Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites

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The dendrites of pyramidal neurons have markedly different electrical properties from those of the soma, owing to the non-uniform distribution of voltage-gated ion channels in dendrites. It is thus possible that drugs acting on ion channels might preferentially alter dendritic, but not somatic, excitability. Using dendritic and somatic whole-cell and cell-attached recordings in rat hippocampal slices, we found that the anticonvulsant lamotrigine selectively reduced action potential firing from dendritic depolarization, while minimally affecting firing at the soma. This regional and input-specific effect resulted from an increase in the hyperpolarization-activated cation current (I_h), a voltage-gated current present predominantly in dendrites. These results demonstrate that neuronal excitability can be altered by drugs acting selectively on dendrites, and suggest an important role for I_h in controlling dendritic excitability and epileptogenesis.

Many central nervous system (CNS) neurons have extensively arborized dendrites on which they receive the majority of their synaptic contacts. Recent advances in electrophysiological techniques have shown that the apical dendrites of hippocampal and neocortical pyramidal neurons have markedly different electrical properties from those of their corresponding somata, and these differing properties are due to non-uniform distributions and kinetics of voltage-gated channels. For example, in hippocampal pyramidal neurons, the A-type transient K^+ current (I_A) and the hyperpolarization-activated cation current (I_h; h-channel) are present in the dendrites at many-fold higher densities than they are in the soma[1–5]. The non-uniform distribution of voltage-gated channels affect signal processing in the dendrites, altering the retrograde propagation (or 'back-propagation') of action potentials (APs), and the integration of synaptic potentials.

We reasoned that some drugs that affect ion channels might act preferentially on dendritic voltage-gated channels, and thus selectively alter excitability in one region of the neuron. One such class of drugs consists of anticonvulsants known to act on Na^+ channels, and includes phenytoin (PHT), carbamazepine (CBZ) and lamotrigine (LTG). LTG is a structurally novel anticonvulsant that is clinically effective on both partial-onset and primarily generalized seizures, as well as in psychiatric illnesses such as bipolar disorder[6,7]. Here we consider whether these drugs that act on the excitability of CNS neurons as studied with somatic recordings[8] might also have some unforeseen effects on dendritic excitability. We found that LTG had a selective effect on the excitability of the dendrites of hippocampal pyramidal neurons, markedly reducing AP firing when initiated from dendritic depolarization, but minimally affecting APs initiated from somatic depolarization. This effect on dendritic excitability was not due to action on Na^+ channels, but rather to an increase in I_h, a voltage-gated current present in high density in the dendrites. These results show that a drug can affect excitability and AP firing regionally within a neuron, and provide evidence that I_h is centrally involved in modulating neuronal excitability and thus may influence epileptogenesis.

Results

Actions of anticonvulsants on dendritic excitability

Using somatic and dendritic recordings in hippocampal CA1 pyramidal neurons, we tested the hypothesis that anticonvulsant drugs differentially affect the excitability of dendrites and somata. Bath application of LTG during prolonged current injection at the soma caused a modest reduction of repetitive AP firing (**Fig. 1a**), consistent with previous findings[9,10]. When a similar rate of AP firing was elicited from a dendritic injection site, however, LTG application markedly reduced or abolished AP firing (**Fig. 1b**). Injection of a range of current steps that produced 5–20 APs in 500 ms under control conditions (**Fig. 1c**) showed that LTG abolished AP firing for all but the highest amplitude current injections in that series. LTG had a disproportionate effect on AP firing elicited from dendritic depolarization as compared to that from the soma. The effect of LTG on dendritic AP firing was reversible with prolonged (>30 min) return to control solution (data not shown).

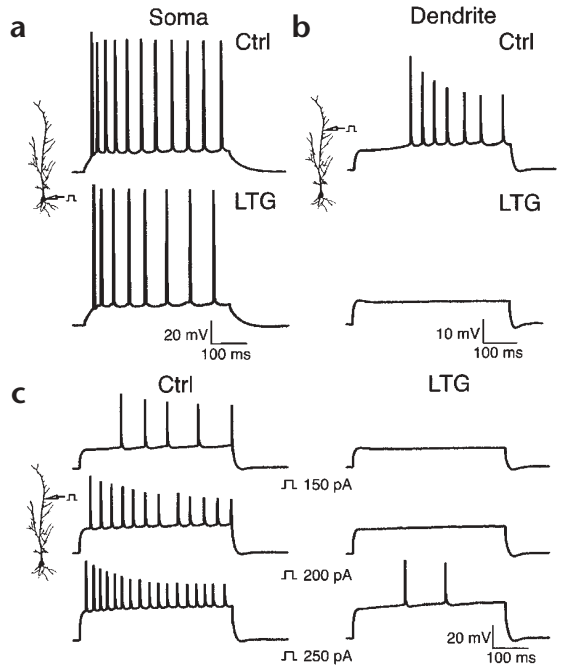

Fig. 1. Lamotrigine (LTG) selectively lowered dendritic excitability. (**a**) Action potential (AP) firing elicited by current injection at the soma under control conditions (Ctrl) was modestly reduced by bath application of LTG (50 M). Resting potential, –68 mV. (**b**) AP firing elicited from dendritic current injection under control conditions was abolished by LTG (50 M). Resting potential, –64 mV. Dendritic recording distance, 190 m. (**c**) Dendritic current injections (150–250 pA for 500 ms), which under control conditions produced 5–20 APs, led to little or no AP firing in the presence of LTG (100 M). Resting potential, –60 mV. Dendritic recording distance, 180 m.

We similarly tested the effects of PHT and CBZ on dendritic excitability. Bath application of PHT (**Fig. 2a**) or CBZ (**Fig. 2b**) did not significantly affect AP firing elicited from dendritic current injection. We quantified the effect of LTG, PHT and CBZ on AP firing by averaging the number of APs elicited by a series of four 500-ms current injections of progressively graded amplitude producing 5–20 APs under control conditions, then repeating these same current injections under drug conditions (**Fig. 2c**). LTG was found to reduce AP firing from dendritic current injection to 17 ± 5.7% of control values ($n = 8$), compared to a much smaller reduction of AP firing elicited at the soma (83 ± 9.3% of control, $n = 6$; $P < 0.0001$ compared to value at the dendrites). In contrast, PHT and CBZ had no significant effect on AP firing from dendritic depolarization (PHT, 99 ± 4.5% of control, $n = 3$, $P > 0.05$; CBZ, 99 ± 6.2%, $n = 3$, $P > 0.05$).

