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HCN hyperpolarization-activated cation channels inhibit EPSPs by interactions with M-type K+ channels

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The processing of synaptic potentials by neuronal dendrites depends on both their passive cable properties and active voltage-gated channels, which can generate complex effects as a result of their nonlinear properties. We characterized the actions of HCN (hyperpolarization-activated cyclic nucleotide-gated cation) channels on dendritic processing of subthreshold excitatory postsynaptic potentials (EPSPs) in mouse CA1 hippocampal neurons. The HCN channels generated an excitatory inward current (I_h) that exerted a direct depolarizing effect on the peak voltage of weak EPSPs, but produced a paradoxical hyperpolarizing effect on the peak voltage of stronger, but still subthreshold, EPSPs. Using a combined modeling and experimental approach, we found that the inhibitory action of I_h was caused by its interaction with the delayed-rectifier M-type K^+ current. In this manner, I_h can enhance spike firing in response to an EPSP when spike threshold is low and can inhibit firing when spike threshold is high.

Neurons actively process and integrate synaptic potentials through the actions of a wide array of voltage-gated ion channels that are often differentially expressed throughout a neuron's dendritic tree[1]. In some instances, the effects of voltage-gated channels on dendritic processing are relatively straightforward and well understood. For example, dendritic voltage-gated sodium and calcium channels can amplify synaptic potentials[2] through the generation of local or propagated dendritic action potentials[3,4]. In contrast, dendritic voltage-gated or calcium-activated K^+ channels can reduce EPSP amplitude and dampen dendritic excitability[5–7]. In other cases, however, nonlinear interactions between dendritic voltage-gated channels give rise to complex effects that are less easily understood. Here, we focused on the paradoxical effects of the HCN channels on the processing of EPSPs in the apical dendrites of CA1 pyramidal neurons, in which these channels are expressed in a gradient of increasing density with increasing distance from the soma[8–11].

Unlike most voltage-gated channels, HCN channels activate with hyperpolarization and deactivate with depolarization. Their mixed permeability to K^+ and Na^+ ions results in a reversal potential (E_h) of approximately –30 mV, causing these channels to generate an excitatory inward current (I_h) at subthreshold potentials. These biophysical properties underlie the role of I_h as a pacemaker current in cardiac myocytes and thalamocortical relay neurons, in which activation of I_h following action potential repolarization generates a depolarizing current that drives spontaneous, rhythmic firing[12,13]. In neurons that are not spontaneously active, I_h contributes a 5−10-mV depolarizing influence on the resting membrane potential (RMP) and increases the resting membrane conductance (that is, it lowers the input resistance), thereby regulating the spatial and temporal integration of synaptic inputs[10,14–16].

Despite the fact that I_h provides a depolarizing current at subthreshold potentials, results from several studies have indicated that it has a paradoxical inhibitory effect on the ability of an EPSP to trigger an action potential. Thus, enhancement of I_h by the anticonvulsant lamotrigine[17], application of dopamine[18] or induction of long-term potentiation[19,20] decreases excitability and spike firing. Conversely, downregulation of I_h via genetic deletion of HCN1 (ref. 21), pharmacological blockade using cesium[15,22] or the organic antagonist ZD7288 (refs. 15,16), or following induction of long-term depression[23] or seizures[24] increases EPSP amplitude, temporal summation and spike firing.

The inhibitory effects of I_h, by which we mean the inhibition that is seen when I_h is enhanced, have generally been attributed to its ability to increase the resting membrane conductance. This so-called 'shunting effect' on the excitatory postsynaptic current decreases the amplitude of an EPSP[10,22], with EPSP amplitude (ΔV_{EPSP}) being defined as the difference between the peak voltage of an EPSP (V_{peak}) and the resting potential. However, the effect of an EPSP depends not on its amplitude, but on the voltage reached at its peak, which determines whether an EPSP is suprathreshold[25]. Notably, I_h exerts two opposing influences on V_{peak}: its shunting effect decreases EPSP peak voltage and its direct depolarizing effect increases V_{peak} (**Fig. 1a**).

We used a simple computational model to show that, in the absence of other voltage-gated conductances, I_h should always be excitatory for EPSPs that are negative to the I_h reversal potential; that is, the depolarizing action of I_h on V_{peak} is always greater than its shunting effect. This implies that any inhibitory effect of I_h on V_{peak} must be caused by its interactions with other voltage-gated conductances.

One such interaction results in an inhibitory effect of I_h on the duration of Ca^{2+} action potentials in the distal dendrites of CA1

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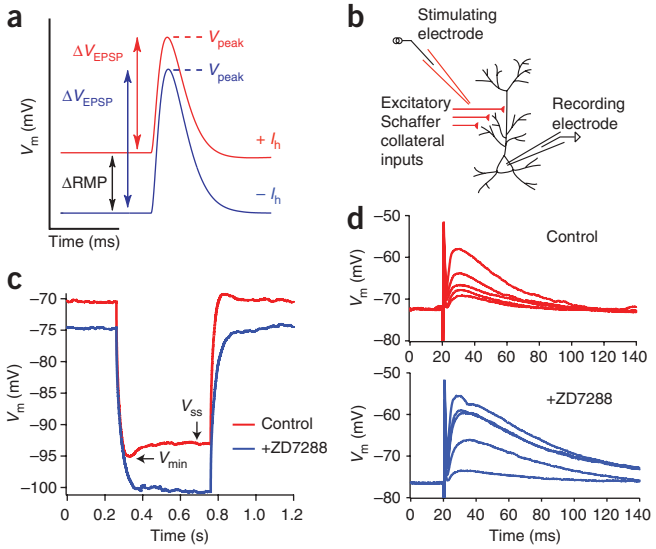

