Activation of $I_h$ and TTX-sensitive sodium current at subthreshold voltages during CA1 pyramidal neuron firing

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Yamada-Hanff J, Bean BP. Activation of $I_h$ and TTX-sensitive sodium current at subthreshold voltages during CA1 pyramidal neuron firing. J Neurophysiol 114: 2376–2389, 2015. First published August 19, 2015; doi:10.1152/jn.00489.2015.—We used dynamic clamp and action potential clamp techniques to explore how currents carried by tetrodotoxin-sensitive sodium channels and HCN channels ($I_h$) regulate the behavior of CA1 pyramidal neurons at resting and subthreshold voltages. Recording from rat CA1 pyramidal neurons in hippocampal slices, we found that the apparent input resistance and membrane time constant were strongly affected by both conductances, with $I_h$ acting to decrease apparent input resistance and time constant and sodium current acting to increase both. We found that both $I_h$ and sodium current were active during subthreshold summation of artificial excitatory postsynaptic potentials (EPSPs) generated by dynamic clamp, with $I_h$ dominating at less depolarized voltages and sodium current at more depolarized voltages. Subthreshold sodium current—which amplifies EPSPs—was most effectively recruited by rapid voltage changes, while $I_h$—which blunts EPSPs—was maximal for slow voltage changes. The combined effect is to selectively amplify rapid EPSPs. We did similar experiments in mouse CA1 pyramidal neurons, doing voltage-clamp experiments using experimental records of action potential firing of CA1 neurons previously recorded in awake, behaving animals as command voltages to quantify flow of $I_h$ and sodium current at subthreshold voltages. Subthreshold sodium current was larger and subthreshold $I_h$ was smaller in mouse neurons than in rat neurons. Overall, the results show opposing effects of subthreshold sodium current and $I_h$ in regulating subthreshold behavior of CA1 neurons, with subthreshold sodium current prominent in both rat and mouse CA1 pyramidal neurons and additional regulation by $I_h$ in rat neurons.

ZD7288; tetrodotoxin; XE991; HCN channels; M current; synaptic integration; dynamic clamp; action potential clamp

VOLTAGE-DEPENDENT ION CHANNELS in mammalian neurons are activated not only during action potentials but also at subthreshold voltages, where such currents have important functions to control resting potential and regulate integration of synaptic potentials. Two types of current active at subthreshold voltages in many types of neurons are TTX-sensitive “persistent” sodium current ($I_{NaP}$; reviewed by Crill 1996; Stafstrom 2007, 2011), carried by members of the Na family of voltage-dependent sodium channels, and $I_h$, carried by HCN channels (reviewed by Robinson and Siegelbaum 2002; Biel et al. 2009; Santoro and Baram 2003; Shah 2014).

Both $I_{NaP}$ and $I_h$ pass inward currents at subthreshold voltages and thus have a depolarizing effect on membrane potential. Both $I_h$ and $I_{NaP}$ can sometimes be significantly activated even in the absence of exogenous depolarizing inputs and thereby help drive spontaneous pacemaking ($I_h$; Aponte et al. 2006; Day et al. 2005; Forti et al. 2006; Maccasferri et al. 1993; Maccasferri and McBain 1996; McCormick and Pape 1990; $I_{NaP}$: Bennett et al. 2000; Bevan and Wilson 1999; Butera et al. 1999; Taddese and Bean 2002). Despite this partial overlap in their function, however, the two currents have very different voltage dependence and kinetics. $I_h$ is activated by hyperpolarization and activates and deactivates with relatively slow kinetics (Aponte et al. 2006; Dougherty et al. 2013; Maccasferri et al. 1993; Maccasferri and McBain 1996; Magee 1998), while $I_{NaP}$ is activated by depolarization and has rapid kinetics of activation and deactivation (Carter et al. 2012; Park et al. 2013; Stafstrom et al. 1985). These differences in voltage dependence and kinetics undoubtedly give the two currents different functional roles in regulating dynamic subthreshold membrane behavior, particularly during rapid changes in membrane potential. However, quantifying the currents during physiological voltage trajectories is difficult. Computer models of channel behavior have given valuable insights into how they contribute to subthreshold behavior (George et al. 2009; Golomb et al. 2006; Hu et al. 2002; Hutcheon et al. 1996; Zemankovics et al. 2010) but of necessity are based on simplified kinetic models.

Here we have quantified the voltage- and time-dependent activation of $I_h$ and $I_{NaP}$ at subthreshold voltages during firing of CA1 pyramidal neurons by performing mixed current-clamp and voltage-clamp experiments. First, we characterized the magnitude and voltage dependence of $I_h$ and $I_{NaP}$ in voltage-clamp experiments using slow voltage ramps spanning the subthreshold voltage range traversed during physiological behavior. We then explored the contribution of the currents to the overall input resistance and membrane time constant of the cells in current-clamp experiments. Then, we quantified the currents through each of the conductances during naturalistic subthreshold voltage trajectories, performing voltage-clamp experiments using command waveforms of recorded voltage trajectories during action potential firing evoked by dynamic clamp simulations of a mixture of excitatory and inhibitory inputs or using experimental records of action potential firing of CA1 neurons in awake, behaving animals. We find that both $I_h$ and $I_{NaP}$ can pass significant currents at subthreshold voltages during naturalistic behavior, but with substantially different kinetic characteristics and different relative contributions in mouse and rat CA1 neurons.

METHODS

Slice preparation. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Harvard Medical School. Acute horizontal brain slices containing the hippocampus were prepared from Sprague-Dawley rats of either sex (postnatal days...
For experiments in Figs. 8 and 9 with in vivo voltage commands, slices were prepared from Swiss-Webster mice of either sex (postnatal days 14–21). Animals were anesthetized with isoflurane and decapitated. The brain was quickly removed and placed in an ice-cold sucrose slicing solution containing (in mM) 87 NaCl, 25 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 7.5 MgCl2, 75 sucrose, and 25 glucose, bubbled with 95% O2–5% CO2. A near-horizontal blocking cut was made along the dorsal side of the cerebral hemispheres, and tissue blocks were glued to the slicing chamber on this surface (Bischoffberger et al. 2006). Slices of 300–μm thickness were cut with a vibratome (DTK-Zero1, DSK, Dosaka, Japan or Campden 7000smz-2, Campden Instruments, Leicester, UK) and incubated for 45 min in a 34°C holding chamber containing artificial cerebrospinal fluid (ACSF) consisting of (in mM) 125 NaCl, 25 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 1 MgCl2, 2 CaCl2, and 15 glucose, bubbled with 95% O2–5% CO2. After incubation, slices were held in bubbled ACSF at room temperature for up to 5 h until recordings.