In addition to affecting AP firing from the dendrites, bath application of LTG caused a depolarization of resting potential which was concentration-dependent. This effect was similar in the soma and dendrites (50 M LTG, 3.1 ± 0.26 mV, $n = 13$; 100 M LTG, 5.1 ± 0.78 mV, $n = 9$). In quantifying the effect of LTG on AP firing, resting potential was compensated to the original pre-drug level. When resting potential was not compensated, the effect of LTG on AP firing from dendritic current injection was somewhat reduced but still significantly different from control (**Fig. 2d**, top traces; AP firing, 53 ± 6.5% of control, $n = 6$, $P < 0.001$). In contrast, LTG had no significant effect on AP firing from somatic current injection when resting potential was uncompensated (**Fig. 2d**, bottom traces; 99 ± 13% of control, $n = 4$, $P > 0.05$). Thus, the steady-state depolarization induced by LTG reduced the magnitude of the effect of LTG on AP firing by moving resting potential closer to threshold. However, even when this resting potential change was uncompensated, LTG continued to selectively affect AP firing elicited in the dendrites.

LTG has been shown to affect Na^+ currents in a use-dependent manner[9,10]. We therefore explored whether the effect of LTG on APs recorded in dendrites might be due to a reduction of the amplitudes of back-propagating APs. Back-propagating APs antidromically activated by 50-Hz trains of stimulation under control conditions showed a progressive decline

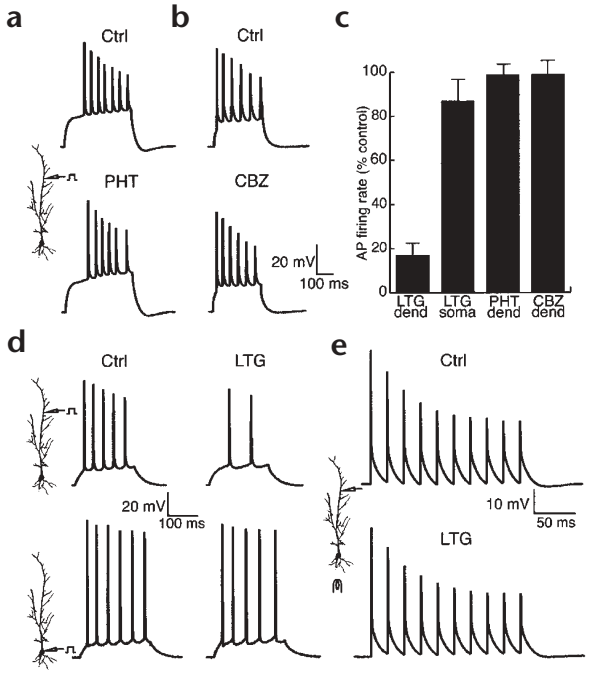

Fig. 2. LTG, but not phenytoin or carbamazepine, lowered dendritic excitability. (**a**) AP firing from dendritic current injection was minimally affected by bath application of phenytoin (PHT, 50 M). Resting potential, –62 mV. Dendritic recording distance, 200 m. (**b**) Carbamazepine (CBZ, 50 M) similarly had a minimal effect on AP firing from dendritic current injection. Resting potential, –67 mV. Dendritic recording distance, 210 m. (**c**) Group data showing the selective effect of LTG on dendritic AP firing compared to its effect on somatic AP firing, and the minimal effects of PHT and CBZ on dendritic AP firing. Drug concentrations and number of observations for each condition: LTG dend, 50–100 M, $n = 8$; LTG soma, 50–100 M, $n = 5$; PHT dend, 50–100 M, $n = 3$; CBZ dend, 25–50 M, $n = 3$. (**d**) Dendritic AP firing shown in response to a 200-pA current injection under control conditions (top traces) was reduced by ~50% in the presence of LTG (100 M) when resting potential was not compensated to control levels. Resting potential, –61 mV (Ctrl) and –58 mV (LTG). Dendritic recording distance, 180 m. When the same experiment was done using somatic current injection (bottom traces), LTG (100 M) had no significant effect on AP firing. Resting potential, –66 mV (Ctrl) and –61 mV (LTG). (**e**) Back-propagating APs in the dendrites elicited by 50-Hz antidromic stimulation under control conditions were not affected by addition of LTG (50 M). Resting potential, –60 mV. Dendritic recording distance, 220 m.

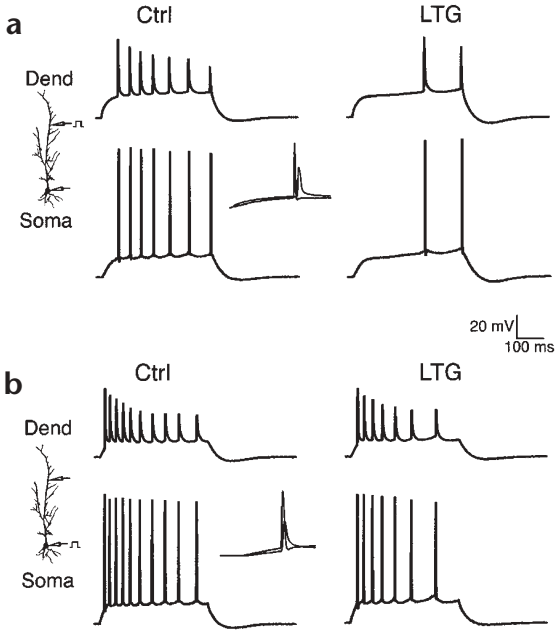

Fig. 3. LTG decreased AP firing elicited from the dendrites without affecting AP back-propagation in dendrites. (**a**) Dual simultaneous whole-cell recordings from soma and dendrites with dendritic current injection. Under control conditions, APs elicited from dendritic current injection initiated near the somatic recording site, then back-propagated to the dendritic recording site, as shown by expanded traces with the first APs from soma and dendrites (inset). In the presence of LTG (100 M), AP firing from dendritic current injection was reduced, but APs continued to initiate near the somatic recording site and back-propagate into the dendrites. Dendritic recording distance, 165 m. Resting potential for all traces, –64 mV. Calibration for insets is same as shown for **Fig. 3**, but with voltage 40 mV and time 12.5 ms. (**b**) Same recording as in (**a**), but with somatic current injection. LTG (100 M) decreased AP firing elicited from the soma to a much lesser extent than when elicited from the dendrites, and AP back-propagation remained unaffected. APs continued to initiate near the somatic recording site (inset).

in amplitude because of the slow inactivation of Na^+ currents[11] (**Fig. 2e**). LTG (50 M) did not significantly affect the amplitudes of antidromic APs throughout the train, including the first or last APs in the train. The amplitude of the first back-propagating AP in response to 50-Hz antidromic activation in the presence of LTG (50–100 M) was 97.3 ± 1.9 % ($n = 6$) of control AP amplitude. Under control conditions, the amplitude of the tenth AP in the train decremented to 39.2 ± 3.4% ($n = 6$) of the amplitude of the first AP, while in the presence of LTG the tenth AP was 40.0 ± 4.5% ($n = 6$) of the first AP amplitude in LTG (not significantly different from control, $P > 0.05$). These data show that LTG did not affect the amplitude of back-propagating APs in the dendrites, nor their decrement due to dendritic Na^+ current entering into a slow inactivated state. Because the amplitudes of back-propagating APs in the dendrites are sensitive to small changes in Na^+ currents[11], these results suggest that the action of LTG on reducing AP firing elicited in the dendrites was not due to an effect on AP back-propagation *per se*, and was independent of actions on Na^+ channels.

