Lidocaine Blocks the Hyperpolarization-activated Mixed Cation Current, I_h, in Rat Thalamocortical Neurons

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ABSTRACT

Background: The mechanisms that underlie the supraspinal central nervous system effects of systemic lidocaine are poorly understood and not solely explained by Na^+ channel blockade. Among other potential targets is the hyperpolarization-activated cation current, I_h, which is blocked by lidocaine in peripheral neurons. I_h is highly expressed in the thalamus, a brain area previously implicated in lidocaine's systemic effects. The authors tested the hypothesis that lidocaine blocks I_h in rat thalamocortical neurons.

Methods: The authors conducted whole cell voltage- and current-clamp recordings in ventrobasal thalamocortical neurons in rat brain slices *in vitro*. Drugs were bath-applied. Data were analyzed with Student *t* tests and ANOVA as appropriate; $\alpha = 0.05$.

Results: Lidocaine voltage-independently blocked I_h, with high efficacy and a half-maximal inhibitory concentration (IC_{50}) of 72 μM. Lidocaine did not affect I_h activation kinetics but delayed deactivation. The I_h inhibition was accompanied by an increase in input resistance and membrane hyperpolarization (maximum, 8 mV). Lidocaine increased the latency of rebound low-threshold Ca^{2+} spike bursts and reduced the number of action potentials in bursts. At depolarized potentials associated with the relay firing mode (>−60 mV), lidocaine at 600 μM concurrently inhibited a K^+ conductance, resulting in depolarization (7–10 mV) and an increase in excitability mediated by Na^+-independent, high-threshold spikes.

Conclusions: Lidocaine concentration-dependently inhibited I_h in thalamocortical neurons *in vitro*, with high efficacy and a potency similar to Na^+ channel blockade. This effect would reduce the neurons' ability to produce intrinsic burst firing and δ rhythms and thereby contribute to the alterations in oscillatory cerebral activity produced by systemic lidocaine *in vivo*.

What We Already Know about This Topic

- The mechanisms underlying the supraspinal central nervous system effects of lidocaine are poorly understood and not solely explained by Na^+ channel blockade, but may involve the hyperpolarization-activated cation current, I_h, which is blocked by lidocaine in peripheral neurons

What This Article Tells Us That Is New

- Lidocaine concentration-dependently inhibited I_h in rat thalamocortical neurons *in vitro*, with high efficacy and a potency similar to Na^+channel blockade
- A resultant reduction of intrinsic burst firing and δ rhythms may contribute to the alterations in oscillatory cerebral activity produced by systemic lidocaine *in vivo*

LIDOCAINE is a widely exploited local anesthetic exerting its main peripheral therapeutic effects by blocking voltage-gated Na^+ channels. It also is useful systemically in the management of acute postoperative and chronic neuropathic pain syndromes, in the maintenance of general anesthesia, and as a class IB antiarrhythmic.[1–5] In addition, systemic lidocaine exhibits concentration-dependent central nervous system (CNS) toxicity that begins with alterations in sensorium at low plasma concentrations that overlap with those associated with the therapeutic effects (in humans, typically less than 5 μg/ml or approximately 20 μM) and progresses to generalized seizures, coma, and death at higher levels (approximately >15–50 μg/ml or 60–200 μM).[6,7]

Though poorly understood, the mechanisms that underlie lidocaine's complex concentration-dependent supraspinal CNS effects are not solely explained by its classic action on Na^+ channels.[8–10] Among the list of other possible targets is the hyperpolarization-activated mixed Na^+/K^+ current, I_h,

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Received from the Department of Anesthesiology, Pharmacology & Therapeutics, The University of British Columbia, Vancouver, British Columbia, Canada. Submitted for publication December 3, 2010. Accepted for publication July 5, 2011. Supported in part by the Canada Foundation for Innovation (Ottawa, Ontario, Canada), the British Columbia Knowledge Development Fund (Victoria, British Columbia, Canada), and a Pfizer Neuropathic Pain Research Award (independently peer-reviewed public operating grant competition sponsored by Pfizer Canada Inc.; Kirkland, Quebec, Canada). Presented in abstract form at the Canadian Anesthesiologists' Society 2010 Annual Meeting; Montreal, Quebec, Canada; June 25–29, 2010, and the Society for Neuroscience 40th Annual Meeting ("Neuroscience 2010"); San Diego, California; November 13–17, 2010.

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 Anesthesiology 2011; 115:822–35

which is blocked by lidocaine in peripheral sensory neurons.[11] I_h, predominantly its underlying channel isoform, HCN2,[12] is highly expressed in the thalamus,[13–15] a brain area that plays an important role in the generation of the different physiologic conscious states and associated cerebral rhythms; in drug-induced sedation, anesthesia, and analgesia; and in epileptogenesis.[16–19] In mammals, lidocaine at subconvulsive doses has long been known to produce slow-wave electroencephalographic rhythmic activity and "spindling" associated with sedation and reduced responsiveness to noxious stimuli,[20–23] implicating the thalamus as a site of action. More recently, *in vitro*[24,25] and human *in vivo*[26] reports have focused on lidocaine's actions in the ventrobasal thalamus, the main supraspinal relay station for somatosensory and nociceptive signals.[27] However, lidocaine's effects on I_h in ventrobasal thalamocortical neurons are unknown.

I_h, whose activation produces a depolarizing noninactivating inward current,[12] is crucial for controlling excitability in thalamocortical neurons in multiple ways. First, it contributes to the setting of the resting membrane potential (RMP), as a significant fraction of I_h channels is active near rest.[12,28–30] Second, because of its leak and negative-feedback properties, I_h operates as a "voltage-clamp," passively shunting incoming impulses and actively opposing hyperpolarization and depolarization.[12,31,32] As a result, I_h is critical for determining the distinct voltage-dependent firing mode of these neurons. At depolarized potentials positive to approximately −60 mV, they exhibit a "relay" or "tonic" mode that is associated with vigilance and wakefulness *in vivo* and characterized by tonic repetitive firing of singleton Na^+-dependent action potentials.[16,33] At hyperpolarized potentials, neurons switch to the "oscillatory" or "burst" mode that occurs in states of slow-wave electroencephalographic activity (*e.g.*, nonrapid eye movement sleep) and features action potential bursts mediated by the low-threshold Ca^{2+} current, I_T.[12,16,33] Third, through interaction with I_T to generate rhythmic burst firing, I_h serves as a pacemaker current and is central to the generation of slow intrinsic neuronal[28] and network oscillations in the thalamocortical system during nonrapid eye movement sleep and drowsiness.[34–36]

Here, we tested the hypothesis that lidocaine blocks I_h in rat ventrobasal thalamocortical neurons *in vitro* and explored the functional consequences of I_h blockade by lidocaine in these neurons.

Materials and Methods

Preparation of Brain Slices

Ethics approval for all animal experiments was obtained from the Committee on Animal Care of The University of British Columbia (Vancouver, British Columbia, Canada). Wistar rats of postnatal age P13–P16 were deeply anesthetized with isoflurane (Abbott Laboratories, Montreal, Canada) and decapitated. The cerebrum was rapidly removed and placed in oxygenated (5% CO_2/95% O_2), cold (1–4°C), artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl, 124; KCl, 2.5; NaH_2PO_4, 1.25; $CaCl_2$, 2; $MgCl_2$, 2; $NaHCO_3$, 26, dextrose, 10 (pH, 7.3–7.4; 290 mOsm). After trimming the chilled brain, a block containing the ventrobasal thalamus was glued onto a tissue-slicer stage with cyanoacrylate adhesive. Coronal slices of the thalamus were cut at 250–300 μm on a Leica VT1200S vibratome (Leica Biosystems, Nussloch, Germany) while the block was submerged in oxygenated, 1–4°C ACSF. Immediately after cutting, the slices were incubated at room temperature (22–24°C) in oxygenated ACSF.

Electrophysiological Recordings

For recording, slices were submerged in a Perspex chamber with a volume of 1.5 ml, fixed between two pieces of polypropylene mesh, and maintained at room temperature. The slices were continuously perfused by gravity with oxygenated ACSF at a flow rate of 2.5 ml/min controlled by a FR-50 flow valve (Harvard Apparatus, St. Laurent, QC, Canada). Individual neurons were visualized with the aid of differential interference contrast infrared videomicroscopy (Zeiss Axioskop FS, Carl Zeiss, Göttingen, Germany). The images were recorded with a Hamamatsu C2400 video camera system (Hamamatsu Photonics K.K., Hamamatsu, Japan). Patch pipettes were pulled from borosilicate glass (World Presicion Instruments, Inc., Sarasota, FL) using a PP-83 two-stage electrode puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan) and filled with a solution containing (mM): K-gluconate, 139; EGTA, 10; KCl, 6; NaCl, 4; $MgCl_2$, 3; HEPES, 10; $CaCl_2$, 0.5; adenosine-5′-triphosphate (disodium salt), 3; guanosine-5′-triphosphate (sodium salt), 0.3, titrated to pH 7.3–7.4 with 10% gluconate. Typical electrode resistances were 5–6 MΩ and access resistance ranged from 10 to 20 MΩ. Whole cell patch-clamp recordings from ventrobasal thalamic neurons were performed in both current- and voltage-clamp modes with a HEKA EPC-7 amplifier (HEKA Elektronik Dr. Schulze GmbH, Lambrecht, Germany) *via* a Digidata 1322A 16 bit data acquisition system (Axon Instruments, Inc., Foster City, CA) using pCLAMP software (Axon Instruments, Inc.). The membrane currents were low-pass filtered (three-pole Bessel filter) at a frequency of 3 kHz and digitized at 10 kHz. Data were collected more than 10 min after whole cell access to allow the internal pipette solution to equilibrate with the neuron. Membrane potentials were corrected off-line for a liquid junction potential of −8 mV.[37] No leak subtraction was performed.