**Electrophysiological recordings.** For recording, slices were placed in a submerged slice chamber continuously perfused with ACSF at a rate of 1–3 ml/min and maintained at a bath temperature of 34°C. Neurons in the CA1 pyramidal layer were visualized with infrared-differential interference contrast imaging. CA1 pyramidal neurons were distinguished from other neurons in the CA1 region by size, shape, the presence of persistent sodium current, and a maximal firing rate below 50 Hz. Recordings were rejected if the series resistance was >14 MΩ, the input resistance was >200 MΩ, the resting potential was more depolarized than −62 mV, or the resting potential varied by >3 mV during the initial 3 min after breakthrough. To block synaptic transmission, all external solutions contained 10 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydronbenzol[1]quinoxaline-7-sulfonamide (NBQX), 5 μM 3-(R)-2-carboxypiperazin-4-yl-propyl-1-phosphonic acid [(R)-CPP], 100 μM picrotoxin, and 1 μM (2S)-3-[[1S]-1(3,4-dichlorophenyl)ethyl]-2-hydroxypropyl]([phenylmethyl) phosphinic acid hydrochloride (CGP55845).

Whole cell current-clamp and voltage-clamp recordings were made with a Multiclamp 700B amplifier (Molecular Devices) using borosilicate patch electrodes (1–3 MΩ). The internal solution consisted of (in mM) 122 K-methanesulfonate, 9 NaCl, 1.8 MgCl2, 4 Mg-ATP, 0.3 Na-GTP, 14 phosphocreatine, 0.09 EGTA, 0.018 CaCl2, and 9 HEPES, adjusted to pH 7.3 with KOH. Reported voltages were corrected for a −8 mV liquid junction potential between this solution and the ACSF in which the pipette current was zeroed at the beginning of the experiment.

Pipette capacitance was reduced by wrapping pipettes with Parafilm; residual capacitance was corrected with the capacitance compensation and neutralization features of the amplifier. Pipette series resistance (4–12 MΩ) was corrected with bridge balance in current-clamp experiments and compensated by 70% during voltage-clamp experiments. Current and voltage signals were filtered at 10 kHz and sampled at 20–50 kHz with either a Digidata 1322A data acquisition interface and pCLAMP 10 software (Molecular Devices) or a Power 1401-mk2 data acquisition interface and Signal 5 software (Cambridge Electronic Design).

**Simulated naturalistic synaptic stimulation using dynamic clamp.** To approximate natural synaptic input, we used the dynamic clamp function of the Power 1401-mk2 interface and Signal 5 software to deliver synaptic-like conductance waveforms while recording the cell’s voltage response. Three synaptic-like conductances were generated simultaneously, each with different kinetics and stimulation frequencies. Each conductance was generated based on a double-exponential function (Fricker and Miles 2000):

\[ f(t) = a \cdot (1 - \exp(-t / \tau_{rise}))^3 \cdot \exp(-t / \tau_{decay}) \]

The resulting single activation conductance waveform was delivered repetitively at various average frequencies with Poisson timings generated by the Python NumPy pseudorandom number generator, which was initialized with a known seed for repeatability. Stimulus activations that overlapped in time were summed linearly. Two of the simulated conductances were excitatory conductances with a reversal potential of −10 mV, and one was an inhibitory conductance with a reversal potential of −68 mV. The conductances were presented with a range of average frequencies. Kinetic parameters were based on previous experimental data in CA1 neurons (Fricker and Miles 2000):

- **excitatory synapse 1**, \( \tau_{rise} = 1.5 \) ms, \( \tau_{decay} = 10 \) ms, stimulus frequencies of 20 Hz, 40 Hz, and 60 Hz were increased every 5 s; 
- **excitatory synapse 2**, \( \tau_{rise} = 2.5 \) ms, \( \tau_{decay} = 15 \) ms, stimulus frequency of 40 Hz; inhibitory synapse, \( \tau_{rise} = 1.0 \) ms, \( \tau_{decay} = 5 \) ms, stimulus frequencies of 40 Hz, 60 Hz, and 80 Hz were increased every 5 s. These parameters were designed to create sufficient voltage fluctuations to roughly approximate a membrane under synaptic barrage.

The dynamic-clamp update frequency and the recording sampling frequency were both set to 50 kHz. Injected currents from the dynamic clamp were calculated based on the equation \( i(t) = g(t) \times (V_{m} - E_{rev}) \), where \( g(t) \) is the clamped conductance, \( E_{rev} \) is the reversal potential for the simulated synapse, and \( V_{m} \) is the measured voltage of the cell. During recording, all conductances were scaled in parallel so that a small number of action potentials were activated in each sweep. This strategy maximized the time at membrane potentials near threshold. The resulting voltage traces were then used in the action potential clamp configuration as a voltage clamp command for the same cell, and currents elicited by that voltage trace were recorded and sampled at 50 kHz.

**Voltage clamp using in vivo recorded traces.** Intracellular voltage traces from mouse CA1 pyramidal neurons in awake mice were kindly provided by Christopher Harvey (Harvey et al. 2009, Supplementary Fig. 7) for use as voltage commands during whole cell recordings from mouse CA1 pyramidal neurons in acute slices. The in vivo traces were recorded in current clamp at 20 kHz and consisted of an 11-s region without current injection, followed by an ascending and a descending ramp of current lasting 2 s each. For consistency, a single trace was used as the voltage command across all cells. The trace was selected because of a prominent subthreshold oscillation, stable average membrane potential, and minimal spiking before the current ramp region. The full voltage trace was converted to a voltage command and shifted by +8 mV to compensate for the junction potential correction. Current responses were sampled at 20 kHz. Because of excessive spiking during the current ramp region, data from this region were not included during analysis.

**Data analysis.** Data analysis was performed with IGOR Pro 6.22 (WaveMetrics, Lake Oswego, OR), using DataAccess 9.3 (Bruxton, Seattle, WA) to read pCLAMP data into IGOR. Custom Python routines were used to read Signal data into IGOR.

Resting membrane potential was measured from current-clamp records over a continuous 2-min window starting immediately after break-in. Spike threshold was defined as the voltage at which the spike rise velocity reached 4% of its maximal value (Kalig and Bean 2010). This definition corresponded within 1–2 mV to a sharp inflection in the phase-plane plot of dV/dt vs. voltage.