To explain how LTG could dramatically affect AP initiation from dendritic current injection while minimally affecting APs elicited from the soma, we simultaneously made whole-cell recordings from the soma and distal dendrites. When current was injected into the dendrites under control conditions, APs initiated near the somatic recording site (as determined by comparing the latency to onset of dendritic and somatic APs; **Fig. 3a**, inset), and then back-propagated into the dendrites (**Fig. 3a**, Ctrl), consistent with prior studies[12,13]. In the presence of LTG,

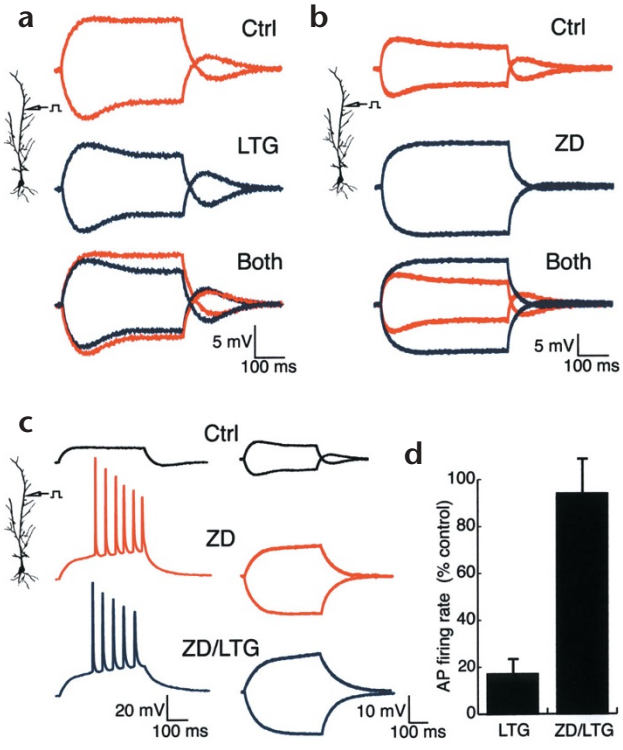

Fig. 4. LTG decreased dendritic excitability by increasing activation of I_h. (**a**) Whole-cell dendritic recordings showing subthreshold response to both hyperpolarizing and depolarizing current injection. Under control conditions (Ctrl), a slow relaxation of membrane potential ('sag') in response to both hyperpolarizing and depolarizing current injection demonstrated the presence of I_h. In the presence of LTG (50 M), the sag was increased in response to both depolarizing and hyperpolarizing current injection. Resting potential, –64 mV. Dendritic recording distance, 190 m. (**b**) Application of ZD-7288 (ZD, 10 M), a specific blocker of I_h, blocked the sag in membrane potential and markedly increased input resistance in response to current injection under control conditions, producing an opposing effect on dendritic excitability compared to LTG. Resting potential, –70 mV. Dendritic recording distance, 240 m. (**c**) Application of ZD-7288 (10 M) caused an initially subthreshold dendritic current injection (left traces, Ctrl) to produce repetitive AP firing (left traces, ZD). Subsequent application of LTG (50 M) produced a minimal effect on dendritic AP firing in the presence of I_h blockade by ZD-7288 (left traces, ZD/LTG). Similarly, the voltage sag produced by I_h with subthreshold hyperpolarizing and depolarizing current injections under control conditions (right traces, Ctrl) was blocked by ZD-7288 (right traces, ZD). Adding LTG (50 M) had no effect on the subthreshold response in the presence of ZD (right traces, ZD/LTG). Resting potential, –68 mV. Dendritic recording distance, 180 m. (**d**) Group data showing that the decrease in dendritic AP firing caused by LTG was largely blocked by previous application of ZD-7288 (ZD/LTG). Drug concentrations and number of observations for each condition: LTG, 50–100 M, n = 8; ZD/LTG, 50 M LTG and 10 M ZD-7288, n = 5.

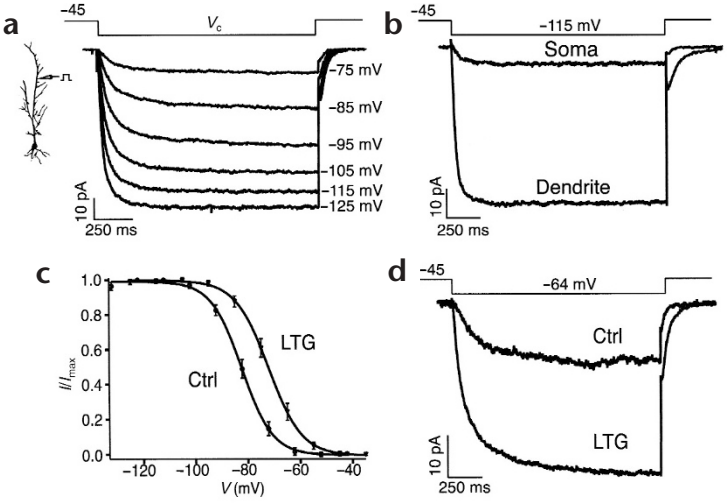

Fig. 5. LTG causes a depolarizing shift in I_h activation. (**a**) Dendritic cell-attached patch recordings of I_h. Hyperpolarizing voltage commands (V_c, labeled at right of current traces) from a –45 mV holding potential produced slowly activating inward currents characteristic of I_h. Dendritic recording distance, 210 m. (**b**) I_h recorded from dendrites was markedly larger than that recorded at the soma. Current traces shown are with a holding potential of –45 mV and a voltage command of –115 mV, and are from different neurons; dendritic recording distance, 200 m. (**c**) I_h activation from dendritic cell-attached patch recordings. Addition of LTG (100 M) to the pipette solution produced an ~11 mV depolarizing shift in I_h activation (LTG) compared to control conditions (Ctrl; $V_{1/2}$ roughly –83 mV; $V_{1/2}$ in LTG, roughly –72 mV). Number of observations for each point in activation curves: control, $n = 7$; LTG, $n = 6$. (**d**) Representative I_h from voltage commands near rest in a neuron under control conditions (Ctrl) and a separate neuron with LTG (100 M) in the recording pipette; to facilitate comparison, control current trace was scaled such that maximal I_h was the same in both control and LTG conditions.