Figure 1 Experimental procedure and the effects of pharmacological blockade of I_h. (**a**) Diagram illustrating the opposing effects of I_h on subthreshold EPSPs, involving a positive shift of the RMP and a decrease in the EPSP amplitude (ΔV_{EPSP}). Red indicates the presence of I_h and blue indicates its absence. $\Delta V_{EPSP} = V_{peak} - RMP$. (**b**) Schematic of experimental setup. Whole-cell current-clamp recordings were obtained from CA1 pyramidal neurons (recording patch electrode). An extracellular stimulating patch electrode was placed ~150 μm away from the soma in stratum radiatum under visual guidance. (**c**) Somatic voltage response to a hyperpolarizing current step in the absence (control) and in the presence of ZD7288 (10 μM). Note the RMP hyperpolarization after application of ZD7288. Under control conditions, the membrane voltage reached an initial minimum value (V_{min}) and then showed a depolarizing sag to a steady-state value (V_{ss}) as a result of activation of I_h. The sag was eliminated by ZD7288. (**d**) Sample EPSPs evoked in response to a range of stimulus strengths under control conditions and after block of I_h using ZD7288.

pyramidal neurons[26]. In this instance, the depolarizing effect of I_h on the resting membrane potential increases the resting inactivation of N- and T-type voltage-gated Ca^{2+} channels, thus inhibiting the Ca^{2+} spikes. In principle, this effect of I_h on resting potential and resting inactivation could also explain how I_h suppresses the firing of Na^+ action potentials. However, it remains unclear whether I_h can exert an inhibitory effect on V_{peak} for subthreshold EPSPs.

We found that I_h, through interactions with voltage-gated K^+ channels, can indeed produce an inhibitory effect on the peak voltage of subthreshold EPSPs. Notably, the influence of I_h on V_{peak} depended on synaptic strength. Thus, although I_h shifted V_{peak} to more positive potentials for weak EPSPs, I_h inhibited V_{peak} for stronger, but still subthreshold, EPSPs. In other words, the effects of I_h on V_{peak} crossed over from depolarizing to hyperpolarizing as a function of increasing synaptic strength, with the 'crossover' potential occurring below both the reversal potential for I_h and the action potential threshold. This indicated that the net effect of I_h is essentially inhibitory, as it made it more difficult for an EPSP to reach threshold. Both our computational and experimental results indicate that the inhibitory effect of I_h is caused by its ability to depolarize the resting membrane, which enhanced the resting activation of the delayed-rectifier M-type K^+ channels. Because the M-channels are under neuromodulatory control[27], the influence of I_h on dendritic integration may switch from inhibitory to excitatory depending on the state of M-channel regulation. Such modulation may have important implications for regulation of long-term synaptic plasticity that contributes to learning and memory[21,28] and for the treatment of epileptic disorders in which both I_h and M-channels may be involved[29–32].

RESULTS

Dual influence of I_h on EPSPs in CA1 pyramidal neurons

We first examined the influence of I_h on neuronal activity in mouse hippocampal CA1 pyramidal neurons by carrying out whole-cell current-clamp recordings of both resting membrane properties and somatic EPSPs that were evoked by stimulation of the Schaffer collateral pathway (**Fig. 1b**). In response to hyperpolarizing current steps injected in the CA1 neuron soma, the membrane voltage showed a depolarizing sag that is characteristic of I_h activation (**Fig. 1c**). We then applied focal synaptic stimulation of increasing strength to elicit EPSPs of increasing amplitude to determine the relationship between V_{peak} and stimulus strength (**Fig. 1d**). In all of these experiments, inhibitory synaptic transmission was blocked using $GABA_A$ and $GABA_B$ receptor antagonists.

Next, we applied the organic antagonist ZD7288 to block I_h and repeated the measurements described above of resting membrane properties, voltage sag and the EPSP input-output curve. Relatively low concentrations of ZD7288 (10 μM) and short exposure times (10–15 min) were used to minimize nonspecific effects of this drug on synaptic transmission[33]. These conditions were sufficient to eliminate the voltage sag in response to hyperpolarizing currents, indicating an effective block of I_h (**Fig. 1c**). The average sag ratio decreased from 10.2 ± 1.0% under control conditions to –3.1 ± 0.5% in the presence of ZD7288 ($n = 7$, $P < 0.001$, paired t test). Application of ZD7288 also shifted the RMP by ~5 mV to more negative voltages (RMP under control conditions equaled –68.9 ± 1.5 mV, RMP in ZD7288 equaled –74.0 ± 1.4 mV, $n = 7$, $P < 0.001$, paired t test) and increased the input resistance by more than twofold (control, 138.6 ± 8.2 MΩ; ZD7288, 287.8 ± 25.1 MΩ; $n = 7$, $P < 0.001$, paired t test), consistent with previous findings[19,23,24,26,34].

A comparison of EPSP input-output curves in the presence and absence of ZD7288 showed that the effects of I_h on peak EPSP voltage depended on EPSP size (**Fig. 2**). For small EPSPs, the presence of I_h increased V_{peak}, shifting it to more positive potentials, as expected for an inward, excitatory current (30-μA stimulus; **Fig. 2a**). However, as the stimulus strength was increased to evoke larger EPSPs, V_{peak} in the absence of I_h approached its value in the presence of I_h (45-μA stimulus; **Fig. 2a**). Eventually, with even stronger stimuli, a crossover occurred, where the presence of I_h decreased the peak EPSP voltage to values that were more negative than those reached in the absence of I_h (60-μA stimulus; **Fig. 2a**). This depolarizing/hyperpolarizing crossover effect was clearly seen when V_{peak} was plotted as a function of stimulus strength in the presence and absence of I_h (**Fig. 2b**). To our surprise, the crossover occurred for subthreshold EPSPs whose peak voltages were well below the I_h reversal potential of −30 mV, that is, at voltages at which I_h provided an inward, depolarizing current.