To better resolve small currents, all current traces were low-pass filtered at 1 kHz. Slow (20 mV/s) voltage ramps were used to determine current-voltage (I–V) curves approximating steady state, taking the mean current value over 0.01–mV intervals after signal averaging over two to four trials. For simplicity, we refer to I–V curves determined by 20 mV/s ramps as “steady-state I–V curves,” although it is shown in Fig. 9 the presence of slow inactivation means that, at least in the case of sodium current, it is difficult or impossible to define a true steady state (cf. Fleidervish and Gutnick 1996). For population average I–V curves and comparison of I–V curves, current values were binned in 2-mV intervals and averaged.

Voltage responses to small current steps in Figs. 3 and 4 were signal averaged over five to seven sweeps. Only sweeps with an average voltage matched within 0.5 mV of each other over the 100 ms before the step were accepted for signal averaging. Input resistance...
was measured with Ohm’s law ($R = \Delta V/\Delta I$), where $\Delta V$ was taken as the difference between the average voltage over 100 ms just before the current step and the average voltage over 100 ms at the end of the 1-s, 5-pA step. Membrane time constant was measured by fitting a single exponential to the first 150 ms of the voltage response at the current step onset.

Currents elicited from voltage-clamp experiments using the cell’s own firing as a voltage command (action potential clamp) were signal averaged over four sweeps. Because the voltage changes during an action potential are too fast for voltage clamp in an extended neuron like CA1 pyramidal neurons in slice, current from action potential clamp recordings was not analyzed from 5 ms before to 30–50 ms (depending on the cell) after each action potential peak. To compare the magnitude of current responses, depolarizing regions were selected from the voltage command after filtering and smoothing to eliminate noisy voltage fluctuations. The magnitude of the current collected from the voltage command after filtering and smoothing to (depending on the cell) after each action potential peak. To compare the magnitude of the current responses, depolarizing regions were selected from the voltage command after filtering and smoothing to eliminate noisy voltage fluctuations. The magnitude of the current response was taken as the absolute value of the difference between the current at the start of the depolarizing event and the maximum current recorded until the start of the next depolarizing event. Statistics are reported as means ± SE.

Drugs: All drugs were diluted in ACSF to the indicated final concentration and were bath applied. Drugs were obtained from Sigma Chemical, except for CGP55845, (R)-CPP, and NBQX, which were obtained from Tocris Bioscience, as well as XE991 and ZD7288, which were obtained from Ascent Scientific.

RESULTS

Voltage dependence of subthreshold $I_{\text{NaP}}$, $I_h$, and $I_{\text{M}}$. We first examined the voltage dependence of $I_{\text{NaP}}$ and $I_h$ at subthreshold voltages in rat CA1 pyramidal neurons, using slow (20 mV/s) ramps to measure quasi-steady-state voltage dependence. Figure 1 shows a typical experiment. With a slow voltage ramp (20 mV/s) from $-108$ mV to $-28$ mV, TTX-sensitive current was first evident around $-75$ mV and grew steeply with depolarization to a peak around 1 nA at $-30$ mV (Fig. 1A). Action potential currents that escaped voltage clamp control were present in many cells, presumably reflecting an inability to voltage clamp the axon initial segment. However, the trajectory of steady-state current was generally continuous before and after the escaped spikes, suggesting that the majority of the recorded steady-state current was under good voltage control. In recordings from 14 cells, TTX-sensitive subthreshold sodium current was $-10 \pm 3$ pA at $-78$ mV where it was first evident, grew to $-35 \pm 8$ pA at $-68$ mV, and reached a maximum of $-526 \pm 75$ pA at $-38$ mV, with a half-activation voltage of $-53 \pm 0.1$ mV and a slope factor of $6.0 \pm 0.1$ mV ($n = 14$; Fig. 2).

Using the same approach, we also defined the steady-state voltage dependence of $I_h$, using application of 10 μM ZD7228, applied in the presence of 1 μM TTX, to define $I_h$ (Fig. 1B). As expected, ramp-evoked $I_h$ was largest at hyperpolarized potentials and became smaller with depolarization until it was no longer detectable positive to $-50$ mV (Fig. 1B). On average, $I_h$ reached a maximum of $-229 \pm 61$ pA at $-108$ mV, was $-93 \pm 26$ pA at $-78$ mV, and was not measurable above $-48$ mV ($n = 9$; Fig. 2).

Figure 2 shows collected results for the voltage dependence of ramp-evoked $I_{\text{NaP}}$ and $I_h$, averaged over multiple cells (14

![Fig. 1. Steady-state current-voltage relationships for persistent sodium current, HCN channel current ($I_b$), and M current ($I_m$) in representative rat CA1 pyramidal neurons. A: persistent sodium current defined by TTX. Left: slow voltage ramps ($-108$ to $-28$ mV at 20 mV/s, from steady holding potential of $-88$ mV) were applied before and after application of 1 μM TTX. Spikes in control trace are uncontrolled action potentials. Right: TTX-sensitive current obtained by subtraction. B: steady-state $I_h$ defined by application of 10 μM ZD7288. C: steady-state $I_m$ defined by application of 10 μM XE991. In B and C, 10 μM TTX was present in both control and test solutions to eliminate uncontrolled spiking.]
subthreshold voltages is inward current (Fig. 2B ramps, using 10 nM ZD7288 in 2015). XE991-sensitive current below persistent sodium current (Fig. 2). Population averages for steady-state current-voltage relationships of subthreshold voltage range, near maximal at more depolarized voltages. In the middle of I_B2 region, defined as the region of membrane potentials bounded on an expanded scale focusing on the subthreshold voltage rest, dashed line) and mean spike threshold (V_{threshold}, dotted line).

Another voltage-dependent current that can be active at subthreshold voltages, using current-clamp recordings. Effective input resistance and time constant were defined by the voltage change in response to a small (5 pA) current injection, with input resistance measured from the steady-state change in voltage (measured at end of 1-s current injection) and membrane time constant defined from a single-exponential fit to the change in voltage. To explore voltage-dependent effects of I_{NaP} and I_h, we tested the effect of the current injection while varying the steady holding potential in 5-mV increments (Fig. 3A). The effective membrane resistance defined in this way showed a strong voltage dependence, with resistance increasing with depolarization. Input resistance increased modestly from 48 ± 6 MΩ at −88 mV to 60 ± 4 MΩ at −78 mV and then increased steeply at more depolarized voltages, to an apparent resistance of 274 ± 34 MΩ at −68 mV (n = 20; Fig. 3B).

The steep increase in apparent input resistance was eliminated by application of 1 μM TTX (n = 7; Fig. 3B), suggesting that persistent sodium current is responsible for the large increase in resistance with depolarization. Although sodium channels are opening over this voltage range, and thus reducing the passive resistance of the membrane, the inward current provided by the opened sodium channels at steady state acts to enhance depolarizations. Therefore, the effective membrane resistance measured from voltage changes in response to depolarizing current injection is higher when subthreshold sodium current is activated.