AP firing was significantly reduced; however, APs still initiated near the somatic recording site and back-propagated into the dendrites (**Fig. 3a**, LTG). When current was instead injected at the soma (**Fig. 3b**), APs continued to initiate near the soma (**Fig. 3b**, inset) but LTG had a much smaller effect on AP firing rate, and did not affect the back-propagation of APs into the dendrites. (Note that the initial dendritic AP in the train was of similar amplitude in both control and drug conditions, but that the amplitude of subsequent APs varied with firing frequency, reflecting decrement due to frequency-dependent slow inactivation of Na^+ channels[11].) These results confirmed that LTG was not altering the back-propagation of APs into the dendrites, but instead was decreasing the transmission of current injected from the dendrites.

LTG affects subthreshold response of dendrites

Prolonged hyperpolarizing and depolarizing subthreshold current pulses injected into the dendrites under control conditions produced voltage waveforms with a slowly activating relaxation (or 'sag') that indicated the presence of the I_h current[14,15]. I_h is a slowly activating, non-inactivating inward current which is active at resting potential and increases its activation with hyperpo-

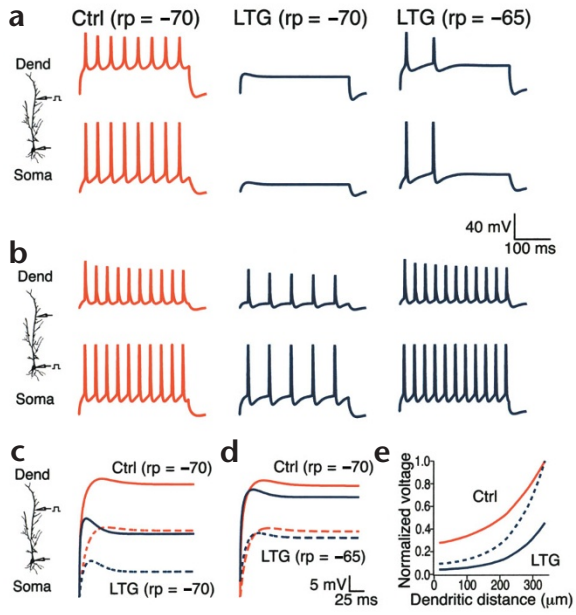

Fig. 6. I_h activation decreased AP firing in a computational model. (**a**) Simulated AP firing in the dendrites (upper traces) and at the soma (lower traces) in response to dendritic current injection. Responses are shown under control conditions with resting potential (rp) set at –70 mV, and during increased I_h activation (10-mV shift) with rp set at –70 mV or –65 mV to simulate the depolarizing action of a 10-mV shift in I_h activation. A simulated increase in I_h activation abolished AP firing from dendritic current injection when resting potential was held at control levels, and produced a significant decrease in AP firing when resting potential was allowed to depolarize to –65 mV. (**b**) Same conditions as in (**a**), but with somatic current injection. A simulated increase in I_h activation produced a much smaller effect on AP firing from somatic current injection than with dendritic current injection when resting potential was held at –70 mV. When resting potential was held at –65 mV, increased I_h activation no longer reduced AP firing from somatic current injection. Dendritic distance, 200 m. (**c**) Simulated decay of voltage transients in response to a 700-pA step current injection at 350 m in the dendrites. When resting potential was held at –70 mV, the steady-state voltage recorded in the dendrites under control conditions (Ctrl; solid red line) was markedly reduced by a 10-mV shift in I_h activation (LTG; solid blue line). The corresponding responses at the soma (dashed lines) show an even larger proportional reduction in the steady-state voltage. (**d**) Same responses as in (**c**), but with resting potential held at –65 mV to simulate the depolarization occurring with a 10-mV shift in I_h activation. Under these conditions, the steady-state membrane potential at the soma resulting from current injection in the dendrites was still reduced compared to control. (**e**) Plot shows the steady-state depolarization from resting potential occurring along the dendrite produced by a 650-pA current injection at 350 m in the dendrites. Solid lines show depolarization under either control (Ctrl) or increased I_h activation conditions (LTG; 10-mV shift) normalized to the peak depolarization at the site of current injection under control conditions. Dashed line shows depolarization during increased I_h activation normalized to peak depolarization in that condition. Resting potential, –70 mV in all traces.