We observed subthreshold hyperpolarizing effects of I_h on V_{peak} in six out of the seven cells that we examined. The one exception occurred in a cell whose resting potential in the presence of I_h was unusually positive ;(–64 mV) and spikes were thus evoked with small current stimuli (35 μA). In some cells, crossover occurred well below the action potential threshold, providing clear evidence that I_h can exert an unambiguously inhibitory influence on subthreshold EPSP peak voltage (**Fig. 2b**). In other cells, the crossover from depolarizing to hyperpolarizing effects occurred near threshold (**Fig. 2c**). In such cells, V_{peak} in the presence of ZD7288 approached or overlapped with V_{peak} in the absence of drug up to potentials very near spike threshold. A slight increase in stimulus intensity could then evoke spikes in

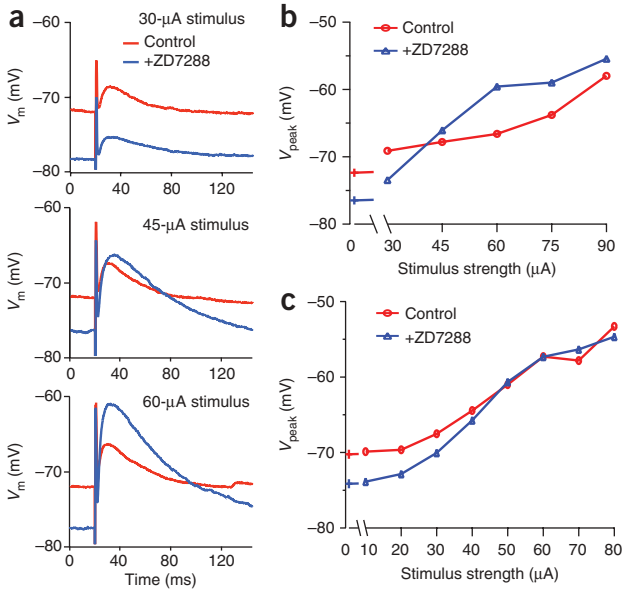

Figure 2 Dual effects of I_h on peak voltage of subthreshold EPSPs. (**a**) Representative EPSP responses for three stimulus strengths recorded at the soma under control conditions (red) and in the presence of ZD7288 (10 μM, blue). Top, I_h had a depolarizing effect on V_{peak} for a small EPSP that was elicited by a weak stimulus. Middle, for an intermediate strength stimulus, V_{peak} was similar in the presence or absence of I_h. Bottom, with a stronger stimulus, I_h had an inhibitory effect and its blockade thus led to a more depolarized V_{peak}. (**b**) Relationship between V_{peak} and stimulus current strength in the presence (control, red) or absence (+ZD7288, blue) of I_h. All EPSPs were subthreshold. The 0 μA points (dashes) denote the resting potential. Note that I_h had an inhibitory effect on V_{peak} over a large range of subthreshold stimulus strengths. (**c**) V_{peak} versus stimulus strength plot for a cell in which I_h had a depolarizing effect on V_{peak} for a large range of weak stimuli. An inhibitory effect of I_h was only observed for a strong stimulus that was just subthreshold.

the presence but not absence, of ZD7288. Such results are consistent with previous findings that I_h has a paradoxical effect of inhibiting spiking[17–19,23].

I_h is purely excitatory when it is the sole active conductance

How can we explain the inhibitory effect of I_h to reduce the peak EPSP voltage at potentials that are negative to the I_h reversal potential? As discussed above, although the depolarizing current carried by I_h should make V_{peak} more positive, the shunting effect of the I_h conductance is expected to decrease V_{peak}. To determine whether these two opposing effects of I_h could yield a net inhibitory influence on V_{peak}, we first examined a single-compartment computational model containing only a passive leak conductance, a physiologically realistic model of I_h[10,17] and a linear excitatory synaptic conductance modeled as an alpha function[35] (**Fig. 3a**). We determined the V_{peak} that was attained for different strengths of synaptic input when either the maximal conductance of I_h or the voltage at which I_h was half-maximally activated ($V_{1/2}$) was varied across a range of physiologically and experimentally relevant values[10,17].

When we increased I_h by increasing its maximal conductance, the RMP was shifted to more positive values, as expected. Similarly, depolarizing shifts of $V_{1/2}$, which also increased resting I_h, also depolarized the membrane (**Fig. 3b**). Enhancing I_h by either method diminished the EPSP amplitude (ΔV_{EPSP}) for all of the synaptic strengths that we examined (**Fig. 3c**). These results confirmed previously reported findings for I_h[10,17,21].

Despite the effect of I_h to decrease ΔV_{EPSP}, the increase in I_h always had a depolarizing effect on the peak EPSP voltage as long as the membrane potential was negative to the I_h reversal potential of –30 mV (**Fig. 3d,e**). As expected, I_h did shift V_{peak} to more negative potentials for very large synaptic conductances that drove V_{peak} positive to E_h, where I_h provided an outward current that hyperpolarized the membrane. Because the threshold for firing a spike is negative to –30 mV (typically around –50 mV), our results indicate that I_h, in the absence of other voltage-gated conductances, should always be excitatory for subthreshold EPSPs. This excitatory effect of I_h persisted even when its maximal conductance was varied 100-fold (**Fig. 3e**), mimicking the range of I_h conductances that have been reported along the somatodendritic gradient in CA1 and layer V neocortical pyramidal neurons[8–11,36]. We also observed an excitatory effect on V_{peak} when the