Blocking I_h with 10 μM ZD7288 increased input resistance over the entire range of subthreshold voltages tested, from −88 mV to −68 mV (Fig. 3C). Tested with depolarizing 5-pA current injections, input resistance at −88 mV increased from 52 ± 8 MΩ in control to 173 ± 28 MΩ after block of I_h and input resistance at −73 mV increased from 97 ± 16 MΩ to 349 ± 66 MΩ. Five of seven cells were so excitable at −68 mV after addition of ZD7288 that it was impossible to measure input resistance because of spiking. These results are consistent with previous work (Gasparini and DiFrancesco 1997; Magee 1998; Surges et al. 2004) showing that I_h acts to reduce the membrane resistance and reduce overall excitability.

Because I_h has fairly steep voltage dependence, typically increasing e-fold with hyperpolarizations of between 4 and 13 mV (Aponte et al. 2006; Dougherty et al. 2013; Maccaferri et al. 1993; Robinson and Siegelbaum 2003) we were surprised that the effect of blocking I_h to increase input resistance showed little voltage dependence, increasing input resistance about threefold over the entire voltage range tested. However, some voltage dependence of the effects of blocking I_h by ZD7288 was evident if expressed as changes in membrane

The requirement for relatively large depolarizations for significant activation of I_M in somatic recordings likely reflects localization of K_7 channels in the axon initial segment (Battelfeld et al. 2014; Pan et al. 2006; Shah et al. 2008.).

These results show that both I_{NaP} and I_h are active throughout the range of subthreshold membrane potentials between resting potential and spike threshold. There is no voltage between the resting potential and the spike threshold at which one or both currents is not sizable. This implies that the electrical behavior of CA1 pyramidal neurons between rest and threshold is never purely passive.
conductive, which decreased by 15 nS at −88 mV (from 21.4 ± 3.2 nS in control to 6.3 ± 0.7 nS in ZD7288) and by 8.4 nS at −73 mV (from 12.02 ± 1.77 nS in control to 3.6 ± 0.7 nS in ZD7288). The effects of ZD7288 to increase input resistance over a wide voltage range are consistent with a previous study in CA1 pyramidal neurons (Surges et al. 2004), although the results cannot be directly compared since the effects of ZD7288 in that study were studied on a background of TTX.

Blocking I_M with 10 μM XE991 had little or no effect on membrane resistance at voltages below −68 mV (n = 7; Fig. 3D), consistent with the lack of measurable I_M at these voltages under our conditions.

Together, these results indicate that persistent sodium current has a large voltage-dependent boosting effect on membrane resistance, whereas the conductance from I_h reduces membrane resistance in a weakly voltage-independent manner.

Another important intrinsic membrane property is the membrane time constant, which controls the input integration time. To measure the time constant, we fit a single exponential to the rising phase of the voltage response at the current pulse onset (Fig. 4A). Consistent with the dependence of time constant on membrane resistance, we found that the voltage-dependent effects on membrane time constant largely mirrored the effects we saw on membrane resistance. Membrane time constant was strongly voltage dependent (Fig. 4, B and C). At −88 mV the time constant was 17 ± 3 ms, and it remained similar with depolarization up to −73 mV at 22 ± 2 ms, then rose sharply to 62 ± 9 ms at −68 mV (n = 20). TTX largely eliminated the voltage-dependent change in membrane time constant (n = 7; Fig. 4B), whereas ZD7288 caused a largely non-voltage-dependent increase in time constant (n = 6, Fig. 4, D–F). There was no detectable change in time constant from blocking I_M with XE991 (n = 7; Fig. 4G). These effects on membrane time constant suggest that subthreshold sodium current acts to lengthen integration time at depolarized potentials and that I_h acts to decrease integration time at most subthreshold potentials.

Subthreshold currents during naturalistic stimuli. The preceding results show that both I_{NaP} and I_h strongly influence intrinsic membrane properties over a wide range of subthreshold voltages, modifying both effective input resistance and time constants as assayed by step current injections. To explore how the currents are activated during more physiological voltage trajectories, we used a modified “action potential clamp” technique, illustrated in Fig. 5. First, in current-clamp recordings we used a dynamic clamp system to evoke subthreshold membrane activity using barrages of synaptic-like conductances (Fig. 5A, top) and recorded the cell’s voltage response (Fig. 5A). We tuned the magnitude of the conductance stimuli for each cell to evoke just a few action potentials because we were most interested in the behavior of the currents at subthreshold voltages. After recording voltage responses to the injection of synaptic conductances simulated by dynamic clamp, we then switched the amplifier to voltage-clamp mode and in the same cell used this recorded voltage trace as the voltage clamp command waveform and serially applied 1 μM TTX and 10 μM ZD7288 to measure sodium current and I_h (Fig. 5A).

Figure 5, B–E, illustrate the behavior of subthreshold sodium current and I_h in more detail. When the voltage is relatively stable slightly below the average voltage of the stimulus for this cell (−69 mV; Fig. 5B, top), sodium current contributes a small standing inward current around −20 pA and I_h contributes a larger current around −100 pA (Fig. 5B). Notably, even small depolarizing excursions activate and deactivate sodium current and I_h, respectively. When the voltage sits even a few millivolts more hyperpolarized than the average voltage of −69 mV, sodium current is much smaller, but it activates quickly with depolarizations and follows voltage changes closely in time (Fig. 5C). Conversely, I_h is less “peaky” and responds more slowly to voltage changes, filtering out the fast voltage changes in the voltage trace.
Recruitment of sodium current is especially obvious at subthreshold voltages just below the action potential threshold, contributing about $\frac{1}{10}$ pA during a depolarized plateau just prior to a spike (Fig. 5D). This perithreshold recruitment of sodium current was particularly clear in cases where the voltage was just subthreshold without actually eliciting a spike (Fig. 5E). On average, wave-elicited sodium current provided $\frac{1}{10}$ pA at $-76$ mV, $\frac{1}{10}$ pA at $-68$ mV, and $\frac{1}{10}$ pA at $-58$ mV ($n = 7$).