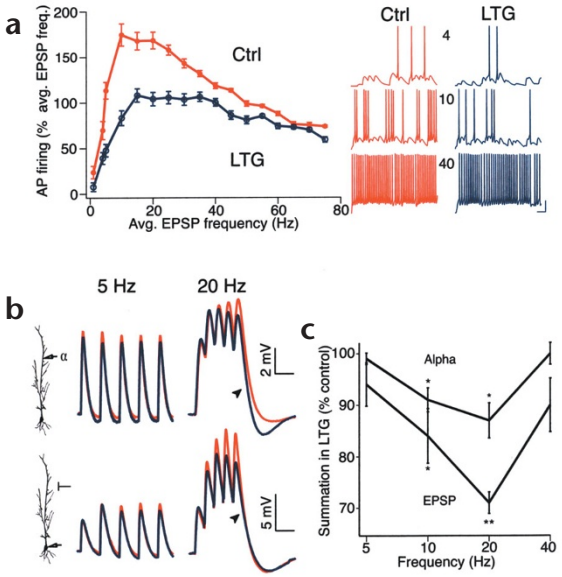

Fig. 7. LTG decreased synaptic activation of pyramidal neurons by reducing temporal summation in a frequency-dependent manner. (**a**) Simulated somatic AP firing in response to synaptic stimulation in the dendrites. At low stimulus frequencies (<5 Hz), AP firing rate was modestly affected by increased I_h activation (10-mV shift; LTG). As the stimulus rate increased, AP firing rate under control conditions increased rapidly due to temporal summation, but increased at a much slower rate with increased I_h activation, with the greatest effect of increased I_h occurring between 10 and 30 Hz. At higher frequencies (>30 Hz), the effect of increased I_h diminished. Traces at right show sample AP firing in control and increased I_h conditions at 4-, 10- and 40-Hz stimulation rates. Resting potential, –65 mV for all traces. Calibration is 20 mV, 100 ms. (**b**) Experimental data showing superimposed dendritic responses to current injection of alpha waveforms (upper traces) under control (red traces) and LTG (100 M; blue traces) conditions. LTG had a minimal effect on a 5-Hz train of stimuli, but decreased temporal summation for a 20-Hz train (arrowhead). Dendritic recording distance: 5 Hz, 240 m; 20 Hz, 210 m. Resting potential, –60 mV for both. Somatic recordings of EPSPs resulting from stimulation of Schaffer collaterals (lower traces) showed that LTG had a similar frequency-dependent effect on temporal summation as for alpha waveforms, minimally affecting low frequency EPSPs (5 Hz) but significantly reducing the postsynaptic response to 20-Hz stimulation (arrowhead). Resting potential: 5 Hz, –61 mV; 20 Hz, –60 mV. Time calibration, 200 and 100 ms for 5 Hz and 20 Hz, respectively. (**c**) Plot of the reduction in temporal summation caused by LTG (100 M) as measured with alpha waveform dendritic current injection or synaptic stimulation. LTG significantly decreased temporal summation at 10 and 20 Hz, but not at 5 or 40 Hz. (Statistical significance compared to control, $^*P < 0.05$ or $^{**}P < 0.005$.)

larization. Because of these unique properties, I_h tends to stabilize membrane potential towards rest, producing either a depolarizing sag in membrane potential with prolonged hyperpolarizing current injection (reflecting activation of an inward current), or a hyperpolarizing sag in response to prolonged depolarizing current injection (due to deactivation of an inward current; **Fig. 4a** and **b**, Ctrl). This voltage sag thus reduced steady-state input resistance in response to prolonged current injection. I_h is distributed in the hippocampal pyramidal neuron in a non-uniform gradient pattern, with a low density at the soma, and a density that increases up to sevenfold with increasing distance in the apical dendrites[16]. The high dendritic density of I_h contributes significantly to local integration properties, reducing the input resistance, time constant and temporal summation of EPSPs in the dendrites[17,18].

In the presence of LTG, subthreshold current injection in the dendrites produced responses with greater voltage sag, particularly in response to depolarization, suggestive of a larger I_h at rest (**Fig. 4a**). LTG thus reduced input resistance in the dendrites: when measured with 500-ms current injections producing <15 mV voltage excursions from rest, LTG (50–100 M) lowered steady-state input resistance by 23.3 ± 5.2% compared to control ($n = 7$, $P < 0.005$) in response to depolarizing current injections, and by 13.6 ± 6.7% in response to hyperpolarizing current injections (not statistically different from control, $n = 5$, $P > 0.05$). Consistent with the smaller density of I_h at the soma, however, LTG had a much smaller effect at the soma on the response to subthreshold current injection (data not shown).

Application of the specific I_h blocker ZD-7288 at near-saturating doses (10 M)[19] had the opposite effect (**Fig. 4b**). ZD-7288 abolished the slowly activating voltage relaxation present under control conditions in response to both hyperpolarizing and depolarizing current injection, and markedly increased input resistance. When ZD-7288 was pre-applied before LTG, it largely blocked the effect of LTG on dendritic excitability: subthreshold current injections in the dendrites under control conditions became suprathreshold with repetitive AP firing in the presence of ZD-7288; when LTG was added, there was a minimal effect on AP firing (**Fig. 4c**). Similarly, ZD-7288 abolished the effect of LTG on subthreshold current injection in the dendrites (**Fig. 4c**). Group data (**Fig. 4d**) shows that I_h blockade by ZD-7288 prevented the effect of LTG on dendritic AP firing (94 ± 14% of control, $n = 5$, $P > 0.05$). These results suggest that LTG acted via I_h, increasing its steady-state activation at membrane potentials near rest. This would have the effect of diminishing the dendritic input resistance, time constant and temporal summation of excitatory inputs.

LTG increases activation of I_h

We examined the mechanism of action of LTG on I_h using cell-attached patch recordings in the dendrites. This technique allowed us to study I_h from a localized region of the dendrites, while leaving intact the modulation of this current by the intracellular milieu[20]. Using a pipette solution that isolated I_h, hyperpolarizing voltage commands from a holding potential of –45 mV produced slowly activating inward currents which were non-inactivating (**Fig. 5a**). The magnitude of these currents was small for voltage commands near rest (–75 mV) and progressively increased with larger hyperpolarizing commands, as is typical for I_h. The currents at the distal dendrites were far larger in peak amplitude than those at the soma (**Fig. 5b**). We plotted the voltage-dependent activation of I_h by measuring the peak inward tail current upon return to holding potential after a 1-s voltage command. I_h activation near resting potential (–60 to –70 mV) was small under control conditions, and had a $V_{1/2}$ of roughly –83 mV (**Fig. 5c**). Addition of LTG to the pipette solution produced an 11-mV depolarizing shift in the I_h activation curve, with a $V_{1/2}$ of roughly –72 mV. This shift in I_h activation caused a substantial increase in the amount of I_h active around resting potential (**Fig. 5d**). LTG did not increase maximal I_h measured at the most hyperpolarized potentials (I_h at –115 mV in control, 30.0 ± 5.3 pA, $n = 6$; in LTG, 34.0 ± 6.3 pA, $n = 8$, $P > 0.05$). LTG

also depolarized the resting potential, with an average depolarization of ~5 mV upon break-in at the end of the experiment, compared to control neurons. (This apparent shift in resting potential is included in the 11-mV shift of I_h activation; that is, without accounting for the resting potential change, there was roughly a 6-mV activation shift.) We attribute this effect to probable LTG entry into the dendritic membrane, affecting local I_h and thus local measurements of resting potential.

LTG reduces synaptic activation of pyramidal neurons

How does increased I_h activation affect AP firing in response to repetitive synaptic stimulation, as would occur during epileptiform firing? To study how modulation of I_h would alter not only the postsynaptic response to trains of EPSPs in the dendrites but also the transmission of voltage transients in the dendrites, we used a computational model (Methods) of the CA1 hippocampal pyramidal neuron. We first simulated AP firing from current injection in control conditions and then with I_h activation shifted in a depolarized direction by 10 mV. This simulated increase in I_h activation significantly lowered the rate of AP firing from dendritic current injection, both when resting potential was held at its control level (**Fig. 6a**, middle traces) and when resting potential was set 5 mV more depolarized, simulating the effects of LTG on resting potential (**Fig. 6a**, right traces). Conversely, simulated current injection at the soma showed only modest reduction of AP firing when I_h activation was increased and resting potential was fixed at control levels (**Fig. 6b**, middle traces); and when resting potential was held depolarized by 5 mV, increased I_h activation caused no reduction (or a small increase) in AP firing at the soma (**Fig. 6b**, right traces). This model replicated our experimental findings of a selective effect of increased I_h activation on dendritic excitability.