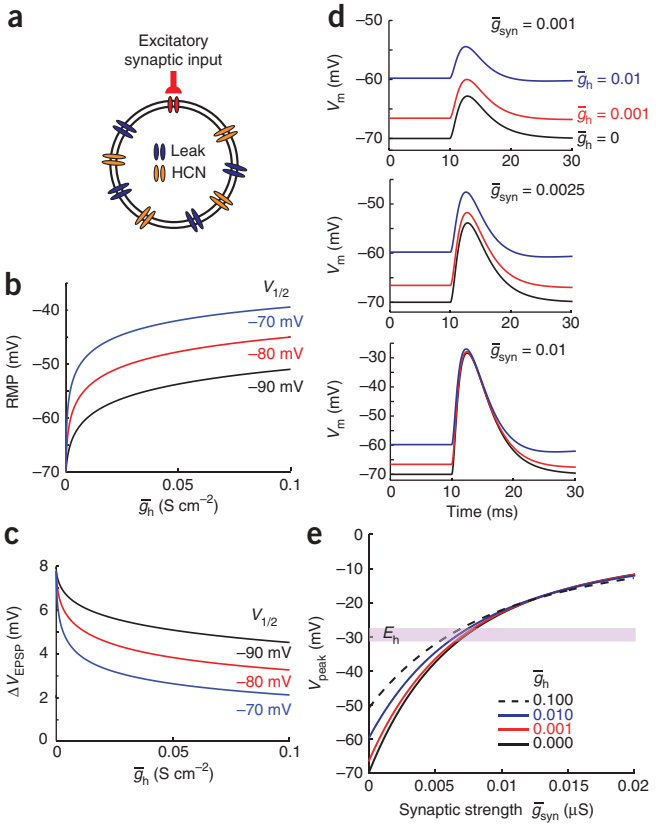

Figure 3 Computational model predicts excitatory role for I_h in the absence of other voltage-gated channels. (**a**) Diagram of the single-compartment model containing I_h (HCN channels), a linear leak conductance and a synaptic conductance (alpha function). (**b**) Effect of I_h on RMP. Increasing maximal I_h conductance ($\bar{g}_h$) or depolarizing shifts of the I_h $V_{1/2}$ produced positive shifts in RMP. (**c**) Effects of I_h on EPSP amplitude, defined as the difference between V_{peak} and RMP. Increasing $\bar{g}_h$ or depolarizing shifts of $V_{1/2}$ caused a reduction in EPSP amplitude. Synaptic strength was set to produce an ~8-mV EPSP in the absence of I_h. (**d**) Sample EPSPs from our model for three synaptic input strengths ($\bar{g}_{syn}$ in μS) in the absence of I_h (black traces) and in the presence of I_h for two levels of $\bar{g}_h$ (in units of S cm^{-2}). (**e**) The effect of I_h on the relationship between V_{peak} and synaptic strength ($V_{1/2}$ = –90 mV in **d** and **e**). Increasing $\bar{g}_h$ to the indicated values had a depolarizing effect on V_{peak} for all membrane potentials below E_h (–30 mV). The *y* intercept shows the effect of I_h on RMP.

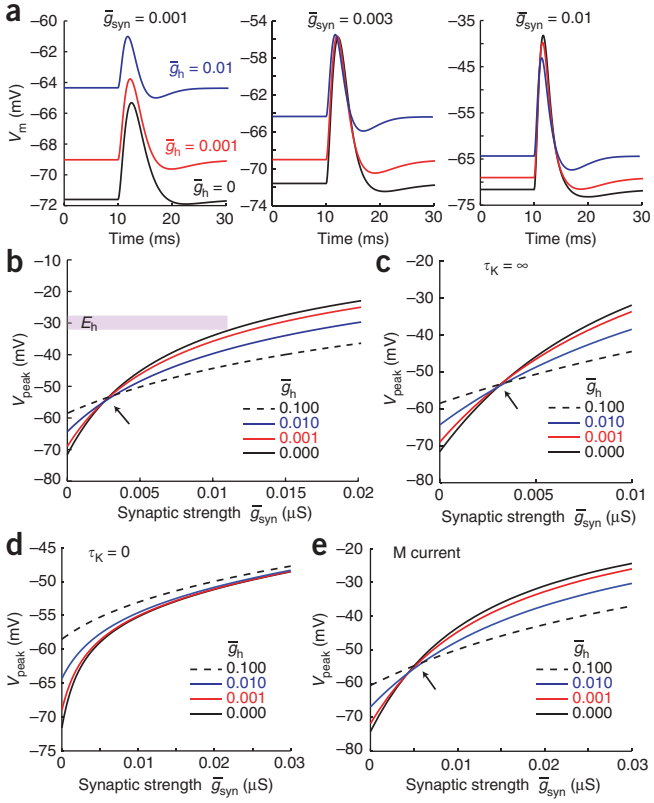

Figure 4 Computational model including a Hodgkin-Huxley voltage-gated K^+ conductance predicts subthreshold inhibitory effects of I_h on peak voltage of strong EPSPs. (**a**) Model EPSPs for three synaptic input strengths ($\bar{g}_{syn}$) in the absence or presence of I_h for two levels of $\bar{g}_h$ as indicated (I_h: $V_{1/2}$ = –90 mV; I_K: $\bar{g}_K = 0.036$ S cm^{-2}, $V_{1/2} = -52$ mV, where I_K is the delayed-rectifier K^+ current and $\bar{g}_K$ is its maximal conductance). (**b**) Effect of I_h on the relationship between V_{peak} and synaptic strength. Increases in $\bar{g}_h$ had a depolarizing effect on V_{peak} for weak synaptic inputs and an inhibitory effect for strong inputs. Notably, the inhibitory regime occurred negative to the I_h reversal potential E_h (–30 mV). The arrow indicates the crossover voltage at which I_h changed from a depolarizing to a hyperpolarizing influence. (**c**) Effects of I_h on V_{peak} when the kinetics of delayed-rectifier K^+ conductance activation were made infinitely slow. (**d**) Effects of I_h on V_{peak} when the kinetics of delayed-rectifier K^+ conductance activation were made infinitely fast. (**e**) Effects of I_h on V_{peak} in a model containing the mammalian M-type K^+ current ($V_{1/2} = -35$ mV). Increasing $\bar{g}_h$ in this model also produced mixed depolarizing and hyperpolarizing effects on V_{peak} with a crossover voltage (arrow).

magnitude of I_h was increased by a shift in its voltage-dependent activation to more positive potentials (**Supplementary Fig. 1** online).