Data Analysis

Data were analyzed using ORIGIN 7 (OriginLab Corporation, Northampton, MA) and Prism 5 software (GraphPad, La Jolla, CA). To determine the IC_{50} and Hill coefficient, concentration-response curves were normalized and fitted using the Hill equation as follows:

$$I/I_{max} = [C]^h/([IC_{50}]^h + [C]^h) \quad (1)$$

where I represents the current measured in the presence of a given drug concentration; I_{max} is the control current measured in the absence of the drug; [C] is the drug concentration; IC_{50} is the half-maximal inhibitory concentration; and h is the Hill coefficient.

The conductance-voltage relationship for I_h steady-state activation was fitted by the Boltzmann equation:

$$G_h/G_{h(max)} = 1/(1 + \exp[(V - V_{0.5})/k]) \quad (2)$$

where G_h is the I_h conductance (calculated as $G_h = I/[V - V_r]$: I, amplitude of the I_h tail current following a hyperpolarizing step [V]; V_r, estimated I_h reversal potential); $G_{h(max)}$ is the maximum conductance obtained after the most hyperpolarizing step; $V_{0.5}$ is the half-maximal activation potential; and k is the slope factor.

We estimated the reversal potential of I_h from the intersection of extrapolated linear regression fits of instantaneous voltage-current relationships at two different holding membrane potentials.[38] The slopes of the plots were assumed to vary depending on the degree of activation of I_h and to intersect at the reversal potential of I_h where there is no driving force.

Data are presented as mean ± SEM unless mentioned otherwise; baseline membrane properties of all included neurons are given as mean ± SD as indicated. We used one-way ANOVA to test for concentration-dependent drug effects and comparisons of more than two groups. Comparisons between two groups were conducted with the use of a paired Student t test; a one-sample Student t test was used to test for differences of normalized data from baseline (*i.e.*, a hypothetic mean of 1.0). Statistical tests were two-tailed and results were considered significant at $\alpha = 0.05$.

Drugs and Chemicals

Lidocaine HCl, tetrodotoxin, and CsCl were purchased from Sigma–Aldrich Canada Ltd. (Mississauga, ON, Canada). ZD7288 was obtained from Ascent Scientific (Princeton, NJ). $BaCl_2$ was obtained from ICN Biomedicals (Aurora, OH). Lidocaine, tetrodotoxin, and ZD7288 were dissolved in fresh ACSF to prepare concentrated stock solutions stored at 4°C. Before application, required aliquots of the stock solutions were dissolved in ACSF to obtain the respective concentrations. All drugs were applied to the bath by switching from the control perfusate to ACSF containing a desired drug concentration. Recordings were conducted after 6 min of perfusion (approximately 2 ml/min) of the slices with a test solution except ZD7288 (20 min). All results reported reflect steady state responses.

Results

We investigated n = 62 thalamocortical neurons of the ventrobasal complex (ventral posterior lateral/medial nuclei). The neurons had an average (± SD) RMP of −67.2 ± 3.1 mV, consistent with the results of previous studies.[24,39,40] When voltage-clamped at −68 mV, the neurons had an average (± SD) input resistance (R_i) of 271 ± 84 MΩ, determined from the responses to a 5 mV hyperpolarizing voltage step. Their average (± SD) membrane capacitance ($C_i = \tau_m/R_i$) was 197 ± 50 pF. All neurons voltage-dependently exhibited both the relay and oscillatory modes of operation characteristic for thalamocortical relay neurons (see Introduction, third paragraph).[33] Accordingly, they responded with tonic repetitive firing to depolarizing current pulses from membrane potentials positive to approximately −60 mV, and, when depolarized from hyperpolarized membrane potentials less than approximately −70 mV, responded with burst firing, generated by a low-threshold spike (LTS; known to be mediated by I_T; see Introduction, third paragraph) crowned by a burst of action potentials.

Lidocaine Concentration-dependently Blocked I_h in Thalamocortical Neurons

Hyperpolarization of neurons voltage-clamped at −68 mV induced an inwardly rectifying, noninactivating current consisting of an instantaneous component and a slow-activating component (fig. 1A), known to be generated by the inwardly rectifying K^+ current, I_{Kir}, and the hyperpolarization-activated mixed cation current, I_h, respectively.[41,42] Extracellular application of 600 μM lidocaine inhibited I_h (calculated as the difference between the instantaneous current [I_{inst}] and the steady-state current [I_{ss}] at the beginning and end of the voltage step, respectively) without affecting I_{Kir} (n = 4; fig. 1, A and B). Lidocaine's effects were mirrored by the specific I_h antagonist, ZD7288 (50 μM),[43,44] which similarly blocked only the I_h component as predicted (n = 4; fig. 1, C and D), whereas Cs^+ (CsCl, 2 mM), a nonspecific I_h blocker,[28] inhibited both I_h and I_{Kir} (n = 4; fig. 1, E and F). Conversely, application of the I_{Kir} blocker, $BaCl_2$ (0.1 mM)[28,45] almost completely abolished this current and effectively unmasked I_h (fig. 1, G and H).[46] In neurons recorded in the presence of extracellular Ba^{2+} at −128 mV, the average magnitude of I_h was 233 ± 97 pA (n = 16). The estimated I_h reversal potential (V_r) was −43.4 ± 2.4 mV (n = 5; fig. 2). Lidocaine reversibly blocked I_h in a concentration-dependent manner, with an IC_{50} of 72 ± 7 μM (n = 4; ANOVA, $P < 0.001$) and an estimated Hill coefficient of 1.19 ± 0.12 (fig. 3, A and B). The I_h block was not voltage-dependent at 100 μM (n = 4; ANOVA, $P = 0.57$; fig. 3C).

Effects of Lidocaine on Biophysical Properties of I_h

Because intrinsic and network thalamocortical oscillations are critically dependent on the activation and deactivation properties of I_h,[47] we further investigated whether lidocaine would alter these. To examine the conductance-voltage relationship for I_h steady-state activation, we measured I_h tail currents on repolarization to −78 mV (shown in fig. 3A; see Materials and Methods, Data Analysis sections). As illustrated in figure 4A, the activation curve rose between −58 and −128 mV, with a half-maximal activation potential ($V_{0.5}$) of −94.6 ± 1.9 mV and a slope factor of 11.4 ± 1.0 (n = 4), yielding an estimated maximum G_h in the range of