We then used the model to address questions more difficult to study experimentally. Increased I_h activation seemed to have competing influences on somatic AP firing in response to dendritic inputs: a reduction in dendritic input resistance reduced the depolarization seen at the soma from an excitatory dendritic input, whereas the depolarizing change in resting potential brought the soma closer to threshold. We simulated subthreshold current injections in the dendrites and found that, as expected, when resting potential was held at the control level, increased I_h activation caused a significant decrease in the steady-state dendritic potential produced by a dendritic current injection (**Fig. 6c**). However, the decrease in steady-state voltage seen at the soma was proportionately larger than in the dendrites, suggesting that the voltage transient had more decay during its transmission from injection site to soma. When resting potential was set at a depolarized level during increased I_h activation, the resting potential change opposed some of the effects of I_h on dendritic input attenuation by moving closer to threshold. Even under these conditions, the steady-state membrane potential reached at the soma in response to a dendritic input remained lower than control (**Fig. 6d**). This finding showed that the effect of increased I_h on dendritic voltage attenuation outweighed the effect of the resting potential change at the soma, and thus inhibited somatic AP firing from dendritic inputs.

A simulation of the decay of voltage transients along the dendrite clarified how I_h preferentially attenuated dendritic inputs. We simulated a depolarizing current injection at a distal dendritic location (350 m), and measured the steady-state voltage along the length of the dendrite (**Fig. 6e**). A 10-mV shift in I_h activation caused steady-state voltage at the site of current injection to be reduced to ~45% of control, but at the soma, the voltage transient had decayed to ~15% of the control value. A plot of the normalized steady-state voltage (**Fig. 6e**, dashed line) showed that voltage transients had an increased decay with distance under conditions of increased I_h, demonstrating that increased I_h had lowered the length constant of the dendrite. This suggested that the effects of LTG on somatic initiation of AP firing from dendritic current injection were due to a decrease in input resistance at the site of current injection as well as to greater attenuation of voltage transients along the length of the dendrite.

We then used the model to study the effects of LTG on synaptically driven AP firing, using a series of simulated random EPSP trains delivered in the dendrites. Under control conditions, as stimulation frequency increased up to ~20 Hz, the number of APs elicited by EPSPs tended to increase, as temporal summation of EPSPs increased (**Fig. 7a**). With a simulated increase in I_h activation, at low stimulation frequencies (<5 Hz) there was little change in AP firing compared to control. However, at frequencies between 10 and 30 Hz, higher I_h caused a significant reduction in synaptically evoked AP firing compared to control, presumably due to a decrease in temporal summation of EPSPs. At still higher frequencies, there was a decline in temporal summation under control conditions, and a similar decrease in the effect of increased I_h. These results suggest that LTG might decrease the pyramidal neuron response to synaptic stimulation in a frequency-dependent manner, with a peak effect between 10 and 30 Hz.

To investigate these computational phenomena experimentally, we made dendritic whole-cell recordings and used short trains of alpha waveform current injections to simulate EPSPs. When trains of stimuli were delivered at low (5 Hz) frequency, there was little temporal summation, and application of LTG had little effect on the extent of summation (**Fig. 7b**, top traces). At higher frequencies (10–20 Hz), there was more temporal summation, and here LTG caused a decline in temporal summation during the train. This effect declined at higher frequencies (40 Hz; **Fig. 7c**). When trains of synaptically evoked responses were substituted for alpha waveform current injections, and recordings made at the soma, LTG had little effect on low frequency stimulation (5 Hz), but markedly decreased temporal summation at higher frequencies (20 Hz; **Fig. 7b**, bottom traces). (Note that with short trains of EPSPs, temporal summation was superimposed on synaptic facilitation, unlike the case with alpha waveform current injections.) Increased I_h caused a frequency-dependent decrease in temporal summation which was of greatest magnitude around 20 Hz, and had little effect on low-frequency synaptic activity (**Fig. 7c**). These results were in agreement with those obtained by computational modeling, and were similar whether experimentally obtained with postsynaptic current injection or synaptic stimulation, suggesting that the frequency-dependent effects of increased I_h on synaptic activation of pyramidal neurons were largely mediated postsynaptically.

Discussion

These results demonstrate a new mode of action for CNS drugs. LTG exerts an inhibitory effect on AP firing in the hippocampal pyramidal neuron by selectively altering the excitability of the apical dendrites while minimally affecting the excitability of the soma. This regional selectivity is a direct consequence of the drug's action on I_h, which is distributed in a non-uniform gradient along the neuron, with the distal dendrites containing a much higher density of I_h than the soma. Increases in dendritic I_h reduce input resistance, length constant and temporal summation, suppressing the effect of dendritic excitatory synaptic inputs on perisomatic AP initiation in the pyramidal neuron, thus causing an overall reduction in excitability. These regional effects of LTG within a single neuron demonstrate that drugs may have unex-

pected and differing effects on different compartments of the neuron, in particular on the dendritic tree (which by some estimates accounts for >90% of the surface area of pyramidal neurons[21]), underscoring the importance of studying dendritic physiology when evaluating pharmaceutical mechanisms of action.

LTG seems to act by shifting the activation of I_h in a depolarizing direction. Because there is little I_h active at the typical neuronal resting potential, small increases in I_h can lead to significant modulation of subthreshold neuronal behavior. The contributions of I_h to the integrative properties of dendrites include a reduction in input resistance, dendritic length constant, and temporal summation, all of which act to reduce dendritic excitability[16–18]. The reduction of dendritic input resistance and length constant causes a given excitatory input in the dendrites to produce less of a local depolarization which then decays with distance along the dendrites. This produces a smaller voltage change at the soma, ultimately reducing AP firing in response to dendritic depolarization. Increases in I_h also reduce temporal summation, diminishing AP firing from repetitive synaptic inputs in the dendrites. This probably results from the gradual buildup of I_h deactivation during a train of EPSPs, producing a net hyperpolarizing current[18]. The frequency-dependence of this effect may derive from the relatively slow activation and deactivation kinetics of I_h (16–20 ms near rest[16]). Thus, low-frequency EPSPs do not achieve significant steady-state I_h deactivation, whereas higher frequencies (~20 Hz) will lead to increased I_h deactivation and therefore decreased temporal summation. (At still higher frequencies the I_h effect may be overcome by increasing EPSP summation.)