One well-characterized effect of I_h is to reduce temporal summation during a burst of EPSPs, an effect that has been attributed to the action of the I_h conductance to decrease the membrane time constant and to the hyperpolarization caused by the deactivation of I_h during the burst of EPSPs[14,15]. Our simulations confirmed that I_h did decrease the extent of temporal summation during a 100-Hz burst of five EPSPs. Even during the burst, however, the net effect of I_h on membrane voltage was still excitatory, with the peak EPSP voltage during the burst reaching more positive potentials in the presence of I_h than in its absence (**Supplementary Figs. 2** and **3** online).

Previous studies reported a particularly strong inhibitory effect of I_h on EPSP peak voltage when its action when its ability to depolarize the resting membrane is compensated by injection of hyperpolarizing current or a reduction in external K^+ concentration[17–19,23,34]. Such results are not surprising, because when changes in resting potential are prevented, the inhibitory effect of I_h to enhance the membrane conductance should predominate. Indeed, when we simulated this protocol by adjusting the leak conductance reversal potential to keep the resting potential constant, an increase in I_h had a marked inhibitory effect on V_{peak} (**Supplementary Fig. 1**). The more important question, however, is how does I_h exert its inhibitory effect on V_{peak} when the resting potential is free to adopt its intrinsic value, as occurs under physiological conditions.

I_h can inhibit EPSPs by interactions with a K^+ current

Our computational results indicate that I_h alone has a purely excitatory effect on subthreshold EPSPs and thus suggest that the inhibitory effect of I_h on the large EPSPs that was observed in our experiments must involve an interaction with other voltage-gated conductances. One possibility that we considered is that the resting membrane depolarization caused by the presence of I_h may increase the activation of a low-threshold voltage-gated K^+ conductance and that this interaction may have a net inhibitory effect on the peak voltage of the EPSP. To test this, we extended our computational model by adding a Hodgkin-Huxley delayed-rectifier K^+ conductance[35,37] and repeated our simulations.

In the presence of the delayed-rectifier K^+ conductance, an increase in I_h always depolarized the resting membrane and diminished EPSP amplitude (ΔV_{EPSP}), as seen in our single-conductance model (**Supplementary Fig. 4** online). For small EPSPs, I_h still exerted a net depolarizing influence on V_{peak}, as observed in the model with I_h alone. However, inclusion of the voltage-gated K^+ conductance revealed a clear inhibitory effect of I_h on the peak voltage of larger, but still subthreshold, EPSPs (**Fig. 4a**). This inhibitory effect was observed even when the peak EPSP voltage was negative to both the reversal potential of I_h (–30 mV) and typical values for action potential threshold (–50 mV) (**Fig. 4b**). Thus, these computational results reproduced the major findings of our synaptic stimulation experiments, including the presence of a subthreshold crossover voltage at which I_h shifted from having a depolarizing effect on V_{peak} to having a hyperpolarizing influence (**Fig. 4b**). As observed in the model with I_h alone, when the resting potential was held fixed (by altering the leak conductance reversal potential), I_h had a purely inhibitory effect, reducing V_{peak} at all synaptic strengths (**Supplementary Fig. 4**).

Which biophysical properties of the voltage-gated K^+ conductance are required to enable the subthreshold inhibitory effects of I_h? We first examined the importance of the K^+ conductance kinetics by making the rate of activation infinitely slow, such that the K^+ conductance remained at its initial equilibrium value set by the resting potential during the entire time course of the EPSP. Under these conditions, I_h still exerted a dual depolarizing/hyperpolarizing influence (**Fig. 4c**), increasing V_{peak} for small EPSPS, but reducing V_{peak} for larger subthreshold EPSPs. In contrast, when we made the activation rate infinitely fast, so that the K^+ conductance attained its steady-state level of activation instantaneously throughout the EPSP, I_h now exerted a purely excitatory effect (**Fig. 4d**), shifting V_{peak} to more depolarized values for all subthreshold EPSP sizes. Thus, the ability of I_h to inhibit V_{peak} requires that the I_h-dependent enhancement in steady-state resting K^+ conductance persists throughout the EPSP.

Next, we examined how shifting the steady-state voltage dependence of K^+ current activation affects the ability of I_h to influence the EPSP. We found that the crossover voltage at which I_h changes from having a depolarizing influence on V_{peak} to having a hyperpolarizing influence became more negative as the voltage dependence of K^+ conductance