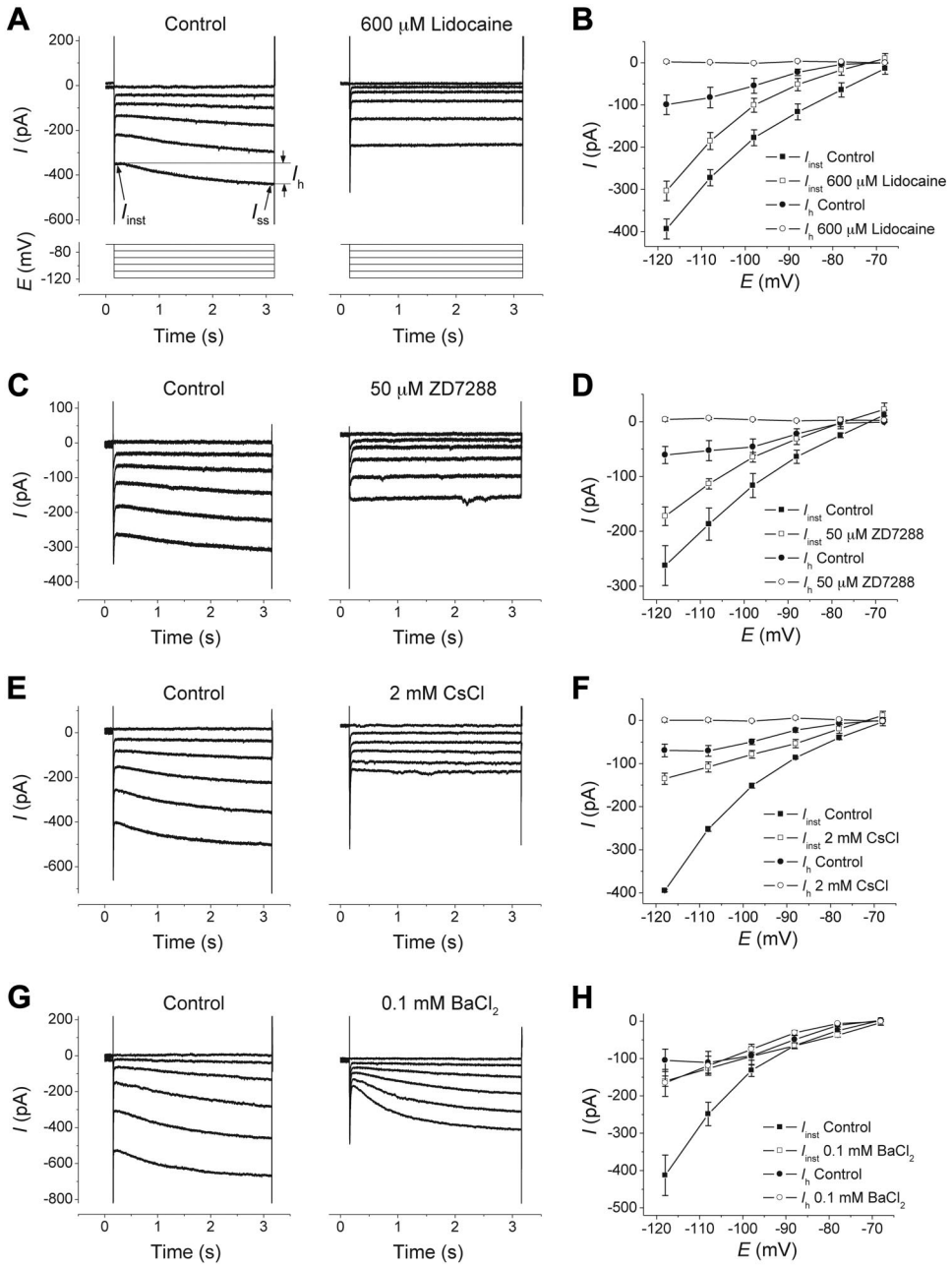

Fig. 1. Lidocaine blocked the hyperpolarization-activated cation current, I_h, in ventrobasal thalamocortical relay neurons without affecting the inwardly rectifying K^+ current, I_{Kir}. (*A*, *C*, *E,* and *G*) Representative current responses (*I*) of neurons voltage-clamped at −68 mV to 3-s hyperpolarizing voltage pulses injected in 10-mV increments (in *A*, ordinate labeled *E*) under control conditions and following application of either 600 μM lidocaine, 50 μM ZD7288, 2 mM CsCl, or 0.1 mM $BaCl_2$. Note the nonlinearly increasing amplitudes of current responses to successive hyperpolarizing voltage injections in neurons under control conditions, indicative of activation of an inwardly rectifying current. This current consisted of an instantaneous component, generated by I_{Kir}, and a slow-activating component, generated by I_h. The magnitude of I_h was calculated as the difference between the instantaneous current at the beginning of each pulse (I_{inst}) and the steady-state current (I_{ss}) at the end of the pulse (*A*, *arrows*). (*A*) Lidocaine robustly blocked I_h without affecting I_{Kir}. (*C*) The effects of lidocaine were mirrored by the specific I_h antagonist, ZD7288. (*E*) In contrast, CsCl nonspecifically blocked both I_h and I_{Kir}. (*G*) $BaCl_2$ almost completely blocked I_{Kir}, thereby unmasking I_h at hyperpolarized potentials. Neurons exhibited recovery after washout (not shown) except after application of ZD7288. (*B*, *D*, *F,* and *H*) show the neurons' I_{inst} and I_h currents plotted against the membrane potential at baseline (Control) and in the presence of either 600 μM lidocaine (*B*), 50 μM ZD7288 (*D*), 2 mM CsCl (*F*), or 0.1 mM $BaCl_2$ (*H*).

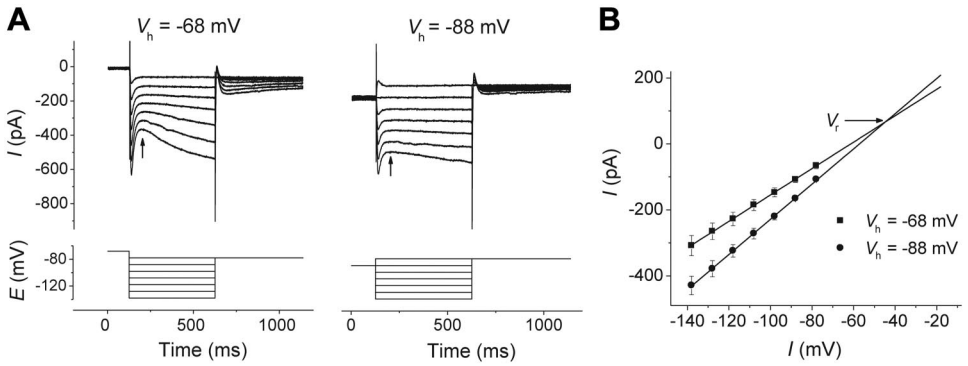

Fig. 2. Reversal potential of I_h in thalamocortical relay neurons. (*A*) Current responses (*I*) to 500-ms voltage pulses injected in 10-mV increments (*E*) of a neuron held at potentials (V_h) of −68 mV and −88 mV, respectively. Recordings were conducted in the presence of extracellular Ba^{2+} (0.1 mM $BaCl_2$) to block the inwardly rectifying K^+ current, I_{Kir}. The voltage-current relationships of the instantaneous current at the beginning of each pulse (I_{inst}, Fig. 1A; *arrows*) were determined at both potentials. (*B*) The I_{inst} voltage-current data (n = 5 neurons) were fit with linear regression and the I_h reversal potential (V_r) was estimated from the intersection of the two regression lines.

3–12 nS. Lidocaine (100 μM) did not significantly shift the $V_{0.5}$ (−90.5 ± 3.1 mV; n = 4; P = 0.11), but decreased the slope factor to 7.6 ± 1.1 (P = 0.02; fig. 4A). These minor effects possibly reflected an improved quality of the voltage-clamp due to an increase in neuronal R_i.

We examined the rate of I_h activation by stepping neurons to potentials from −98 to −128 mV (fig. 3A). The resulting kinetics were examined by fitting the activation phase of the current with a double-exponential function. The fast time constant decreased from 1,553 ± 510 ms at −98 mV to 274 ± 33 ms at −128 mV (n = 4; ANOVA, P = 0.02). Lidocaine (100 μM) had no significant effect on the fast time constant of I_h activation in the voltage range from −98 to −128 mV (n = 4; each voltage tested, $P > 0.05$; fig. 4B).

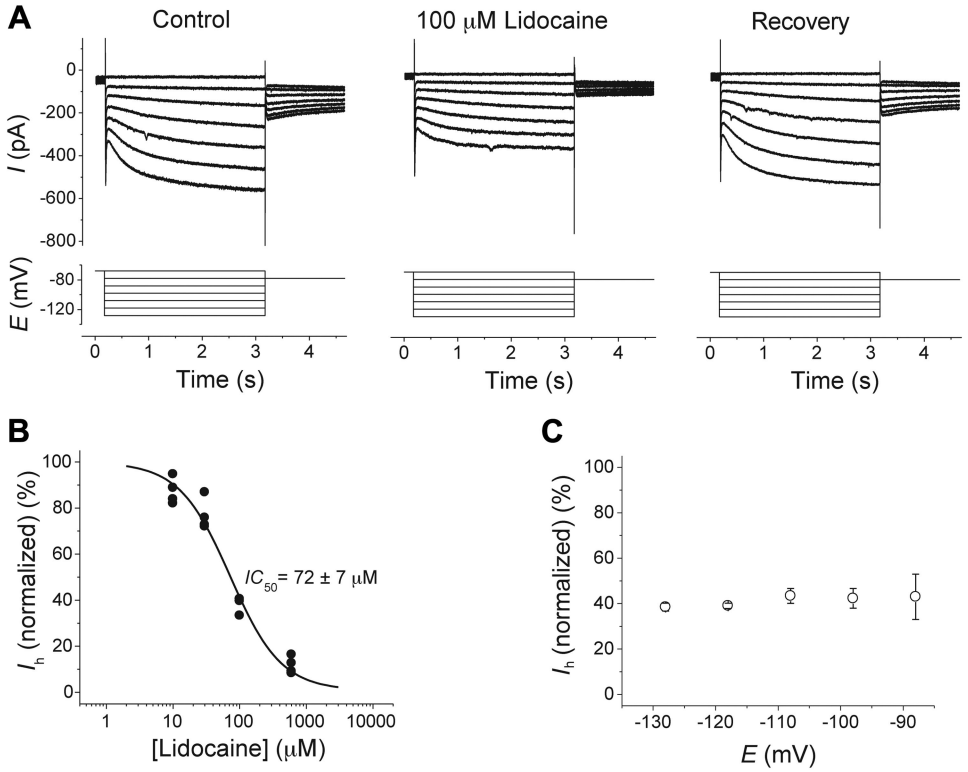

Fig. 3. Characteristics of lidocaine block of I_h in thalamocortical relay neurons. (*A*) Representative current responses (*I*) of a neuron voltage-clamped at −68 mV to 3 s voltage pulses injected in 10-mV increments (*E*) under control conditions, after application of 100 μM lidocaine, and after 20 min washout (Recovery). Concentration (*B*) and voltage (*C*) dependences of lidocaine inhibition of I_h. Amplitudes of I_h in *B* (each concentration, n = 4; n = 16 neurons total; ANOVA, $P < 0.001$) and *C* in the presence of lidocaine (100 μM in *C*; n = 4; ANOVA, $P > 0.05$) were normalized to those in control at the same test voltage (−128 mV in *C*). All recordings were performed in the presence of 0.1 mM $BaCl_2$.

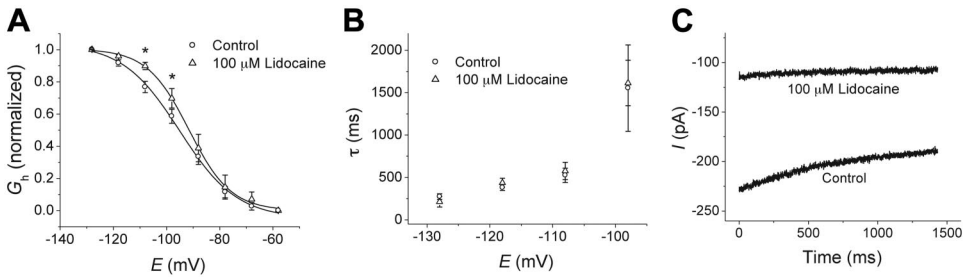

Fig. 4. Effects of lidocaine on biophysical properties of thalamocortical relay neurons. (*A*) Effects of 100 μM lidocaine on the voltage dependence of activation of the I_h conductance (G_h; ordinate), calculated from the amplitudes of I_h peak tail currents evoked by a repolarization to −78 mV. Tail current amplitudes were normalized to the maximum current levels obtained after the most negative prepulse (−128 mV) and plotted as a function of step potential (*E*). Lidocaine decreased the slope factor but had no significant effect on the half-maximal activation potential (details, see Results, third paragraph); * = $P < 0.05$ (Student *t* test). Effects of lidocaine on the kinetics of I_h activation (*B*) and deactivation (*C*). (*B*) Fast-time constants (τ) of activation plotted as a function of test voltages (*E*) in control and in the presence of 100 μM lidocaine. (*C*) Representative I_h tail current (*I*) relaxations upon repolarization to −78 mV after a hyperpolarization to −128 mV in control and in the presence of 100 μM lidocaine. All recordings were performed in the presence of 0.1 mM $BaCl_2$.