Increases in I_h also depolarize resting potential, moving it towards threshold for AP firing, thus counteracting some of the inhibitory effect on excitatory inputs. Our experiments and simulations showed that the balance of these two influences is different in the two compartments of the neuron. In response to somatic inputs, when the change in resting potential was experimentally uncompensated (as would occur *in vivo*), LTG had little or no effect on AP firing, suggesting that the small change in local input resistance was counterbalanced by the depolarization of resting potential (which was more uniform throughout the neuron). Increased I_h had a more dramatic influence on dendritic inputs and their ability to drive somatic firing, and this remained inhibitory even when the resting potential change was uncompensated. This is because increased I_h not only had a much larger effect on the local depolarization caused by a dendritic input (owing to its increased dendritic density), but also further attenuated that potential during its spread from the dendrites to the soma. These two effects combined to substantially reduce the effects of dendritic inputs on somatic AP firing, while minimally affecting somatic inputs. Thus it appears that increased activation of I_h by LTG reduces the overall excitability of hippocampal pyramidal neurons by attenuating their response to dendritic inputs.

The action of LTG on I_h may constitute an important new anticonvulsant mechanism. The frequency-dependent effect of LTG on temporal summation of EPSPs allows for the selective reduction of AP firing that results from pathologically high levels of excitatory synaptic activity in the dendrites, while preserving AP firing from lower frequency inputs. This fulfills a criterion for ideal anticonvulsant action: that normal brain function be unaffected while excessive firing is suppressed. Other mechanisms of action have been ascribed to LTG that may contribute to its anticonvulsant effect, including reduction of Na^+ currents, Ca^{2+} currents and glutamate receptor activation[9,10,22,23]. However, the action of LTG on I_h, and the role of I_h in modulating oscillatory behavior in other neurons[15] may provide a unique explanation of its efficacy against primarily generalized epilepsy (such as absence epilepsy), a feature not shared by other anticonvulsants with actions on Na^+ channels such as PHT or CBZ[24]. A further implication of these results is that I_h may have an important role in epileptogenesis, as is supported by recent evidence demonstrating alterations in I_h in an animal model of febrile seizures[25], and the involvement of I_h in maintaining the spontaneous activity of CA1 hippocampal interneurons[26] and modulating presynaptic excitability[27]. These multiple actions of I_h, some of which may increase rather than decrease neuronal excitability, imply that its effects on epileptogenesis may be complex. Nonetheless, our results, along with the growing appreciation for the important role of I_h in regulating neuronal excitability, suggest that I_h may be a useful target for further CNS drug development.

Methods

Electrophysiology. Hippocampal slices (400 m) were prepared from 6–10 week-old male Sprague-Dawley rats using standard procedures[4]. Animal protocols were approved by the Animal Research Committee at Baylor College of Medicine. Neurons were visualized with differential interference contrast microscopy using a Zeiss Axioskop (Oberkochen, Germany). Recordings were made at 31–33°C. The extracellular recording solution contained 125 mM NaCl, 25 mM $NaHCO_3$, 10 mM dextrose, 2.5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$ and 1.25 mM NaH_2PO_4. CNQX (10 M) and bicuculline methiodide (20 M) were added to the extracellular solution except when synaptic stimulation was used. The whole-cell recording pipette solution contained 120 mM potassium gluconate, 20 mM KCl, 10 mM HEPES, 4 mM Na_2-ATP, 2 mM $MgCl_2$, 0.3 mM Tris-GTP and 0.2 mM EGTA (pH 7.3 with KOH). For cell-attached patch recordings, the pipette solution contained 120 mM KCl, 20 mM TEA-Cl, 10 mM HEPES, 5 mM 4-aminopyridine, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mM $BaCl_2$ (pH 7.3 with KOH). Recorded neurons had resting potentials between –60 and –72 mV (mean –64 ± 0.7 mV, $n = 36$).

Whole-cell current-clamp recordings were made using a Dagan BVC-700 amplifier (Minneapolis, Minnesota), were sampled at 10 KHz, and filtered at 2 KHz. Patch recordings used an Axon Instruments AxoPatch 1C amplifier (Foster City, California), and were sampled at 2 KHz and filtered at 500 Hz. Data acquisition used custom software written for the Igor Pro analysis environment (Wavemetrics, Lake Oswego, Oregon). Extracellular stimulation in the alveus with a tungsten electrode (A-M Systems, Everett, Washington) was used to antidromically activate APs, and stimulation in the stratum radiatum was used to elicit EPSPs. Dendritic current injections simulating EPSPs were modeled by an alpha function of the form: $I = I_{max}(t/\alpha)e^{-\alpha t}$ where $\alpha = 0.1$.

Drugs were applied in the bath made either from aqueous stock solutions (LTG, ZD-7288) or dissolved in DMSO such that the final DMSO concentration was <1% (PHT, CBZ). All drugs were obtained from Sigma (St. Louis, Missouri) except as noted. LTG was a gift from GlaxoSmithKline (Research Triangle Park, North Carolina); ZD-7288 was obtained from Tocris (Balwin, Missouri). Concentration ranges of anticonvulsants used in this study were chosen with reference to the established peak therapeutic free cerebrospinal fluid concentrations in humans[28]: 39 M LTG, 7 M PHT and 13 M CBZ. Synaptic stimulation experiments were performed with addition to the bath of 50 M APV, 20 M MK-801, 100 M CGP-35348 (Tocris) and 20 M bicuculline methiodide (to block NMDA and GABAergic currents). Area CA3 was isolated from the rest of the slice by a knife cut to diminish repetitive firing from reduced GABAergic inhibition. In addition, EGTA (10 mM) was added to the pipette solution to reduce potentiation of postsynaptic responses from repetitive synaptic stimulation. Temporal summation was measured as the ratio of fifth response amplitude (measured from baseline) to the first response amplitude.

Group data are expressed as mean ± s.e.m. Statistical significance was calculated using Student's *t*-test.

Modeling. All simulations were carried out using the NEURON simulation program[29]. The realistic model of a hippocampal CA1 pyramidal neuron was based on that used in a previous work[30] and included Na^+, DR- and A-type K^+ voltage-gated conductances. A non-inactivating, non-

specific cation current $I_h = g_h * n * (V - E_{rev})$, with $E_{rev} = -30$ mV was inserted in the soma and the apical dendrites. Its dendritic distribution and activation kinetics were consistent with the available experimental data on CA1 neurons[16]. Thus, the voltage dependence of the activation gate variable was modeled as $n = 1/(1 + \exp(0.151(V - V_{1/2})))$, with $V_{1/2} = -82$ mV in the soma and proximal dendrites (<100 m), and $V_{1/2} = -90$ mV for locations >100 m from the soma; its time constant was approximated as $\alpha_n = \exp(0.033(V - V_t))/(0.011(1 + \exp(0.083(V - V_t))))$, with $V_t = -75$ mV. A peak conductance density of $g_h = 3$ pS/ m^2 was used at the soma, and linearly increased with distance, d (m), as $g_h * (1 + 1.5d/100)$. LTG application was modeled with a 10-mV depolarizing shift of the activation curve. The resting potential change resulting from this shift in I_h activation was modeled as a fixed 5-mV depolarization. Synaptic conductances were represented by a double exponential function with rise and decay time constants of 3 and 30 ms, respectively, a peak conductance of 16 nS, and a reversal potential of 0 mV. For the simulations of **Fig. 7a**, five independent synapses were randomly placed and randomly (poisson) activated on dendritic compartments 150–250 m from the soma. The average AP firing rate at each average frequency was calculated from 50 simulations, each 1 s long. The model and simulation files are publicly available on the ModelDB database of Senselab (http://senselab.med.yale.edu).