To better understand the current behavior during naturalistic voltages, we compared the voltage dependence of the wave-elicited current with that of steady-state current obtained from slow ramps recorded in the same cell (Fig. 6). We removed uncontrolled currents during action potentials for this analysis. We found that wave-elicited sodium current was similar to steady-state sodium current in being steeply voltage dependent, but the wave-elicited current was larger at all voltages (Fig. 6A, top). By contrast, while wave-elicited $I_h$ retained its hallmark hyperpolarization-activated voltage dependence, it was far smaller than steady-state $I_h$ at most voltages (Fig. 6A, bottom). On average, wave-elicited sodium current was $\frac{1}{10}$ pA larger (more inward) than steady-state current at $-76$ mV while wave-elicited $I_h$ was $\frac{1}{10}$ pA smaller (less inward) than steady-state current at the same voltage ($n = 5$; Fig. 6C).

These differences between wave-elicited current and steady-state current can be explained by considering the time-dependent behaviors of sodium current and $I_h$. Sodium channels gate rapidly, in less than a millisecond, so that they can respond...
maximally to voltage changes almost instantaneously (Carter et al. 2012). The larger wave-elicited sodium current compared with steady-state current likely reflects the additional contribution of transient subthreshold sodium current, which is activated by fast voltage changes but not by the slow changes used to evaluate steady-state current (Carter et al. 2012). By contrast, \( I_h \) channels gate slowly, with time constants two or three orders of magnitude slower than sodium channels (Hu et al. 2002; Magee 1998), so they cannot follow fast voltage changes and contribute less current during natural voltage changes than at steady state.

Figure 7 illustrates the currents carried by sodium channels and HCN channels for depolarizing events over a range of voltages in a typical cell. More sodium current than \( I_h \) is recruited when the maximum depolarization is greatest and when voltage changes are fastest (Fig. 7A, left 2 panels), while \( I_h \) is greater for events that are slower and reach less depolarized voltages (Fig. 7A, right 2 panels). Figure 7B shows data from a representative cell comparing the change in sodium current vs. the change in \( I_h \) for all subthreshold voltage fluctuations, illustrating that recruitment of additional sodium current is most commonly larger than changes in \( I_h \), with a general trend of a larger difference for larger depolarizing events, resulting in a positive slope for the scatterplot. Such a positive slope was prominent in four of six cells in which the comparison was done (Fig. 7C). The heterogeneity among cells may reflect heterogeneity previously described in the size and kinetics of \( I_h \) among different CA1 neurons (Dougherty et al. 2013).

Sodium current and \( I_h \) during in vivo membrane behavior. Our dynamic clamp stimuli were designed to elicit behavior that roughly approximates the characteristics of in vivo-like synapse activations (dynamic clamp). We obtained recordings of in vivo voltage trajectories made from mouse CA1 pyramidal neurons while animals ran on a spherical treadmill during a virtual navigation task (Harvey et al. 2009) and used them as voltage commands in in vitro recordings from mouse CA1 pyramidal neurons in acute slices. The slice recordings allow a sequence of solution changes to dissect out the influence of sodium current and \( I_h \) on subthreshold voltages recorded during normal in vivo cell behavior.

Figure 8 illustrates such an experiment. Using an in vivo voltage trace (from Harvey et al. 2009, Supplementary Fig. 7) as a voltage command (Fig. 8A), we recorded the current response and then serially applied 1 \( \mu M \) TTX and 10 \( \mu M \) ZD7288.
ZD7288 to define the contributions of sodium current (Fig. 8A) and \( I_h \) (Fig. 8A). Strikingly, the recordings showed that substantial subthreshold sodium current flowed throughout the natural subthreshold voltage trajectory and that the current magnitude closely followed fast voltage changes (Fig. 8, B–E), increasing with depolarization and decreasing with hyperpolarization (but never to zero). In collected results from six neurons, sodium current evoked by the natural in vivo voltage trajectory was \(-47 \pm 6\) pA at \(-62\) mV and grew to \(-170 \pm 19\) pA at \(-52\) mV (\( n = 9 \)).

By contrast to the substantial sodium current throughout the natural subthreshold voltage trajectory, there was no measurable \( I_h \) elicited by the in vivo trace (Fig. 8, A and B). There was no wave-elicited ZD7288-sensitive inward current over the entire subthreshold voltage range (\(+6.1 \pm 2.4\) pA at \(-62\) mV and \(-0.7 \pm 2.6\) pA at \(-52\) mV; \( n = 9 \)). However, \( I_h \) was present in the cells, as evident by large steady-state current ZD7288-sensitive current at more hyperpolarized potentials when tested with a ramp voltage-clamp command (Fig. 9C).

**Fig. 6.** Comparison of naturalistic wave-elicited current with steady-state current in rat CA1 pyramidal neurons. A: current-voltage relationships of \( I_{Na} \) (top) and \( I_h \) (bottom) in a representative cell. Steady-state current was obtained with slow voltage ramps as in Fig. 1. Wave-elicited current was obtained with naturalistic voltage trajectories as in Fig. 5. Graphed currents omit current during uncontrolled spikes. Sodium current was defined as current sensitive to 1 \( \mu M \) TTX; \( I_h \) was defined as current sensitive to 10 \( \mu M \) ZD7288. B: population average current-voltage relationship for sodium current (\( n = 7 \)) and \( I_h \) (\( n = 5 \)) elicited by naturalistic voltage command waveforms. C: average difference between wave-elicited and steady-state sodium current (\( n = 7 \)) and \( I_h \) (\( n = 5 \)). Data are means \( \pm \) SE.

**Fig. 7.** Comparison of sodium current and \( I_h \) flow during simulated excitatory events at different subthreshold voltages. A: changes in sodium current (\( \Delta I_{Na} \)) and \( I_h \) (\( \Delta I_h \)) during excitatory events of various sizes and from varying voltages (top). Currents are aligned to the baseline at the beginning of each event (top), and to facilitate comparison changes in current are plotted as absolute values. B: \( \Delta I_{Na} \) subtracted by the \( \Delta I_h \) for each synaptically-like event for the cell shown in A, graphed against the magnitude of the membrane potential change (\( \Delta V_m \)) for each event. Thus events where \( \Delta I_{Na} \) was greater than \( \Delta I_h \) are positive, whereas events where \( \Delta I_h \) was greater are negative. Events that elicited action potentials are excluded. As \( \Delta V_m \) grows, the tendency for \( \Delta I_{Na} \) to dominate also grows, as quantified by a linear fit with a positive slope (1.84 \( \pm \) 0.19 pA). C: slope of the linear fit for the analysis shown in B for all cells (\( n = 6 \)).
In the slice recordings using the in vivo recordings as commands, there were occasional large stereotyped action currents representing loss of voltage control and firing of action potentials in the initial segment. These occurred in tight correlation with the presence of spikes in the voltage command from the original in vivo recording. This shows good correspondence in the voltage-dependent behavior of the cells at subthreshold-to-threshold voltages between the in vivo and in vitro conditions, despite the very different conditions.