Because the I_h was still increasing slightly even at the end of a 3-s activation pulse, the slow time constant, especially at less negative potentials (fig. 3A), could not be estimated accurately; longer activation pulse durations compromised the whole cell patch.

We determined the rate of I_h deactivation by examining its tail current relaxation kinetics upon repolarization to −78 mV after a 3-s hyperpolarizing pulse to −128 mV. Repolarization to more depolarized potentials resulted in contamination of I_h tail currents with T type, low-threshold Ca^{2+} conductances (see Results section, first paragraph). Fitting the kinetics of I_h depolarization with a single-exponential function produced a deactivation time constant of 991 ± 17 ms (n = 4). Lidocaine (100 μM) substantially delayed I_h deactivation (fig. 4C), such that the deactivation time constant could not be estimated correctly in three of four neurons.

Implications of Lidocaine's Actions on I_h for Membrane Electrical Properties of Thalamocortical Neurons

To investigate the implications of lidocaine's actions on I_h for thalamocortical neurons' membrane electrical properties, we performed a series of current-clamp experiments in neurons pretreated with the tonic Na^+ channel blocker, tetrodotoxin. At 600 nM, tetrodotoxin did not significantly alter passive membrane properties of neurons: the baseline R_i, RMP, and C_i were 303 ± 21 MΩ, −65.9 ± 0.8 mV, and 206 ± 15 pF, respectively, compared with 277 ± 26 MΩ ($P = 0.22$), −66.4 ± 1.1 mV ($P = 0.45$), and 201 ± 26 pF ($P = 0.77$; for all variables, n = 12) in the presence of tetrodotoxin. Application of tetrodotoxin also did not greatly change current-voltage relationships at potentials negative to −60 mV, but reduced the apparent R_i at depolarized potentials (not shown).

Application of lidocaine at concentrations blocking I_h produced a reversible increase in the R_i of neurons and a hyperpolarization of their RMP (table 1). These effects were concentration-dependent with a peak at 600 μM, but diminished in magnitude at 1 mM (fig. 5, A and B). Of note, application of 1 mM lidocaine initially (within the first 2 min) resulted in hyperpolarization of the RMP followed by its eventual depolarization to a steady-state value. The C_i was not significantly different from the baseline values over the range of 0.1–1 mM (data not shown), indicating a primary effect of lidocaine on membrane conductance ($1/R_i$).[24]

Table 1. Effects of Lidocaine Compared with Those of the I_h Blockers, CsCl and ZD7288, on Membrane Electrical Properties of Ventrobasal Thalamocortical Relay Neurons

	Control		Treatment			
	R_i (MΩ)	RMP (mV)	R_i (MΩ)	*P* Value	RMP (mV)	*P* Value
Lidocaine	262 ± 50	−68.0 ± 1.7	556 ± 93 (212%)	0.02	−74.5 ± 1.9	0.005
Lidocaine + TTX*	207 ± 51	−71.0 ± 2.6	377 ± 91 (182%)	0.03	−78.8 ± 1.4	0.001
Lidocaine + $BaCl_2$†	260 ± 71	−62.3 ± 2.2	380 ± 110 (146%)	<0.001	−67.8 ± 2.1	<0.001
CsCl	183 ± 14	−66.3 ± 3.0	312 ± 43 (170%)	0.02	−77.8 ± 1.7	0.003
ZD7288	234 ± 47	−69.7 ± 1.1	417 ± 94 (178%)	0.03	−78.3 ± 1.9	0.03

Summarized are the input resistance (R_i) and resting membrane potential (RMP) of ventrobasal thalamocortical relay neurons under baseline control conditions (with *600 nM tetrodotoxin [TTX] or †0.1 mM $BaCl_2$ present in the superfusing extracellular solution) and following application of lidocaine (600 μM), CsCl (2 mM), or ZD7288 (50 μM). The average magnitude of increase in R_i is given in parentheses. Each row, n = 4 except for lidocaine + $BaCl_2$ (n = 16).

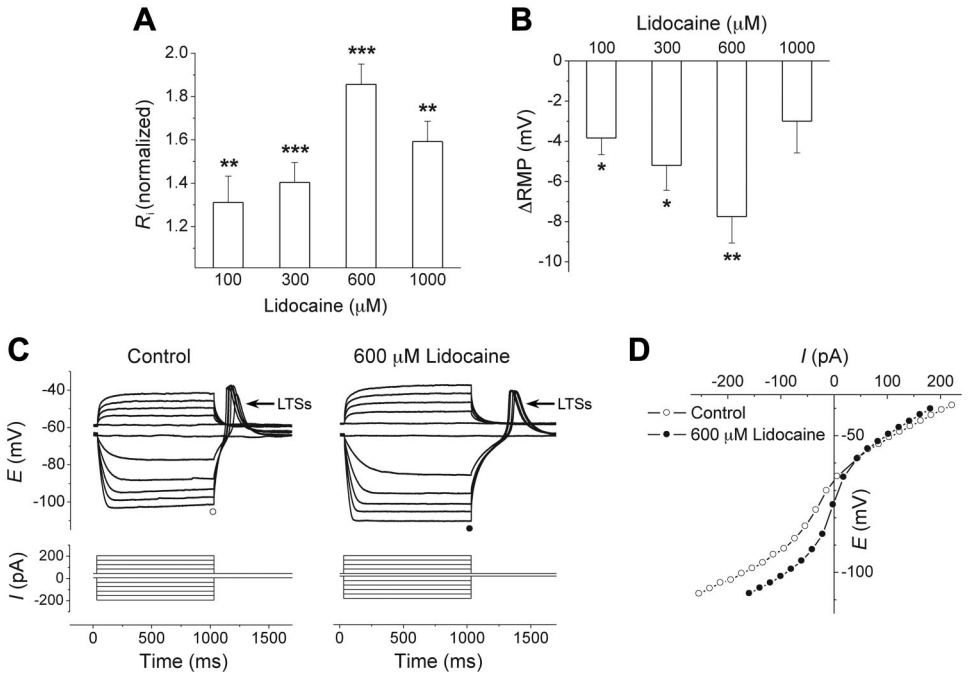

Fig. 5. Lidocaine altered passive and active properties of thalamocortical relay neurons pretreated with tetrodotoxin. (*A*) Lidocaine concentration-dependently increased input resistance (R_i; normalized to control) and (*B*) hyperpolarized the resting membrane potential of neurons pretreated with 600 nM tetrodotoxin; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ (one-sample Student *t* test; each experiment, n = 4–5). (*C*) Representative voltage responses of a neuron pretreated with 600 nM tetrodotoxin and current-clamped at −58 and −64 mV (*E*) to 1-s depolarizing and hyperpolarizing current injections (*I*), respectively, under control conditions and after application of 600 μM lidocaine. (*D*) Current-voltage relationship of the same neuron, constructed by plotting voltage responses measured at the end of the 1-s current injections (*open circle* and *filled circle* in *C*) against the magnitude of the injected current in control and in the presence of 600 μM lidocaine. Note the inward rectification under control conditions in the hyperpolarized voltage range, at potentials negative to approximately −85 mV. Lidocaine increased the slope of the current-voltage curve in the voltage range from approximately −60 to −90 mV, indicative of inhibition of a conductance whose reversal potential is represented by the point of intersection of the curves (here, approximately −57 mV; details, see Results). RMP = resting membrane potential.

To define the effects of I_h blockade by lidocaine on the active membrane properties of neurons, we conducted current-clamp experiments at potentials negative to −45 mV, corresponding to the activation range of I_h. Neurons current-clamped at −62 to −64 mV exhibited in their voltage responses to hyperpolarizing current pulses a typical inward ("anomalous")[41] rectification consisting of instantaneous and time-dependent components (fig. 5C). Bath application of lidocaine (600 μM) inhibited only the time-dependent, I_h-mediated inward rectification and produced an increase in the voltage responses to injected current pulses most pronounced at hyperpolarized potentials (n = 4). Current-voltage relationship analyses of the lidocaine-induced changes (fig. 5D) revealed inhibition of a conductance with an average reversal potential of −58.1 ± 0.9 mV (n = 4) and implicated other conductance(s) in addition to I_h and voltage-gated Na^+ currents. In addition to the previously mentioned effects, lidocaine also increased the latency of rebound LTSs (arrows, fig. 5C; calculated as the time required by the membrane potential to reach the LTS peak following the termination of the hyperpolarizing current pulse; see Results, first paragraph) from 128 ± 5 ms to 279 ± 47 ms (n = 4, $P = 0.04$). This increase occurred despite greater hyperpolarization responses, which would result in a larger population of deinactivated T-type Ca^{2+} channels.

