodulation the transition rates can be considered state variables whose values depend on the phosphorylation state of the channels.

Generality of this mechanism of spike rate adaptation

Adaptation due to long-term inactivation of Na_v channels has been observed in serotonergic raphe neurons (Navarro et al., 2020). Long-term inactivation of Na_v channels has also recently been demonstrated to control not only adaptation, but also entry into depolarization block in midbrain dopamine neurons (Knowlton et al., 2021). We conclude that long-term inactivation of Na_v channels may be quite generally utilized as a mechanism for spike frequency adaptation in the central nervous system.

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Figure 1. Model Schematic and Calibration. A. Morphology as implemented in the simulation package NEURON, showing the location of simulated current injection. Stimulus current was only injected either in the soma or apical trunk (219 μm from the soma). B. Circuit diagram for each compartment. C. Superimposed voltage traces resulting from a 500 ms, 200 pA hyperpolarizing step recorded experimentally (gray) or upon simulation (black) to determine the neuron input resistance. D. Sodium current model and calibration. D1. Five-state Markov model for NaV1.6; transition from the I1 to the I2 state is drawn in gray for emphasis. D2. Data on percent steady state sodium channel inactivation along the apical dendrite in CA1 pyramidal cells, slightly modified with permission from Figure 3B in the complete Elsevier source (Mickus et al., 1999). D3. Simulations were calibrated to fall within the envelope of the data in D2. Scale bars in D2 and D3 are 200 ms (horizontal) and 50 pA (vertical).

Figure 2. Asymmetric responses to symmetric triangular current ramps. A. Somatic injection. A1. Voltage trace recorded in the soma for a two second triangular current ramp injected in the soma. The inset shows the reciprocal of the interspike interval plotted versus the current injected at the midpoint of the interval; filled and open circles indicate interspike intervals on the up- and down-ramps, respectively. A2. Same as A1 but for a ten second ramp. B. Dendritic injection. B1. Voltage trace recorded for a two second triangular current ramp injected in the apical dendrite at about 200 μ m from the soma. B2. Same as B1 but for a ten second ramp. C. Summary data of the ratio of spikes on the up-ramp to spikes on the down-ramp for two second (C1) and 10 second (C2) ramps are represented as box and whisker plots. The

766 number of recordings is indicated for each group; all ratios are greater than one, indicating more
 767 action potentials on the up-ramp. * $p = 0.003$; ** $p < 0.001$

Figure 3. No clear effect of SK or M-type K^+ channel block on asymmetry of ramp responses

in the dendrites. Recordings were obtained at approximately 200 μ m from the soma along the apical dendrite. A. Voltage trace recorded in the apical dendrite for a two second triangular current ramp (shown beneath the voltage trace) injected in the apical dendrite in control (top) and in the presence of apamin (100 nM, bottom). Insets show the reciprocal of the interspike interval plotted versus the applied current at the midpoint of the interval as in Figure 2. B. Same as A for XE-991 10 μ M. C. Same as A for the combined effects of apamin 100 nM and XE-991 10 μ M. D-F. Spike ratio summary data for n = 10 neurons for apamin (D), n = 9 neurons for XE991 (E) and n = 11 for apamin + XE (F). The inset above F shows expanded traces, with first spikes aligned, from the areas indicated by the dotted boxes above (black control, gray in apamin+XE-991; scalebars

779 20 mV vertical and 50 ms horizontal). Dotted lines connect individual cells before and after
 780 addition of drug. Black squares with error bars represent group averages $\pm$ SEM. n.s. not
 781 significant; * denotes $p < 0.05$.

782

Figure 4. Model captures differences in somatic and dendritic responses in terms of Nav availability. A. Simulated voltage traces and the resulting occupancy in each state of the Nav Markov model for current ramps injected at the soma. A1. Somatic voltage response to a two

787 second current ramp (shown beneath the voltage trace). A2. Somatic voltage response to a ten
788 second current ramp (shown beneath the voltage trace). Insets are as in previous figures. Color
789 plots depict the state occupancy for the corresponding two second ramp (A3) and ten second
790 ramp (A4) and are color coded to match the Markov model schematic at right. B. Simulated
791 voltage traces and resulting state occupancy for current ramps injected in the apical trunk at 219
792 microns from the soma. B1. Dendritic voltage response to a two second current ramp. B2.
793 Dendritic voltage response to a ten second current ramp. Color plots of state occupancy (B3 and
794 B4) correspond to the two second dendritic injection and ten second dendritic injection,
795 respectively.