We compared the natural trajectory-evoked sodium current to the steady-state current evoked by slow depolarizing ramps. In an initial series in which ramps were delivered from a steady holding potential of −88 mV, as in the protocol in Fig. 1, the sodium current at any given voltage during the fluctuating natural trajectory command was considerably smaller than the current at the corresponding voltage during persistent sodium current measured by a slow (20 mV/s) depolarizing ramp. In this initial series of experiments, natural trajectory-evoked sodium current at −60 mV was 28 ± 4 pA (n = 5) smaller than steady-state sodium current measured with a depolarizing ramp delivered from a holding potential of −80 mV, and natural trajectory-evoked sodium current at −52 mV was 69 ± 6 pA smaller than the corresponding ramp-evoked current.

We hypothesized that the smaller natural trajectory-evoked sodium current could be explained by slow inactivation of sodium channels, which could reduce the overall availability of sodium current—including persistent sodium current—after relatively long periods spent at depolarized voltages (Fleidervish and Gutnick 1996). In the in vivo voltage recordings, the membrane potential fluctuates around an average value of −58 mV, which could produce slow inactivation relative to the steady holding potential of −88 mV used for the ramp delivery in this initial series. To test this, we did a second series of experiments in which the ramp was integrated into a single command trace incorporating the in vivo voltage trajectory (Fig. 8A), using a steady holding voltage of −58 mV before and after the ramp. With this protocol, the difference between ramp-evoked current and natural trajectory-evoked current was much smaller. In this protocol, we also added a second descending ramp following the ascending ramp. The current evoked by the descending ramp was strikingly smaller than that evoked by the ascending ramp (Fig. 9A), consistent with substantial slow inactivation of persistent sodium current. The natural trajectory-evoked current at any given voltage was generally between the current evoked by the ascending and descending ramps (Fig. 9A), except for the most depolarized subthreshold voltages, between −55 and −50 mV, where the natural trajectory-evoked current was sometimes larger than current evoked by both ramps. Our interpretation is that rapid voltage subthreshold fluctuations induce extra transient sodium current, as in the rat neuron experiments, but that there is significant slow inactivation of sodium current present.

Fig. 8. Contributions of sodium current and I_h during in vivo voltage trajectory in mouse CA1 pyramidal neurons. A: voltage trace from a CA1 pyramidal neuron in an awake, behaving mouse (black trace) (Harvey et al. 2009, Supplementary Fig. 7) was played as a voltage command to a mouse CA1 pyramidal neuron in an acute slice. Ascending and descending slow voltage ramps (20 mV/s) were applied immediately after this trace to assay steady-state current over the same voltage range. Serial application of 1 μM TTX and 10 μM ZD7288 defined the contribution of sodium current (red trace) and I_h (blue trace). Steady holding potential before and after the command waveform was set at the average voltage of the in vivo voltage trace (−58 mV, gray dotted line). B–E: expanded views of the voltage command (black trace) and elicited sodium current (red trace) and I_h (blue trace) from A. Gray dotted line indicates the average membrane potential of the voltage trace.

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substantially smaller during the descending ramp, apparently some recovery from slow inactivation. The sodium current is substantially smaller during the descending ramp, likely reflecting additional slow inactivation that develops during the most depolarizing parts of the ascending-descending ramp sequence. We conclude that the dynamic slow inactivation in mouse CA1 pyramidal neurons thus makes it very difficult to accurately define true “steady-state” persistent sodium current for comparison with the natural trajectory-evoked current.

**DISCUSSION**

We investigated the voltage- and time-dependent flow of TTX-sensitive sodium current and \( I_h \) at subthreshold voltages during naturalistic firing in CA1 pyramidal neurons. We find that both currents can be activated at subthreshold voltages during naturalistic firing and that there is no voltage at which membrane behavior is truly passive, without substantial contribution of one or both conductances.

**Contrasting effects of \( I_h \) and \( I_{NaP} \) on input resistance and time constant.** Although both persistent sodium current and \( I_h \) provide inward current at subthreshold voltages, they have contrasting properties, in both voltage dependence and kinetics. Persistent sodium current is activated by depolarization in a steeply voltage-dependent manner, requiring voltages positive to about \(-75\) mV, and increasing e-fold in about \(6\) mV. The kinetics of activation of persistent current are extremely rapid (hundreds of microseconds; Carter et al. 2012; Park et al. 2013). Activation of persistent sodium current therefore acts nearly instantaneously to amplify depolarizing events. Thus, for currents causing depolarizing events [whether excitatory postsynaptic currents (EPSCs) or steady injected current] the effect of persistent sodium current is to enhance the resulting depolarization, appearing as an increase in apparent input resistance. This effect is large positive to \(-75\) mV (Fig. 3B), resulting in a threefold increase in apparent input resistance at \(-68\) mV. The increase in apparent input resistance is accompanied by an increase in apparent time constant of the same magnitude (Fig. 4, B and C). A priori, it might seem strange that the nearly instantaneous activation of persistent sodium current can result in a longer apparent time constant. Although the activation of sodium current is very rapid, its effect on membrane voltage still requires charging of the membrane capacitance. The net effect is a time-dependent sequence of progressive depolarization and activation of increasing sodium current until a new steady state is reached. The overall change in membrane voltage can be approximated by an exponential—and thus measured as an apparent time constant—but is a much more complicated process than if the membrane conductance were not voltage dependent.

The effect of voltage-dependent inward persistent sodium current is to produce a region of “negative” conductance in the membrane \(I-V\) curve, where increasing depolarization results in a shift to more inward current. At any point on the \(I-V\) curve where net membrane current is still outward (i.e., the contribution of persistent sodium current is outweighed by other currents, mainly from the background potassium conductance), the voltage will relax back to the resting potential near \(-80\) mV once any depolarizing current (whether EPSC or injected current) is removed. The rate of relaxation will be slower when the net outward current at the starting voltage is smaller (i.e., when the effect of the persistent inward current is larger), so that the effect of persistent current is to slow the decay of excitatory postsynaptic potential (EPSPs) or the decay after injected currents—effects that can be viewed as an increase in