Effects of Extracellular Cs^+ and ZD7288

We compared the effects of lidocaine on the passive and active membrane properties of thalamocortical neurons with those of Cs^+ and ZD7288. Extracellular application of both CsCl (2 mM) and ZD7288 (50 μM) led to an increase in the R_i of neurons, comparable with that produced by 600 μM lidocaine, as well as a significant hyperpolarizing shift in their RMP (table 1). In the current-clamp mode, extracellular Cs^+ reversibly inhibited both components of the inward rectification in the voltage responses of neurons current-clamped at −62 to −66 mV (n = 4; fig. 6) whereas ZD7288 irreversibly abolished only the time-dependent, I_h-mediated component (n = 4; fig. 7). Both Cs^+ and ZD7288 increased the voltage response magnitudes at potentials negative to approximately −50 mV. More depolarized holding potentials, from −60 to −63 mV and from −55 to −56 mV, were required to trigger rebound LTSs in three of four and in two of four neurons in the

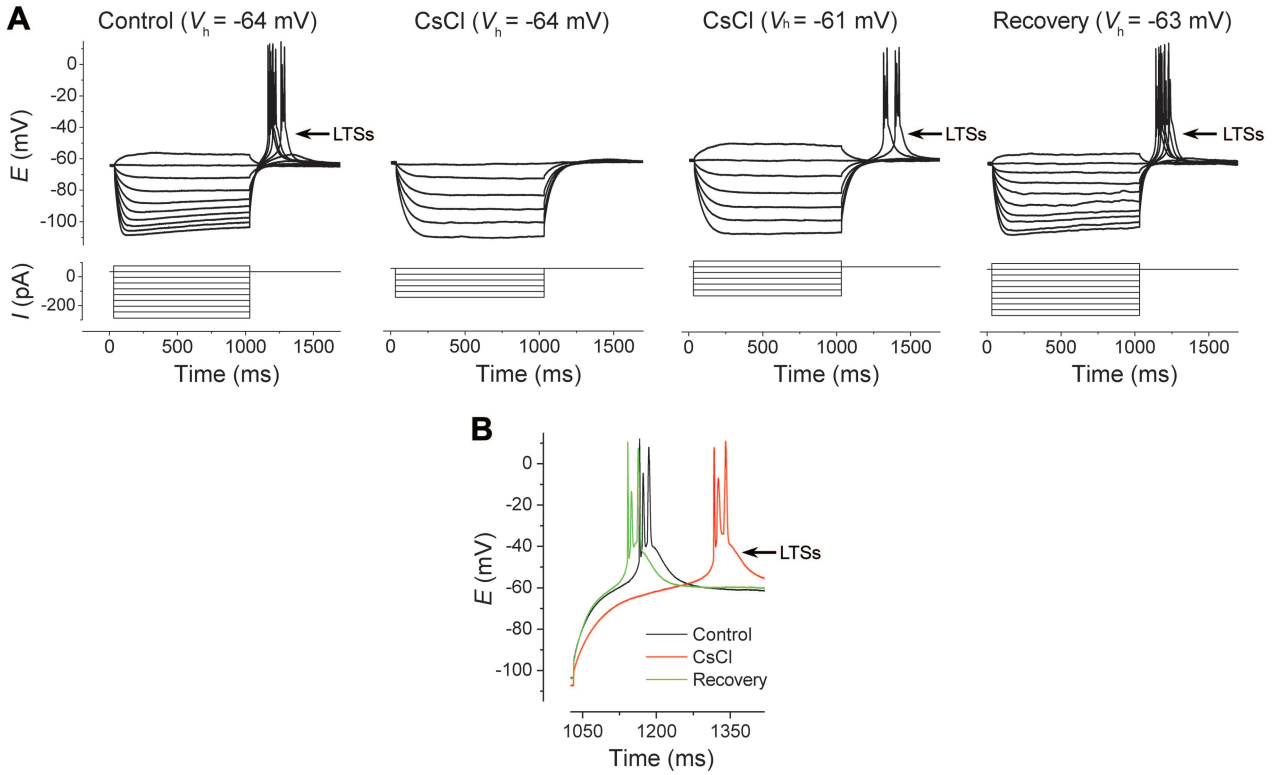

Fig. 6. Extracellular Cs^+ altered active properties of thalamocortical relay neurons. (*A*) Representative voltage responses (*E*) a neuron held at potentials (V_h) of −61 and −64 mV to 1-s hyperpolarizing current injections (*I*) under control conditions, after application of 2 mM CsCl and after washout. Cs^+ reversibly increased input resistance (reflected by increased voltage response magnitudes in the hyperpolarized range) similar to lidocaine and inhibited instantaneous and time-dependent inward rectification in the voltage responses of neurons. (*B*) Expanded portions of the responses containing rebound low-threshold Ca^{2+} spikes (LTSs; *arrow*) crowned by action potential bursts. Cs^+ reversibly increased LTS latencies, delaying the activation of rebound burst firing without affecting the number of action potentials in the LTS-evoked bursts.

presence of Cs^+ and ZD7288, respectively. However, even in those neurons whose holding potentials were depolarized, Cs^+ and ZD7288 application both increased the LTS latencies (figs. 6 and 7). In addition to delaying the activation of rebound LTSs, ZD7288 also decreased the number of action potentials in the LTS-evoked bursts from 3.8 ± 0.6 to 2.0 ± 0.4 (n = 4, $P = 0.006$). In contrast, Cs^+ had no effect on LTS burst firing.

Effects of Lidocaine on Firing Properties of Thalamocortical Neurons

We also examined the effects of lidocaine at concentrations blocking I_h on firing properties of neurons not pretreated with tetrodotoxin. For this purpose, we used a current-clamp protocol to generate both tonic and rebound burst firing,[39] applied in 2-min intervals to neurons constantly injected with a depolarizing current required to shift their membrane potential from RMP to −58 mV (associated with the tonic mode of firing that occurs in states of vigilance and wakefulness *in vivo*; see Introduction, third paragraph). As expected from its action on voltage-gated Na^+ channels, lidocaine, at 100 μM, abolished tonic firing of Na^+-dependent action potentials. However, the number of action potentials in the rebound LTS-evoked bursts decreased only slightly at this concentration, from 5.3 ± 0.3 to 4.3 ± 0.3 (n = 3; $P < 0.001$) (fig. 8A1). The burst discharges disappeared only at 600 μM, with the exception of the first spike (n = 4; fig. 8A2), which, consistent with our previous findings,[24] was resistant to lidocaine in all neurons tested (but blocked by 600 nM tetrodotoxin; fig. 5C), raising the possibility that lidocaine's potency for voltage-gated Na^+ channel blockade in thalamocortical neurons might be lower than for its blockade of I_h. At 600 μM, lidocaine produced a significant (7–10 mV) depolarization of the holding membrane potential and triggered repetitive firing of high-threshold spikes in response to the depolarizing current pulses. Application of 1 mM lidocaine led neither to a depolarizing shift in the holding potential nor to firing of high-threshold spikes (n = 5; fig. 8A3), although the latter could be evoked by increasing the amplitude of the depolarizing pulse (not shown). Similar to the findings in neurons pretreated with tetrodotoxin (fig. 5C), lidocaine at all three concentrations (100 μM, 600 μM, and 1 mM) concentration-dependently and reversibly increased LTS latencies (fig. 8A1–3). Consistent with previous findings of others,[48] we observed little effect of lidocaine on LTS magnitude.

Also similar to our results obtained in tetrodotoxin-pretreated neurons, application of lidocaine produced a revers-

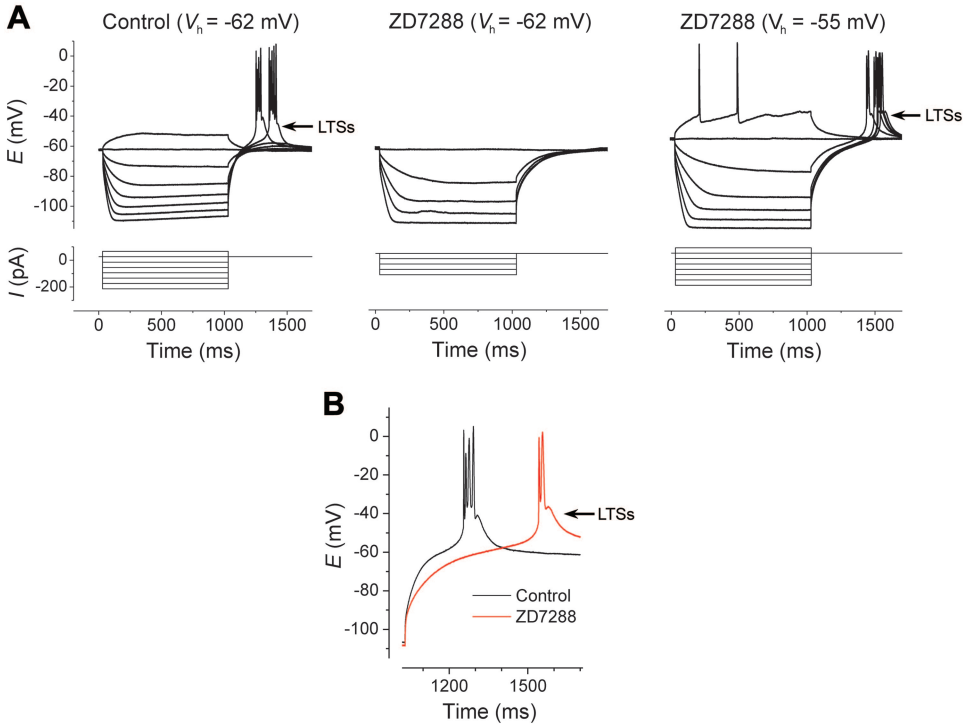

Fig. 7. ZD7288 altered active properties of thalamocortical relay neurons. (*A*) Representative voltage responses (*E*) of a neuron held at potentials (V_h) of −55 and −62 mV to 1-s hyperpolarizing current injections (*I*) under control conditions and after application of the I_h antagonist, ZD7288 (50 μM). ZD7288 reversibly increased input resistance (reflected by increased voltage response magnitudes in the hyperpolarized range) similar to lidocaine. In contrast with Cs^+ (fig. 6), ZD7288 inhibited only the time-dependent, I_h-mediated component of inward rectification in the voltage responses of neurons. (*B*) Expanded portions of the responses containing rebound low-threshold Ca^{2+} spikes (LTSs; *arrow*) crowned by action potential bursts. Similar to Cs^+, ZD7288 reversibly increased LTS latencies, delaying the activation of rebound burst firing. In addition, ZD7288 (but not Cs^+; fig. 6) decreased the number of action potentials in the LTS-evoked bursts.

ible increase in slope resistance in the range from approximately −50 to −85 mV, a hyperpolarization of the RMP (table 1), and suppression of the time-dependent inward rectification. The lidocaine-induced changes (600 μM) in the current-voltage relationships reflected inhibition of a conductance with a reversal potential of −66.5 ± 2.4 mV (n = 3; fig. 8B shows the current-voltage curves of a representative neuron). Application of Ba^{2+} (0.1 mM; fig. 8C) shifted this value above −55 mV (n = 4), toward the reversal potential of I_h. We found that the lidocaine-induced increase in R_i was smaller in the presence of Ba^{2+} than that observed at baseline or in the presence of tetrodotoxin (table 1). Collectively, these data implicate a K^+ conductance (other than I_{Kir}) besides I_h in the actions of lidocaine at 600 μM.