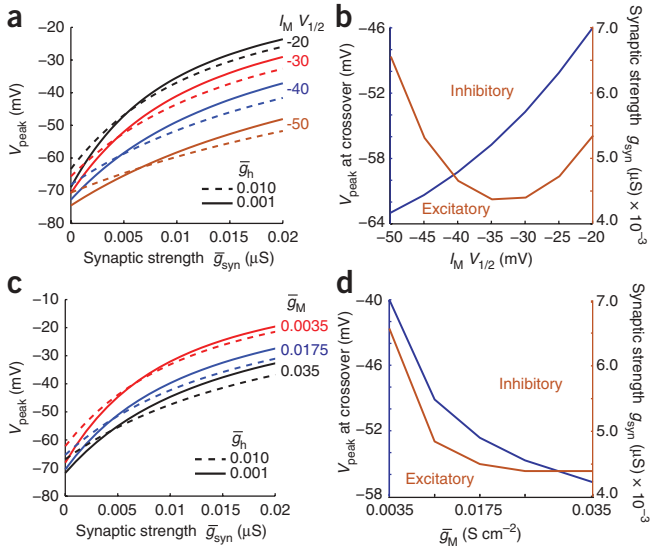

Figure 5 Computational results demonstrating that changes in M-current properties alter the crossover voltage at which I_h first exerts an inhibitory influence on EPSP V_{peak}. (**a**) The effect of I_h on the relationship between V_{peak} and synaptic strength depends on M-current $V_{1/2}$ values (the maximal condutance for I_M equals 0.035 S cm^{-2} throughout). The crossover voltage in response to an increase in $\bar{g}_h$ was shifted to more negative values as M-current $V_{1/2}$ was made more negative. (**b**) The effects of M-current $V_{1/2}$ values on synaptic conductance magnitude (orange line, right axis) or V_{peak} (blue line, left axis) at which crossover occurred (from data in **a**). Regions above the blue or orange lines correspond to V_{peak} or synaptic strength values at which I_h was inhibitory; regions below the line correspond to V_{peak} or synaptic strengths for which I_h depolarized V_{peak}. (**c**) The effect of I_h on the relationship between V_{peak} and synaptic strength depends on M-current maximal conductance values (in S cm^{-2}; M-current $V_{1/2}$ = –35 mV). The crossover voltage in response to an increase in $\bar{g}_h$ was shifted to more negative potentials as I_M was increased. (**d**) The effects of varying the maximal M-conductance on V_{peak} (blue line) or synaptic conductance (orange line) at which crossover occurred.

activation was shifted to more hyperpolarized potentials. Conversely, depolarizing shifts in K^+ current activation properties moved the crossover voltage to more positive potentials (data not shown). Notably, a subthreshold crossover voltage, and hence an inhibitory effect of I_h, was observed over a wide voltage range of K^+ current activation parameters, indicating a robust effect.

I_h interacts with the M-type K^+ current to inhibit EPSPs

Because our computational results relied on the squid axon K^+ conductance, it was important to explore whether a model incorporating a mammalian voltage-gated K^+ conductance could also interact appropriately with I_h to yield subthreshold inhibitory effects. We reasoned that the K_V7 M-type K^+ current was a good candidate for mediating the inhibitory effects of I_h, as the M current is present in CA1 pyramidal neurons, activates at subthreshold voltages, has a slow time course of activation and shows non-inactivating gating properties[38–41]. We therefore examined a model containing I_h, a passive leak conductance, an excitatory synaptic input and a model of the M current based on previous studies in mammalian pyramidal neurons[40,42,43].

We found that the M current also enabled I_h to exert a dual depolarizing/hyperpolarizing effect on the peak voltage of subthreshold EPSPs. As observed with the Hodgkin-Huxley K^+ conductance model, I_h interacted with M current to depolarize the peak voltage of weak EPSPs and to hyperpolarize V_{peak} for stronger, but still subthreshold, EPSPs (**Fig. 4e**). Also similar to the Hodgkin-Huxley K^+ conductance, shifts in the $V_{1/2}$ of M-current activation (**Fig. 5a,b**) or changes in M-current maximal conductance (**Fig. 5c,d**) altered the crossover voltage and synaptic strength at which I_h began to exert an inhibitory influence. In the presence of M current, I_h was also able to exert a net inhibitory effect on peak voltage during a burst of strong EPSPs, as observed previously[14,15], although the influence of I_h by the final EPSP was small as a result of its deactivation during the burst (**Supplementary Figs. 2** and **3**).

In contrast with the crossover in the EPSP input-output curves in response to changes in I_h, a comparison of EPSP input-output curves with or without M current revealed that this K^+ current exerted a purely inhibitory effect, shifting peak EPSP voltage to more negative potentials at all synaptic strengths. This inhibitory action of M current was seen in either the absence or presence of a fixed level of I_h (data not shown). Such an effect is in accord with previous results showing that the M current consistently inhibits neural activity[40,41].

So far we have considered the interaction of I_h and M current in the context of a single-compartment model. However, I_h is present in a gradient of increasing density along the apical dendritic tree of both CA1 and layer 5 pyramidal neurons, where I_h density at the distal tips of the dendrites is up to 50-fold larger than that in the soma[8,9,11]. In contrast, the precise subcellular localization of the M-type K^+ channels is less clear, with some studies reporting dendritic M currents[38,41] and others claiming only somatic and/or axonal localization[40,44]. To examine the importance of the subcellular localization of these channels, we incorporated the I_h dendritic gradient into a multicompartment model of a CA1 neuron in which excitatory inputs were targeted to the apical dendrites. Notably, an inhibitory effect of I_h on the somatic peak EPSP voltage was still observed, regardless of whether M current was present in dendrites or restricted to the soma (**Supplementary Fig. 5** online). When M current was restricted to the soma, dendritic I_h exerted a purely depolarizing effect on the local dendritic EPSPs recorded at the site of synaptic input, 250 μm from the soma. However, the dendritic I_h still was able to inhibit the peak somatic voltage for large, subthreshold EPSPs. Conversely, in multicompartment models lacking an M current, I_h produced a purely depolarizing effect on both local dendritic EPSPs and somatic EPSPs (**Supplementary Fig. 5**). These results indicate that the inhibitory effects of I_h on somatic EPSPs require the presence of M-type K^+ channels, but are not very sensitive to the subcellular distribution of either I_h or M current.