Figure 5. Differential regulation of spike amplitude and frequency arises from differences in Nav availability during ten second current ramp. Different parameters measured during

799 somatic injection are shown on the left and dendritic injection on the right. A. Hysteresis in
800 occupancy in the available pool of C1+C2 is visible during the ten second ramp, with availability
801 generally lower on the down-ramp (black) than on the up-ramp (gray). B. Occupancy in the
802 available pool (C1+C2) at spike threshold is strongly predictive of spike height, measured from
803 threshold to peak, irrespective of ramp direction (up, filled circles; down, open circles). C.
804 Hysteresis in occupancy in the slow, unavailable pool (I2) shows that inactivation is generally
805 higher on the down-ramp (black) than on the up-ramp (gray). D. Hysteresis in occupancy in the
806 open state, with higher occupancy on the up-ramp than the down-ramp, explains the lower
807 frequencies on the down-ramp. Values of occupancy during the spike were truncated for clarity
808 to better show the very small open fraction during the interspike intervals.

Figure 6. The model predicts and experiments confirm that reducing long-term inactivation reduces adaptation and asymmetry. A. Simulations of somatic current injection in control and with the I1 to I2 transition rate set to zero, meaning there is no occupancy in the long-term inactivated state. The insets show that adaptation and asymmetry are essentially eliminated by this manipulation, producing a linear f/I curve. B. Simulations of dendritic current injection in

control and with the I1 to I2 transition rate set to zero. Controls in A and B are repeated from Figure 4; stimulus current was injected either in the soma or apical trunk (219 μm from the soma). C. Example experimental traces showing the voltage response to somatic current ramp injection in control and with 5 μM PDBu. D. Example experimental traces for dendritic current injection before and after addition of 5 μM PDBu. E. Summary spike ratio data for soma ($n = 11$). F. Summary spike ratio data for dendritic ramps ($n = 10$). Dotted lines connect individual cell averages before and after addition of drug. Black squares with error bars represent group averages $\pm$ SEM. ** $p < 0.001$; * $p = 0.002$.

Figure 6-1. Extended data for Figure 6 with an alternate morphology. A. Simulations of somatic current injection in control (A1) and with the I1 to I2 transition rate set to zero (A2). The insets show that adaptation and asymmetry are essentially eliminated by this manipulation, producing a linear f/I curve. B. Simulations of dendritic current injection (at 192 μm from the soma) in control (B1) and with the I1 to I2 transition rate set to zero (B2). C) Morphology (http://neuromorpho.org/neuron_info.jsp?neuron_name=c80761 from (Ishizuka et al., 1995) as implemented in the simulation package NEURON, showing the location of simulated current injection. The point where the apical dendrite branches into the tuft is at 388 μm from the soma in this case. Stimulus current was injected either in the soma or apical trunk.

Figure 6-2. Extended data for Figure 6 with a second alternate morphology. A. Simulations of somatic current injection in control (A1) and with the I1 to I2 transition rate set to zero (A2). The insets show that adaptation and asymmetry are essentially eliminated by this manipulation, producing a linear f/I curve. B. Simulations of dendritic current injection (at 208 μm from the soma) in control (B1) and with the I1 to I2 transition rate set to zero (B2). C) Morphology

839 (http://neuromorpho.org/neuron_info.jsp?neuron_name=pc1a from Megías et al., 2001) as
 840 implemented in the simulation package NEURON, showing the location of simulated current
 841 injection. The point where the apical dendrite branches into the tuft is at 343 μm from the soma
 842 in this case. Stimulus current was injected either in the soma or apical trunk. In this case, the
 843 percent of inactivation in the model (see Figure 1D3) was uniformly decreased by 5% to increase
 844 firing with dendritic injection; the resulting plot was still in the envelope of the experimental data
 845 (see Figure 1D2).

Figure 7 PKC phosphorylation of Kv4 channels does not mediate the reduction in adaptation and asymmetry due to phorbol ester. Top. Two second dendritic ramps. Voltage traces, current ramps and f/I curve are as in Figures 2-4. A Control. B. AmmTx3 (300 nM) added to the bath. C. PDBu (5μM) in addition to AmmTx3. D. Traces at the bottom show the first temporal derivative of the voltage trace. E. Summary data for spike ratios. ** p < 0.001; * p < 0.05; n.s. not significant. F. Normalized peak first temporal derivative for seventh spike. G. AmmTx3 broadens the AP by slowing repolarization.

Figure 8. Summary diagram of the contributions of the various occupancy states to spike height (left) and adaptation/asymmetry. Details are in the discussion.