Discussion

Here, we have demonstrated that lidocaine reversibly and voltage-independently inhibited the hyperpolarization-activated mixed cation current, I_h, in rat ventrobasal thalamocortical relay neurons. Lidocaine blocked I_h with high efficacy (producing near-complete blockade; fig. 3B) and a potency (IC_{50}, 72 μM) similar or higher in comparison with that associated with its best-known effect, voltage-gated Na^+ channel blockade.[10,49] Our findings in the thalamus are overall comparable with previous observations in the periphery, *i.e.*, rat dorsal root ganglion neurons (IC_{50}, 99 μM)[11] and also cardiac (sinoatrial) myocytes (IC_{50}, 38 μM).[50]

The biophysical and pharmacologic properties of I_h in our experiments were similar to those previously reported in thalamocortical neurons[42,51] and correspond well to those characteristic for the underlying HCN2 channel isoform dominant in these neurons.[12] Lidocaine blocked the I_h-mediated time-dependent inward rectification without affecting the instantaneous inward rectification due to the K^+ current, I_{Kir}. In addition, lidocaine substantially delayed I_h deactivation while exhibiting no effects on the rate and voltage dependence of I_h activation. These observations suggest that lidocaine's action is unlikely to reflect a primary effect on channel gating.

Functional Consequences of I_h Inhibition for Thalamocortical Neurons

Consistent with the identified role of I_h in determining membrane electrical properties,[12,28,29,52] its blockade by lidocaine was accompanied by a concentration-dependent hyperpolarization and led to large increases in the voltage responses to hyperpolarizing current pulses. The magnitude of lidocaine's effects declined at 1 mM, suggesting that other

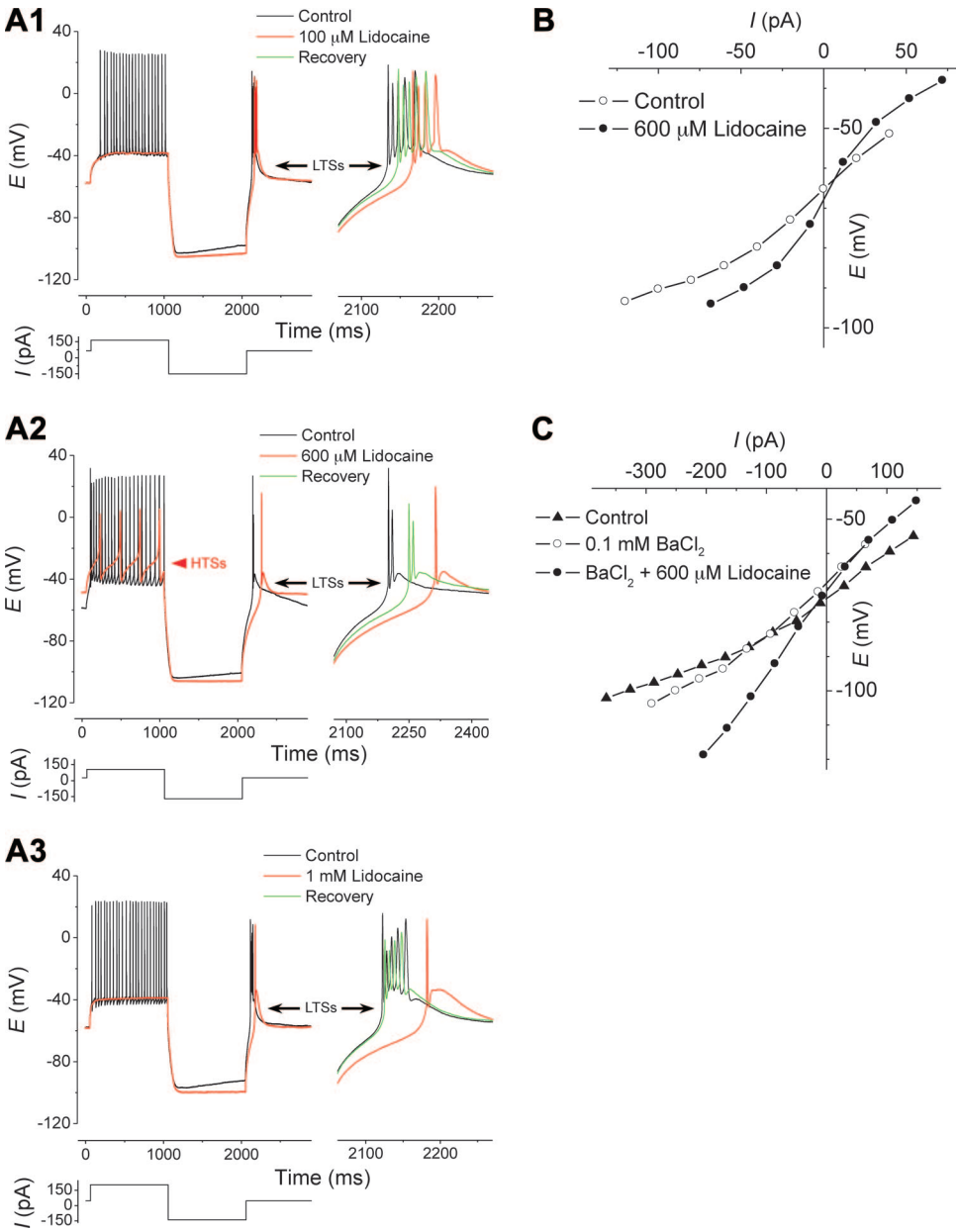

Fig. 8. Lidocaine altered firing properties of thalamocortical relay neurons. (*A*) Voltage responses (*E*) of neurons current-clamped at −58 mV to injection of a 1-s depolarizing current pulse followed by a 1-s hyperpolarizing pulse (*I*) under control conditions (*black traces*) and in the presence of 100 μM (*A1*), 600 μM (*A2*), and 1 mM lidocaine (*A3*) (*red traces*). The right panels in *A1–3* depict portions of the voltage responses at an expanded scale, together with the corresponding responses showing recovery after washout (*green traces*), containing rebound low-threshold Ca^{2+} spikes (LTSs; *arrows*) crowned by action potential bursts. Lidocaine at 100 μM abolished tonic firing of Na^{+}-dependent action potentials and increased LTS latencies while only slightly decreasing the number of action potentials in the rebound LTS-evoked bursts (*A1*). At 600 μM, lidocaine depolarized the neuron from the holding potential of −58 mV and triggered repetitively firing high-threshold spikes (HTSs; *red traces/arrow*) in response to the depolarizing current pulses, while further increasing LTS latencies and abolishing rebound burst discharges with the exception of the first spike in a burst (*A2*). 1 mM lidocaine had similar effects on the rebound bursts but produced neither a depolarizing shift in the holding potential nor firing of HTSs (*A3*). (*B*) Voltage responses (*E*) of a neuron at rest (−65 mV), measured at the end of the 1-s current injections and plotted against the magnitude of the injected current (*I*) at baseline (Control) and in the presence of 600 μM lidocaine. Lidocaine produced an increase in slope resistance in the range from approximately −50 to −85 mV and a hyperpolarization of the resting membrane potential. The lidocaine-induced current-voltage relationship changes reflected inhibition of a conductance with a reversal potential of approximately −63 mV. (*C*) Voltage responses of a neuron measured at the end of the 1-s current injections and plotted against the magnitude of the injected current at baseline (Control), in the presence of $BaCl_2$ (0.1 mM), and in the presence of 600 μM lidocaine plus $BaCl_2$ (0.1 mM). In the presence of Ba^{2+}, lidocaine almost completely inhibited inward rectification; Ba^{2+} depolarized the reversal potential of the conductance blocked by lidocaine toward the reversal potential of I_h (see Results, last paragraph).

conductances counteracting the hyperpolarization and increase in R_i were activated. This observation is in agreement with the lidocaine-induced depolarization at more than 3 mM previously reported in cultured dorsal root ganglion neurons.[10] The precise mechanisms are unknown and have been speculated to involve blockade of ion channels and pumps playing a role in the maintenance of RMP.