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Competing interests statement

Authors declare competing financial interests: see the Nature Neuroscience website (http://neuroscience.nature.com) version for details.

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1. Hausser, M., Spruston, N. & Stuart, G. J. Diversity and dynamics of dendritic signaling. *Science* **290**, 739–744 (2000).
2. Johnston, D., Magee, J. C., Colbert, C. M. & Christie, B. R. Active properties of neuronal dendrites. *Annu. Rev. Neurosci.* **19**, 165–186 (1996).
3. Magee, J., Hoffman, D., Colbert, C. & Johnston, D. Electrical and calcium signaling in dendrites of hippocampal pyramidal neurons. *Annu. Rev. Physiol.* **60**, 327–346 (1998).
4. Poolos, N. P. & Johnston, D. Calcium-activated potassium conductances contribute to action potential repolarization at the soma but not the dendrites of hippocampal CA1 pyramidal neurons. *J. Neurosci.* **19**, 5205–5212 (1999).
5. Johnston, D. *et al.* Dendritic potassium channels in hippocampal pyramidal neurons. *J. Physiol.* (*Lond.*) **525**, 75–81 (2000).
6. Messenheimer, J. in *The Treatment of Epilepsy: Principles and Practice* (ed. Wyllie, E.) 899–905 (Williams and Wilkins, Baltimore, 1996).
7. Calabrese, J. R. Lamotrigine and the treatment of bipolar disorder. Introduction. *Eur Neuropsychopharmacol.* **9** (Suppl. 4), S107–108 (1999).
8. White, H. S. in *The Epilepsies 2* (eds. Porter, R. J. & Chadwick, D.) 1–30 (Butterworth-Heinemann, Boston, 1997).
9. Kuo, C.-C. & Lu, L. Characterization of lamotrigine inhibition of Na^+ channels in rat hippocampal neurons. *Br. J. Pharmacol.* **121**, 1231–1238 (1997).
10. Xie, X., Lancaster, B., Peakman, T. & Garthwaite, J. Interaction of the antiepileptic drug lamotrigine with recombinant rat brain type IIA Na^+ channels and with native Na^+ channels in rat hippocampal neurons. *Pflugers Arch.* **430**, 437–446 (1995).
11. Colbert, C. M., Magee, J. C., Hoffman, D. A. & Johnston, D. Slow recovery from inactivation of Na^+ channels underlies the activity-dependent attenuation of dendritic action potentials in hippocampal CA1 pyramidal neurons. *J. Neurosci.* **17**, 6512–6521 (1997).
12. Golding, N. L. & Spruston, N. Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. *Neuron* **21**, 1189–1200 (1998).
13. Stuart, G., Spruston, N., Sakmann, B. & Hausser, M. Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends Neurosci.* **20**, 125–131 (1997).
14. Santoro, B. *et al.* Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. *J. Neurosci.* **20**, 5264–5275 (2000).
15. Pape, H.-C. Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annu. Rev. Physiol.* **58**, 299–327 (1996).
16. Magee, J. C. Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *J. Neurosci.* **18**, 7613–7624 (1998).
17. Stuart, G. & Spruston, N. Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. *J. Neurosci.* **18**, 3501–3510 (1998).
18. Magee, J. C. Dendritic I_h normalizes temporal summation in hippocampal CA1 neurons. *Nat. Neurosci.* **2**, 508–514 (1999).
19. Harris, N. C. & Constanti, A. Mechanism of block by ZD 7288 of the hyperpolarization-activated inward rectifying current in guinea pig substantia nigra neurons *in vitro*. *J. Neurophysiol.* **74**, 2366–2378 (1995).
20. Chen, S., Wang, J. & Siegelbaum, S. A. Properties of hyperpolarization-activated pacemaker current defined by coassembly of HCN1 and HCN2 subunits and basal modulation by cyclic nucleotide. *J. Gen. Physiol.* **117**, 491–504 (2001).
21. Fiala, J. C. & Harris, K. M. in *Dendrites* (eds. Stuart, G., Spruston, N. & Hausser, M.) (Oxford University Press, Oxford, 1999).
22. Stefani, A., Spadoni, F., Siniscalchi, A. & Bernardi, G. Lamotrigine inhibits Ca^{2+} currents in cortical neurons: functional implications. *Eur. J. Pharmacol.* **307**, 113–116 (1996).
23. Wang, S. J., Sihra, T. S. & Gean, P. W. Lamotrigine inhibition of glutamate release from isolated cerebrocortical nerve terminals (synaptosomes) by suppression of voltage-activated calcium channel activity. *Neuroreport* **12**, 2255–2258 (2001).
24. Coulter, D. A. Antiepileptic drug cellular mechanisms of action: where does lamotrigine fit in? *J. Child Neurol.* **12** (Suppl.1), 2–9 (1997).
25. Chen, K. *et al.* Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat. Med.* **7**, 331–337 (2001).
26. Lupica, C. R., Bell, J. A., Hoffman, A. F. & Watson, P. L. Contribution of the hyperpolarization-activated current (I_h) to membrane potential and GABA release in hippocampal interneurons. *J. Neurophysiol.* **86**, 261–268 (2001).
27. Beaumont, V. & Zucker, R. S. Enhancement of synaptic transmission by cyclic AMP modulation of presynaptic I_h channels. *Nat. Neurosci.* **3**, 133–141 (2000).
28. Shorvon, S. D. *Handbook of Epilepsy Treatment* (Blackwell, Oxford, 2000).
29. Hines, M. L. & Carnevale, N. T. The NEURON simulation environment. *Neural Comput.* **9**, 1179–1209 (1997).
30. Migliore, M., Hoffman, D. A., Magee, J. C. & Johnston, D. Role of an A-type K^+ conductance in the back-propagation of action potentials in the dendrites of hippocampal pyramidal neurons. *J. Comput. Neurosci.* **7**, 5–15 (1999).