Inhibitory effects of I_h are prevented by M-current blockade

We used an experimental approach to examine whether the inhibitory effects of I_h in CA1 neurons do indeed arise from its interaction with the M current by applying the specific M-current inhibitor XE991[30,39,40] at a concentration (10 μM) that does not alter synaptic transmission under the conditions of our experiments[44]. Application of XE991 produced a variable depolarization of the resting membrane that was large enough to lead to spontaneous firing in some CA1 neurons, consistent with the inhibitory role of the M current. Such cells were not studied further because the spiking interfered with EPSP measurements. In those cells that did not fire spontaneously, XE991 produced a relatively small 3.4 mV ($P = 0.10$, $n = 7$) depolarization of the resting membrane.

We next examined the effects of I_h on somatic EPSPs with M current blocked by measuring V_{peak} as a function of stimulus strength, first in the presence of I_h and then following I_h blockade with ZD7288. Addition of ZD7288 in the presence of XE991 hyperpolarized the resting membrane by ~8 mV (RMP with XE991 alone = –65.5 ± 1.7 mV, RMP with XE991 and ZD7288 = –73.5 ± 1.8 mV, $n = 7$,

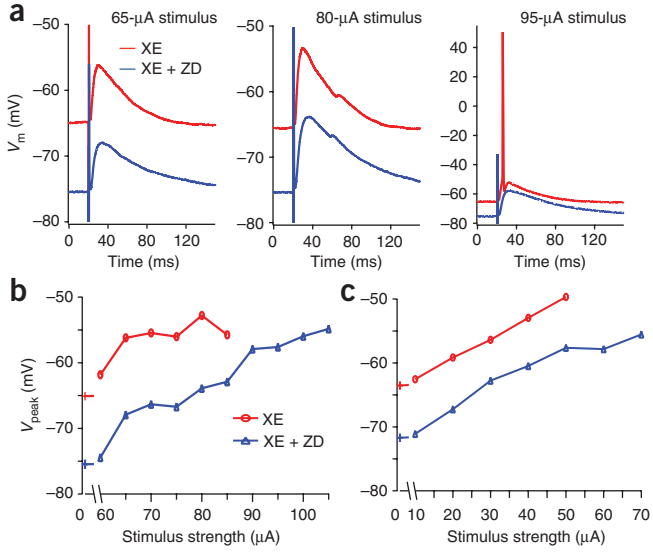

Figure 6 Pharmacological blockade of M current caused I_h to have a purely excitatory influence. (**a**) Experimental EPSPs recorded at the soma in response to three stimulus strengths when M current was blocked with XE991 (10 μM). EPSPs shown in XE991 (XE, red traces) and XE991 with 10 μM ZD7288 (XE + ZD, blue traces). The presence of I_h had an excitatory effect on V_{peak} for weak (left), intermediate (middle) and strong (right) stimuli. Note that the strongest stimulus evoked a spike in the presence of I_h, but not in its absence. (**b**) I_h had an excitatory effect on the relationship between V_{peak} and stimulus strength with M current blocked by XE991. Data are shown in the absence (red) or presence (blue) of ZD7288. The 0 μA data (dashes) denote the RMP. (**c**) Similar results were obtained in a second cell as in **b**.

$P < 0.001$, paired t test), similar to its effect in the absence of XE991. Of particular interest, blockade of the M current abolished the inhibitory effect of I_h on peak EPSP voltage. In the present of XE991, I_h exerted a purely excitatory effect on V_{peak} for both weak and strong stimuli (**Fig. 6a–c**; compare with **Fig. 2**, with normal M current). This result was seen in all of the cells tested ($n = 7$). In addition, when M current was blocked, the presence of I_h now increased the excitability of the cell, as evidenced by spike firing at lower stimulus strengths in the absence of ZD7288 than in its presence (**Fig. 6a**). These findings confirm our modeling results and further support the idea that I_h alone exerts an excitatory influence on neuronal activity and that the inhibitory effects of I_h require an interaction with voltage-gated K^+ currents.

DISCUSSION

We found that I_h exerted dual depolarizing/hyperpolarizing effects on the peak voltage of subthreshold EPSPs as a function of synaptic strength. For weak EPSPs, I_h exerted a depolarizing effect on V_{peak}. In contrast, for strong EPSPs, I_h exerted an inhibitory, hyperpolarizing effect. Although previous studies have described an inhibitory influence of I_h on EPSP amplitude (ΔV_{EPSP}) and firing of both Na^+ and Ca^{2+} spikes[10,15,17,19,23,26], our results provide, to the best of our knowledge, the first demonstration that I_h can also exert an inhibitory effect on subthreshold peak EPSP voltage. This is an important distinction because peak EPSP voltage, rather than EPSP amplitude, determines the effect of the EPSP on neuronal firing[25].

Our results also provide insight into the mechanism of the paradoxical effect by which the depolarizing inward I_h can produce a net hyperpolarizing effect on peak EPSP voltage. First, using a simple computational model, we found that I_h acting in the absence of other voltage-gated channels exerted a purely depolarizing effect on the peak membrane potential achieved by subthreshold EPSPs. This indicates that the direct excitatory effect of I_h to depolarize the membrane predominates over its inhibitory effect to increase resting membrane conductance. This direct excitatory effect of I_h also underlies its classical contribution to the pacemaker depolarization that generates rhythmic firing in both cardiac myocytes and certain CNS neurons, such as thalamic relay neurons[12,13].