In the current study, the effects of lidocaine on membrane potential critically depended on holding voltage. At potentials associated with the relay mode of operation (> approximately −60 mV; see Introduction), lidocaine, at 600 μM, depolarized neurons. Current-voltage analyses showed that the depolarization occurred due to the hyperpolarized reversal potential of the lidocaine-blocked conductance relative to the holding membrane potential. At the same time, 100 μM lidocaine did not depolarize neurons and produced a smaller increase in R_i than expected based on the concentration dependence of the I_h inhibition. Combined with the effects of Ba^{2+} on reversal potential and R_i changes, these observations implicate the contribution of a K^+ conductance (other than the inward rectifier, I_{Kir}) blockade to lidocaine's actions that is substantially increasing at 600 μM. In good agreement is the report that lidocaine inhibits the hTREK1 current underlying leak K^+ conductance, with an IC_{50} of 180 μM.[53] The lidocaine-induced depolarization increased neuronal excitability mediated by (under the conditions of Na^+-dependent action potential blockade) a high-threshold Ca^{2+} conductance.[33,39,46] In this regard, our findings support the hypothesis of Mulle *et al.*,[54] explaining the occurrence of dendritic high-threshold spikes in thalamocortical neurons in the presence of intracellular QX-314 (100 μM), a permanently charged lidocaine analog, as resulting from an increase in R_i due to inhibition of persistent $Na^+$and/or K^+ conductances. With regard to lidocaine's concentration-dependent effects on passive membrane properties, it is of note that an older study with "blind" recordings in the ventral posterior lateral nucleus of Sprague-Dawley rats yielded some results at variance with the current findings on R_i, failing to find a statistically significant effect at 600 μM.[24] Whereas the precise reason is unclear, differences in recording technique and associated quality, species, animal age, neuronal homogeneity, and/or a type II error (an increase in R_i occurred in some neurons) may have contributed.

In the current investigations, we also found that by blocking I_h, lidocaine concentration-dependently altered firing properties of thalamocortical neurons in the burst mode, which *in vivo* is associated with nonrapid eye movement sleep and drowsiness.[16] Specifically, lidocaine increased the latency of rebound LTSs. Most likely, inhibition of I_h tail currents, known to evoke hyperpolarization-activated membrane potential overshoots,[28,55] accounts for these effects. Furthermore, at 100 μM, lidocaine reduced the number of Na^+ action potentials in LTS-evoked bursts. Our findings that the I_h blocker, ZD7288, produced the same effects are indicative of this action being due to inhibition of I_h rather than voltage-gated Na^+ channels. At the same time, the almost complete suppression of burst firing at 600 μM likely is mediated by both I_h inhibition and Na^+ channel blockade. Our results are consistent with previous observations that both pharmacologic (ZD7288) and "electronic" (dynamic clamp) I_h blockade increase LTS latency, partially suppress LTS-evoked bursts, and decrease the propensity of thalamocortical neurons to generate intrinsic δ oscillations.[35,56] We would therefore predict that lidocaine blockade of I_h, despite hyperpolarization, will reduce the ability of thalamocortical neurons to produce intrinsic burst firing and δ oscillations.[57] The fact that intracellular QX-314 (100 μM) inhibits intrinsic slow oscillatory activity in cat thalamocortical neurons *in vivo* supports this prediction.[54]

Clinical Relevance of I_h Inhibition by Lidocaine in Thalamocortical Neurons

In a discussion of the potential clinical relevance of the current findings it is important to note that results from *in vitro* animal investigations obviously cannot easily be translated to the *in vivo* domain without consideration of experimental limitations. For example, whereas our current work has a specific focus on intrinsic properties of single ventrobasal thalamocortical neurons, I_h also is expressed in other neurons involved in thalamocortical networks, such as those in the thalamic reticular nucleus and cortex.[13,42] Future studies using such approaches as multiunit and field potential recordings, imaging, and neural network modeling will help define the I_h-mediated effects of lidocaine on the entire thalamocorticothalamic system and aid in filling the gap between findings from single cells in brain slices and higher levels of organization (and ultimately, the human patient).

These considerations notwithstanding, numerous lines of evidence render it a plausible possibility that the mechanisms of systemic lidocaine's concentration-dependent CNS effects involve varying degrees of thalamic I_h inhibition, thereby affecting neuronal excitability and oscillatory behavior. For example, with regard to the higher (and presumably epileptogenic/CNS-toxic) concentrations producing close to maximal I_h blockade in the current study, an absence of I_h in thalamocortical neurons of HCN2-deficient (−/−) knockout mice produces abnormal synchronized (3–5 Hz) electroencephalographic oscillations and facilitates the occurrence of spike-and-wave discharges.[12] In rat models, a decreased responsiveness of I_h to cyclic adenosine monophosphate in the ventrobasal thalamus promotes epileptogenesis.[19,58] I_h blockade in other brain regions involved in the actions of systemic lidocaine[59,60] obviously may contribute to the complex array of this agent's CNS effects. Finally, given that the pathogenesis of lidocaine neurotoxicity involves an increase in intracellular Ca^{2+},[9,10,61] lidocaine-induced depolarization and Ca^{2+}-mediated increase in excitability at high concentrations may well play a part. Clearly, a body of future research is required to further elucidate these mechanisms.

In addition to its toxic effects on the CNS, systemic lido-

caine, at low, subconvulsive plasma concentrations (ranging from approximately 1–7 μg/ml or approximately 4–30 μM), is efficacious in alleviating acute postoperative as well as chronic neuropathic pain in humans[2,4,5,62,63] and animal models.[64] We recognize that extrapolation of ACSF concentrations from *in vitro* rodent studies to *in vivo* human plasma concentrations (where, among other factors, protein binding occurs and species differences play a role) requires caution. However, the therapeutic lidocaine concentration range in humans is near the lower end of that producing the I_h inhibition in this study (*e.g.*, approximately 23% suppression at 30 μM), which is noteworthy particularly because the current experiments were conducted at room temperature to facilitate stable recording conditions and slice viability. Given the pivotal role of the ventrobasal thalamus in pain and analgesia, our observations raise the possibility that moderate inhibition of thalamic I_h in the low micromolar range might represent a contributing mechanism for lidocaine's systemic analgesic actions. Again, future studies are required to test this hypothesis.

I_h: An Emerging Anesthetic Drug Target in the Thalamus?

In addition to shedding new light on the mechanisms of lidocaine's supraspinal CNS effects, the current findings also emphasize on the role of I_h as an emerging anesthetic drug target. For example, our findings with lidocaine, which has well-known general anesthetic properties (see Introduction),[1,3] share some noteworthy similarities with those recently obtained with propofol.[57] In thalamocortical neurons, propofol inhibited I_h-HCN2 at clinically relevant concentrations (*e.g.*, 36% at 5 μM; 23°C) and slowed I_h activation. Consistent with our predictions on the *in vivo* implications of lidocaine's I_h blockade, propofol's actions resulted in decreased regularity and frequency of δ oscillations in the neurons. Another example is ketamine, recently reported to block I_h-HCN1 in mouse cortical pyramidal neurons (approximately 29% at 20 μM; room temperature); in HCN1 knockout mice, ketamine showed a dramatically decreased hypnotic efficacy.[65] In addition, there is growing evidence that the anesthetic mechanisms of volatile agents involve I_h inhibition.[66,67] A comprehensive review on the role of I_h and HCN channels in anesthesia and other physiologic and pathologic conditions (including pain and epilepsy) has appeared recently.[68]

Summary and Conclusions

In this work, we have shown that lidocaine concentration-dependently inhibited I_h in ventrobasal thalamocortical neurons at micromolar concentrations *in vitro*, with high efficacy and a potency similar or higher compared with that associated with its blockade of voltage-gated Na^+ channels. By inhibiting I_h, lidocaine profoundly altered membrane properties of neurons and reduced their ability to generate the rebound burst firing associated with slow oscillatory cerebral activity. Our findings provide new insight into the multiple overlapping mechanisms that underlie the complex array of concentration-dependent therapeutic and toxic effects that intravenous lidocaine exerts on the CNS and emphasize on the significance of I_h as an emerging anesthetic drug target.

The authors express their gratitude to Christian Caritey (Engineering Technician) and Andy Jeffries (Operations Manager; both, Department of Anesthesiology, Pharmacology & Therapeutics, The University of British Columbia, Vancouver, British Columbia, Canada) for excellent technical assistance; James E. Cooke, Ph.D. (recent doctoral graduate, Department of Anesthesiology, Pharmacology & Therapeutics, The University of British Columbia), for valuable advice; Dietrich W. F. Schwarz, M.D., Ph.D. (Professor Emeritus, Department of Surgery, The University of British Columbia), for generously gifting equipment; and Ernest Puil, Ph.D. (Professor Emeritus, Department of Anesthesiology, Pharmacology & Therapeutics, The University of British Columbia), for his inspiration and constructive comments.