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**Fig. 9.** Comparison of in vivo wave-elicited current and ramp-evoked current in mouse CA1 pyramidal neurons. A: current-voltage relationships for sodium current (red) and \( I_h \) (blue) in a representative cell. Ramp-evoked current was larger during an ascending ramp (rust) than an immediately following descending ramp (red), both applied at \(20\) mV/s. Wave-elicited current was obtained by using a ramp in vivo voltage trajectory, preceding the ramps, as shown in Fig. 8. Wave-elicited current excludes current during spikes. B: population average current-voltage relations for sodium current and \( I_h \) elicited by ascending and descending voltage ramps and in vivo voltage command waveforms (n = 11 for ascending ramps and in vivo voltage command waveforms, n = 6 for descending ramps, recorded in 6 of the 11 cells). C: current-voltage relationships for steady-state current from a representative mouse CA1 pyramidal neuron using ascending slow voltage ramps over a wider voltage range. Note the presence of \( I_h \) at negative voltages. D: average steady-state sodium current (n = 11) and \( I_h \) (n = 11) elicited by ascending voltage ramps show that \( I_h \) is measurable at \(-80\) mV but negligible at the lowest voltage of the in vivo trace (\(-62\) mV) and at perithreshold voltages (\(-58\) mV). Data are means ± SE.
apparent membrane time constant. At a voltage near \(-60\) mV where net membrane current becomes zero with a negative slope conductance, there is a point where in principle the membrane time constant is infinite (see Farries et al. 2010 for a detailed discussion). However, this zero-current point is unstable, because any small outward current produces a repolarization that becomes regenerative by resulting in more outward current while any small inward current produces a depolarization that becomes regenerative by resulting in more inward current. Changes in either direction will be initially very slow, reflecting small currents. The combined effects of persistent sodium current to produce an increase in apparent time constant negative to \(-60\) mV and to result in slow depolarization positive to \(-60\) mV results in long integration windows of injected current or EPSCs, which were evident in our recordings in CA1 pyramidal neurons as small plateaus preceding spikes during dynamic clamp input or during in vivo firing.

**Weakly voltage-independent effects of \(I_h\).** \(I_h\) has the opposite voltage dependence as persistent sodium current, being activated by hyperpolarization. Also, unlike persistent sodium current, \(I_h\) gates slowly, so steady-state conductance at a given voltage is maintained during voltage deflections resulting from EPSPs. As a result, \(I_h\) at subthreshold voltages acts to reduce both input resistance and membrane time constant (Gasparini and DiFrancesco 1997; Magee 1998), effects opposite to that of persistent sodium current.

In our experiments, the effect of \(I_h\) on input resistance and membrane time constant was large over a wide voltage range, with block of \(I_h\) by ZD7288 producing about a threefold increase in both input resistance and time constant over a voltage range from \(-88\) to \(-73\) mV. Similarly, weak voltage dependence of the effects of \(I_h\) on membrane resistance has been reported before (Dougherty et al. 2013; Narayanan et al. 2010). However, when analyzed in terms of conductance, the ZD7288-sensitive conductance did show some voltage dependence, decreasing from 15 nS at \(-88\) mV to 8.4 nS at \(-73\) mV; the equal increases in membrane resistance at the different voltages can be explained by the fact that conductance remaining in ZD7288 is lower at \(-73\) mV than at \(-88\) mV. At least in cloned HCN channels, there can be a voltage-independent component of \(I_h\) (Proenza and Yellen 2006), which could also reduce the voltage dependence associated with the loss of \(I_h\).

**Time-dependent effects of sodium current and \(I_h\).** Subthreshold sodium current gates within 100–300 \(\mu s\) (Carter et al. 2012), and this extremely rapid gating is reflected in its ability to closely track naturalistic voltage trajectories. Its voltage dependence also matched well with that predicted by steady-state sodium current, as would be expected given that the channel gates can respond as fast as or faster than the changes in voltage occur.

In rat CA1 pyramidal neurons, the naturalistic stimulus recruited more sodium current at each voltage than elicited in steady state, which supports the physiological importance of the recently described subthreshold transient current (Carter et al. 2012). This current, carried by the same channels as suprathreshold transient and subthreshold persistent current, is activated readily by rapid voltage fluctuations like those in the naturalistic stimulus. Thus membrane potential trajectories with high variance are expected to elicit some contribution from subthreshold transient sodium current like that observed here.

By contrast, because of the much slower gating of \(I_h\), with time constants around 10–100 ms (Dougherty et al. 2013; Santoro et al. 2000), \(I_h\) during the naturalistic voltage trajectory in rats was much lower than at steady state, showing that \(I_h\) could not follow the fast voltage fluctuations well. This slow gating reduces the effective voltage dependence of \(I_h\) during fast EPSP-like events.

As recognized previously, the divergent kinetics and voltage dependence of subthreshold sodium current and \(I_h\), produce very different effects on membrane behavior, with sodium current acting as an amplifying conductance boosting voltage fluctuations at all frequencies and \(I_h\) acting as a “resonant conductance” (Hutcheon et al. 1996; Hutcheon and Yarom 2000). In CA1 pyramidal neurons, this mechanism explains the resonance peak at theta frequencies generated by \(I_h\) (Hu et al. 2002; Narayanan and Johnston 2008).

During the voltage trajectory from in vivo recordings, subthreshold sodium current in mouse neurons was large, suggesting its importance during physiological membrane behavior. However, somewhat surprisingly, the sodium current elicited by the in vivo mouse voltage trajectory was lower than that measured at steady state in the same cell with slow ramps, in contrast to the larger currents evoked by fluctuating naturalistic stimuli vs. steady state in rat neurons. The simplest explanation is that there is more slow inactivation of sodium channels during the in vivo voltage trajectory (with a mean voltage around \(-60\) mV) compared with the ramp-evoked steady-state current. Slow inactivation describes a condition of sodium channels in which recovery from inactivation is slow (on the order of seconds) and likely occurs by a mechanism distinct from normal fast inactivation. Slow inactivation has been previously identified in CA1 pyramidal neurons, where it mediates activity-dependent changes in backpropagating action potentials (Colbert et al. 1997; Jung et al. 1997; Mickus et al. 1999). Slow inactivation affects subthreshold persistent sodium current as well as transient sodium current (Do and Bean 2003; Fleidervish and Gutnick 1996; Taddese and Bean 2002) and might be expected to be significant at theta frequencies. A slow inactivation mechanism also explains the resonance peak at theta frequencies generated by \(I_h\) (Hu et al. 2002; Narayanan and Johnston 2008).

Interestingly, \(I_h\) appears not to be activated during the in vivo voltage trajectories in mouse CA1 pyramidal neurons, even though \(I_h\) can be activated at more hyperpolarized voltages. This finding contrasts with the much greater contribution of \(I_h\) to naturalistic membrane trajectories in rat neurons and is consistent with previously described \(I_h\) differences between rat and mouse that suggest lower levels of active \(I_h\) at rest in mouse (Routh et al. 2009). These differences between rat and mouse may reflect a different composition of HCN channels, different basal regulation by cAMP, or a combination of both (Santoro et al. 2000), in addition to a simple difference in expression levels in CA1 pyramidal neurons of the two species.