In contrast with the results of our simple model in which I_h is purely excitatory, we found that I_h produced an inhibitory effect on V_{peak} of large, subthreshold EPSPs in models containing delayed-rectifier voltage-gated K^+ channels. The excitatory-to-inhibitory crossover effect of I_h on peak EPSP voltage that occurred in such models provides an example of the nonlinear interplay of voltage-dependent currents. The depolarization of the resting membrane by I_h enhanced the resting voltage-gated K^+ conductance beyond that attained in the absence of I_h. At the peak of a weak EPSP, the outward driving force on K^+ was quite small compared with the large inward driving force on current through I_h channels, causing the direct depolarizing effect of I_h to be dominant. In contrast, at the peak of large EPSPs, the outward driving force on K^+ was increased and the inward driving force on I_h was decreased. As a result, the inhibitory effect of the K^+ current was dominant. Our computational results further suggest that the inhibitory effect of I_h requires that the K^+ current kinetics be relatively slow compared with the time course of the EPSP. This allows the I_h-mediated increase in resting K^+ conductance to persist throughout the EPSP and thus inhibit EPSP peak voltage.

Both our experimental and computational findings implicate the M-type K^+ current as being the probable mediator of the inhibitory effects of I_h in CA1 pyramidal neurons. The relatively negative voltage range of activation of the M current allows it to respond to the small changes in resting potential mediated by I_h; the slow activation kinetics of the M current ensure that such changes in resting activation influence the peak EPSP voltage[30,38,40,41]. Moreover, in experiments in which we blocked the M current with XE991, I_h exerted a purely excitatory effect, confirming that M current is necessary for the inhibitory effect of I_h. One other important computational result is that the inhibitory effect on somatic peak EPSP voltage caused by the interaction of I_h and M current does not depend on channel distributions in the somatodendritic compartments. This is important, as I_h is known to be present in a gradient of increasing density in apical dendrites, whereas it is unclear whether M current is restricted to axosomatic compartments or is present in dendrites[38,40,41].

In contrast to the dual depolarizing/hyperpolarizing effects of I_h on V_{peak}, all manipulations that increased the M current, whether in the absence or presence of I_h, hyperpolarized V_{peak} with no crossover effect. This is consistent with a large number of previous studies that have shown an inhibitory influence of the M current[40,41]. The purely inhibitory nature of the M current arises because its two actions to enhance membrane conductance and to generate a hyperpolarizing outward current both act in the same direction to inhibit peak EPSP voltage and neuronal firing. This is in contrast to I_h, whose direct depolarizing and shunting effects have opposing influences.

The fact that the M current can be modulated by neurotransmitters and second messenger cascades raises the possibility that the mode of action of I_h on dendritic integration can be tuned from inhibition to excitation. For example, the loss of M current that occurs on muscarinic receptor stimulation[45] and phosphatidylinositol (4,5)-bisphosphate depletion[46] should drive I_h into a purely excitatory

mode of action on V_{peak}. In contrast, the large increase in M current that occurs in response to an increase in cAMP[32] should cause I_h to exert a predominantly inhibitory effect on V_{peak} (**Fig. 5**). As changes in both the M current and I_h are thought to occur in epileptic diseases[24,29,31,32], an understanding of how these two currents interact to regulate excitability may be important for the development of new therapeutic approaches.

The dual depolarizing/hyperpolarizing effects of I_h on peak EPSP voltage have interesting implications for how this current may differentially regulate neuronal firing depending on the state of excitability of a neuron. Under conditions in which spike threshold is low and negative to the I_h crossover voltage for inhibition, manipulations that enhance I_h will increase V_{peak} for subthreshold EPSPs and will thus have an excitatory effect on the ability of an EPSP to trigger an action potential. In contrast, when spike threshold is high and positive to the crossover voltage, manipulations that enhance I_h will decrease V_{peak} for EPSPs near threshold and will thus inhibit the ability of an EPSP to elicit an output. Therefore, the polarity of the effect that a change in I_h exerts on neuronal firing will depend on the overall excitability of the cell. Even if I_h and the M current remain constant, the effect of their interaction on neuronal output can shift from excitatory to inhibitory as a result of modulatory changes in other voltage-gated conductances that alter spike threshold. Such nonlinear subthreshold interactions among voltage-gated channels provide a rich variety of mechanisms for fine-tuning the relationship between excitatory synaptic input and neuronal output.

METHODS

Slice electrophysiology. Whole-cell recordings were obtained from hippocampal CA1 pyramidal cells in submerged horizontal brain slices from postnatal day 28–40 mice. Recordings were carried out at 31–33 °C with inhibitory transmission being blocked by $GABA_A$ (2 μM gabazine) and $GABA_B$ receptor antagonists (1 μM CGP-55845). Stimulating current pulses (0.1–0.2 ms) were applied through focal extracellular electrodes with a constant current generator once every 15 s. For graded stimulation, current amplitude was adjusted to evoke an EPSP in control conditions and then incremented until spike threshold was reached. Identical current pulses were reapplied after addition of 10 μM ZD7288 to block I_h. All procedures conformed to US National Institutes of Health regulations and were approved by the Institutional Animal Care and Use Committees of Columbia University and the New York State Psychiatric Institute. See **Supplementary Methods** online for full details.

Statistical analysis. The average sag ratio was expressed as $[(1 - \Delta V_{ss} / \Delta V_{min}) \times 100\%]$, where $\Delta V_{ss} = RMP - V_{ss}$, $\Delta V_{min} = RMP - V_{min}$, V_{ss} is the steady-state potential and V_{min} is the initial minimum potential. Comparisons were made using paired t tests where appropriate. An unpaired t test was used to compare control RMP with the RMP in the presence of XE991. $P < 0.05$ was considered to be statistically significant. Results are expressed as mean ± s.e.m.

Computational modeling. Computational models were implemented and run in NEURON[47] (version 5.9; http://www.neuron.yale.edu/neuron). The I_h[10,17] and M-type K^+ conductance[40,42,43] models were based on experimental studies. See **Supplementary Methods** for full details.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

M.S.G. performed all experiments, wrote the computer programs, carried out the analysis and wrote the initial draft of the manuscript. L.F.A. and S.A.S. participated in the design of the experiments and modeling studies and helped in the preparation of the final manuscript.

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