**Implications for input integration.** Our results show that in CA1 pyramidal neurons of both rats and mice there is substantial activation of TTX-sensitive sodium current well below the threshold voltage for spiking and that subthreshold sodium current is activated in a steeply voltage-dependent manner during EPSPs, even when these remain subthreshold. Our results obtained in voltage-clamp experiments using naturalis-
tic voltage trajectories fit well with previous experiments applying TTX in current-clamp experiments (Deisz et al. 1991; Schwindt and Crill 1995; Stuart and Sakmann 1995) or doing voltage-clamp experiments with small depolarizations (Axmacher and Miles 2004).

CA1 pyramidal neurons use both a rate code and a temporal code during spatial navigation (Harvey et al. 2009; O’Keeffe and Burgess 2005). The lengthening of time constant by persistent sodium current acts to expand the temporal window for synaptic integration (Andreasen and Lambert 1999; Artian et al. 2011; Fricker and Miles 2000; Prescott and De Koninck 2005; Stafstrom et al. 1985; Stuart and Sakmann 1995), which enhances rate coding. By itself, the lengthening of the time of synaptic integration acts to reduce EPSP-spike coupling precision (Prescott and De Koninck 2005), which is relatively limited in CA1 pyramidal neurons (Fricker and Miles 2000). However, EPSP-spike coupling precision is favored by another feature of subthreshold sodium current—greater amplification of EPSPs as the rate of rise in the EPSP increases (Axmacher and Miles 2004). This rate-dependent boosting is likely facilitated by subthreshold transient sodium current (Carter et al. 2012). The relationship between spiking precision and rate of rise in EPSPs appears to support enhanced selectivity to sensory stimuli in cortical neurons in vivo (Azouz and Gray 2000; Wilent and Contreras 2005), where lower spike thresholds correlate with faster preceding depolarizations (Henze and Buzsáki 2001). Farries and colleagues (2010) demonstrated that such dynamic thresholds can facilitate spiking precision even when the membrane time constant is long. In vivo, network mechanisms like feedforward inhibition also likely play a role in enhancing spiking precision (Pouille and Scanziani 2001).

$I_h$ decreases summation of EPSPs (Berger et al. 2001; Magee 1998, 1999; Poolos et al. 2002; Shah et al. 2004; Sheets et al. 2011; Stuart and Spruston 1998; van Welie et al. 2006), an effect consistent with reduction of both input resistance and membrane time constant. This effect improves spike timing precision at the expense of firing rate (Gastrein et al. 2011). The lengthening of time constant by persistent sodium current acts to expand the temporal window for synaptic integration (Andreasen and Lambert 1999; Artian et al. 2011; Fricker and Miles 2000; Prescott and De Koninck 2005; Stafstrom et al. 1985; Stuart and Sakmann 1995), which enhances rate coding. By itself, the lengthening of the time of synaptic integration acts to reduce EPSP-spike coupling precision (Prescott and De Koninck 2005), which is relatively limited in CA1 pyramidal neurons (Fricker and Miles 2000). However, EPSP-spike coupling precision is favored by another feature of subthreshold sodium current—greater amplification of EPSPs as the rate of rise in the EPSP increases (Axmacher and Miles 2004). This rate-dependent boosting is likely facilitated by subthreshold transient sodium current (Carter et al. 2012). The relationship between spiking precision and rate of rise in EPSPs appears to support enhanced selectivity to sensory stimuli in cortical neurons in vivo (Azouz and Gray 2000; Wilent and Contreras 2005), where lower spike thresholds correlate with faster preceding depolarizations (Henze and Buzsáki 2001). Farries and colleagues (2010) demonstrated that such dynamic thresholds can facilitate spiking precision even when the membrane time constant is long. In vivo, network mechanisms like feedforward inhibition also likely play a role in enhancing spiking precision (Pouille and Scanziani 2001).

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Our analysis was confined to measuring currents at the cell body. However, while boosting of EPSPs by subthreshold sodium current occurs mainly in the soma or axon initial segment (Stuart and Sakmann 1995), $I_h$ is expressed at highest density in distal dendrites (Lörincz et al. 2002; Magee 1998) and the effects of $I_h$ are greatest for summation of EPSPs elicited at distal dendrites (Magee 1999). It is not practical to perform voltage-clamp experiments in dendrites, but if they were possible such experiments would almost certainly show much larger currents from $I_h$ than we recorded at the cell body. Therefore, the near lack of $I_h$ seen during subthreshold events recorded at the cell body of mouse CA1 pyramidal neurons does not mean that $I_h$ does not shape EPSPs originating in the dendrites in these neurons. In addition to being present at higher density, HCN channels in dendrites also have a shifted activation curve such that they open for smaller hyperpolarizations (Magee 1998), which would also make their contribution to subthreshold current more prominent than for channels at the cell body.

The spatial separation of the effects of $I_h$ and sodium current, which we did not address experimentally, may provide a further mechanism by which effects of $I_h$ are slower than those of sodium current, since the EPSPs modified by dendritic $I_h$ are likely further slowed by dendritic cable properties before they reach the spike initiation zone, while the rapid effects of sodium current act near the site of spike initiation. These considerations emphasize that the overall integrative properties of CA1 pyramidal neurons involve a complex combination of the effects of subthreshold somatic currents together with the integrative properties of the dendrites, which themselves reflect both cable effects and dendritic expression of voltage-dependent ion channels (reviewed by Magee 2000; Gulledge et al. 2005).

**Passive vs. active membrane properties.** Our results add to data from a variety of neuronal types suggesting that the traditional separation of passive from active membrane properties is often impossible, because in many neurons, including CA1 pyramidal neurons in our results, there is no voltage at which voltage-dependent conductances are completely inactive (Amarillo et al. 2014; Marder and Goaillard 2006). These results emphasize that measurements of “passive” membrane properties such as input resistance and time constant should generally be regarded as phenomenological measurements rather than reflecting a well-defined parameter, and are likely to vary even in a single cell according to the exact voltage range in which the measurement is made. More generally, the results emphasize the importance of voltage-dependent conductances for determining the behavior of neurons even near resting voltages.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: J.Y.-H. and B.P.B. conception and design of research; J.Y.-H. performed experiments; J.Y.-H. analyzed data; J.Y.-H. and B.P.B. approved final version of manuscript.

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