References

1. de Clive-Lowe SG, Desmond J, North J: Intravenous lignocaine anaesthesia. Anaesthesia 1958; 13:138–46
2. Bartlett EE, Hutaserani O: Xylocaine for the relief of postoperative pain. Anesth Analg 1961; 40:296–304
3. Himes RS Jr, DiFazio CA, Burney RG: Effects of lidocaine on the anesthetic requirements for nitrous oxide and halothane. ANESTHESIOLOGY 1977; 47:437–40
4. McCarthy GC, Megalla SA, Habib AS: Impact of intravenous lidocaine infusion on postoperative analgesia and recovery from surgery: A systematic review of randomized controlled trials. Drugs 2010; 70:1149–63
5. Vigneault L, Turgeon AF, Côté D, Lauzier F, Zarychanski R, Moore L, McIntyre LA, Nicole PC, Fergusson DA: Perioperative intravenous lidocaine infusion for postoperative pain control: A meta-analysis of randomized controlled trials. Can J Anesth 2011; 58:22–37
6. Covino BG: Toxicity and systemic effects of local anesthetic agents, Handbook of Experimental Pharmacology, Vol. 81: Local Anesthetics. Edited by Strichartz GR. Berlin, Springer-Verlag, 1987, pp 187–212
7. Wallace MS, Laitin S, Licht D, Yaksh TL: Concentration-effect relations for intravenous lidocaine infusions in human volunteers: Effects on acute sensory thresholds and capsaicin-evoked hyperpathia. ANESTHESIOLOGY 1997; 86:1262–72
8. Sakura S, Bollen AW, Ciriales R, Drasner K: Local anesthetic neurotoxicity does not result from blockade of voltage-gated sodium channels. Anesth Analg 1995; 81:338–46
9. Xu F, Garavito-Aguilar Z, Recio-Pinto E, Zhang J, J Blanck TJ: Local anesthetics modulate neuronal calcium signaling through multiple sites of action. ANESTHESIOLOGY 2003; 98: 1139–46
10. Gold MS, Reichling DB, Hampl KF, Drasner K, Levine JD: Lidocaine toxicity in primary afferent neurons from the rat. J Pharmacol Exp Ther 1998; 285:413–21
11. Bischoff U, Bräu ME, Vogel W, Hempelmann G, Olschewski A: Local anaesthetics block hyperpolarization-activated inward current in rat small dorsal root ganglion neurones. Br J Pharmacol 2003; 139:1273–80
12. Ludwig A, Budde T, Stieber J, Moosmang S, Wahl C, Holthoff K, Langebartels A, Wotjak C, Munsch T, Zong X, Feil S, Feil R, Lancel M, Chien KR, Konnerth A, Pape HC, Biel M, Hofmann F: Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. EMBO J 2003; 22: 216–24
13. Santoro B, Chen S, Luthi A, Pavlidis P, Shumyatsky GP, Tibbs GR, Siegelbaum SA: Molecular and functional heterogeneity

of hyperpolarization-activated pacemaker channels in the mouse CNS. J Neurosci 2000; 20:5264–75

14. Abbas SY, Ying SW, Goldstein PA: Compartmental distribution of hyperpolarization-activated cyclic-nucleotide-gated channel 2 and hyperpolarization-activated cyclic-nucleotide-gated channel 4 in thalamic reticular and thalamocortical relay neurons. Neuroscience 2006; 141:1811–25
15. Kanyshkova T, Pawlowski M, Meuth P, Dubé C, Bender RA, Brewster AL, Baumann A, Baram TZ, Pape HC, Budde T: Postnatal expression pattern of HCN channel isoforms in thalamic neurons: Relationship to maturation of thalamocortical oscillations. J Neurosci 2009; 29:8847–57
16. McCormick DA, Bal T: Sleep and arousal: Thalamocortical mechanisms. Annu Rev Neurosci 1997; 20:185–215
17. Alkire MT, Hudetz AG, Tononi G: Consciousness and anesthesia. Science 2008; 322:876–80
18. Franks NP: General anaesthesia: From molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci 2008; 9:370–86
19. Kuisle M, Wanaverbecq N, Brewster AL, Frère SG, Pinault D, Baram TZ, Lüthi A: Functional stabilization of weakened thalamic pacemaker channel regulation in rat absence epilepsy. J Physiol 2006; 575:83–100
20. Eriksson E, Persson A: The effect of intravenously administered prilocaine and lidocaine on the human electroencephalogram studied by automatic frequency analysis. Acta Chir Scand (Suppl) 1966; 358:37–46
21. Wagman IH, De Jong RH, Prince DA: Effects of lidocaine on spontaneous cortical and subcortical electrical activity. Production of seizure discharges. Arch Neurol 1968; 18:277–90
22. Seo N, Oshima E, Stevens J, Mori K: The tetraphasic action of lidocaine on CNS electrical activity and behavior in cats. ANESTHESIOLOGY 1982; 57:451–7
23. Shibata M, Shingu K, Murakawa M, Adachi T, Osawa M, Nakao S, Mori K: Tetraphasic actions of local anesthetics on central nervous system electrical activities in cats. Reg Anesth 1994; 19:255–63
24. Schwarz SKW, Puil E: Analgesic and sedative concentrations of lignocaine shunt tonic and burst firing in thalamocortical neurones. Br J Pharmacol 1998; 124:1633–42
25. Schwarz SKW, Puil E: Lidocaine produces a shunt in rat thalamocortical neurons, unaffected by $GABA_A$ receptor blockade. Neurosci Lett 1999; 269:25–8
26. Cahana A, Carota A, Montadon ML, Annoni JM: The long-term effect of repeated intravenous lidocaine on central pain and possible correlation in positron emission tomography measurements. Anesth Analg 2004; 98:1581–4
27. Albe-Fessard D, Berkley KJ, Kruger L, Ralston HJ III, Willis WD Jr: Diencephalic mechanisms of pain sensation. Brain Res Rev 1985; 9:217–96
28. McCormick DA, Pape HC: Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. J Physiol 1990; 431:291–318
29. Doan TN, Kunze DL: Contribution of the hyperpolarization-activated current to the resting membrane potential of rat nodose sensory neurons. J Physiol 1999; 514:125–38
30. Lupica CR, Bell JA, Hoffman AF, Watson PL: Contribution of the hyperpolarization-activated current I_h to membrane potential and GABA release in hippocampal interneurons. J Neurophysiol 2001; 86:261–8
31. Nolan MF, Dudman JT, Dodson PD, Santoro B: HCN1 channels control resting and active integrative properties of stellate cells from layer II of the entorhinal cortex. J Neurosci 2007; 27:12440–51
32. Biel M, Wahl-Schott C, Michalakis S, Zong X: Hyperpolarization-activated cation channels: From genes to function. Physiol Rev 2009; 89:847–85
33. Jahnsen H, Llinás R: Ionic basis for the electro-responsiveness and oscillatory properties of guinea-pig thalamic neurones *in vitro*. J Physiol 1984; 349:227–47
34. Destexhe A, Bal T, McCormick DA, Sejnowski TJ: Ionic mechanisms underlying synchronized oscillations and propagating waves in a model of ferret thalamic slices. J Neurophysiol 1996; 76:2049–70
35. Lüthi A, Bal T, McCormick DA: Periodicity of thalamic spindle waves is abolished by ZD7288, a blocker of Ih. J Neurophysiol 1998; 79:3284–9
36. Lüthi A, McCormick DA: H-current: Properties of a neuronal and network pacemaker. Neuron 1998; 21:9–12
37. Zhang L, Krnjević K: Whole-cell recording of anoxic effects on hippocampal neurons in slices. J Neurophysiol 1993; 69:118–27
38. Bal R, Oertel D: Hyperpolarization-activated, mixed-cation current I_h in octopus cells of the mammalian cochlear nucleus. J Neurophysiol 2000; 84:806–17
39. Ries CR, Puil E: Mechanism of anesthesia revealed by shunting actions of isoflurane on thalamocortical neurons. J Neurophysiol 1999; 81:1795–801
40. Kim HS, Wan X, Mathers DA, Puil E: Selective GABA-receptor actions of amobarbital on thalamic neurons. Br J Pharmacol 2004; 143:485–94
41. Williams SR, Turner JP, Hughes SW, Crunelli V: On the nature of anomalous rectification in thalamocortical neurones of the cat ventrobasal thalamus *in vitro*. J Physiol 1997; 505:727–47
42. Rateau Y, Ropert N: Expression of a functional hyperpolarization-activated current (I_h) in the mouse nucleus reticularis thalami. J Neurophysiol 2006; 95:3073–85
43. BoSmith RE, Briggs I, Sturgess NC: Inhibitory actions of ZENECA ZD7288 on whole-cell hyperpolarization activated inward current (I_f) in guinea-pig dissociated sinoatrial node cells. Br J Pharmacol 1993; 110:343–9
44. Harris NC, Libri V, Constanti A: Selective blockade of the hyperpolarization-activated cationic current I_h in guinea pig substantia nigra pars compacta neurones by a novel bradycardic agent, Zeneca ZM 227189. Neurosci Lett 1994; 176:221–5
45. Travagli RA, Gillis RA: Hyperpolarization-activated currents, I_H and I_{KIR}, in rat dorsal motor nucleus of the vagus neurons in vitro. J Neurophysiol 1994; 71:1308–17
46. Tennigkeit F, Schwarz DWF, Puil E: Mechanisms for signal transformation in lemniscal auditory thalamus. J Neurophysiol 1996; 76:3597–608
47. Bal T, McCormick DA: What stops synchronized thalamocortical oscillations? Neuron 1996; 17:297–308
48. Sun H, Varela D, Chartier D, Ruben PC, Nattel S, Zamponi GW, Leblanc N: Differential interactions of Na^+ channel toxins with T-type Ca^{2+} channels. J Gen Physiol 2008; 132:101–13
49. Docherty RJ, Farmer CE: The pharmacology of voltage-gated sodium channels in sensory neurones. Handb Exp Pharmacol 2009; 519–61
50. Rocchetti M, Armato A, Cavalieri B, Micheletti M, Zaza A: Lidocaine inhibition of the hyperpolarization-activated current (I_f) in sinoatrial myocytes. J Cardiovasc Pharmacol 1999; 34:434–9
51. Munsch T, Pape HC: Modulation of the hyperpolarization-activated cation current of rat thalamic relay neurones by intracellular pH. J Physiol 1999; 519:493–504
52. Meuth SG, Kanyshkova T, Meuth P, Landgraf P, Munsch T, Ludwig A, Hofmann F, Pape HC, Budde T: Membrane resting potential of thalamocortical relay neurons is shaped by the interaction among TASK3 and HCN2 channels. J Neurophysiol 2006; 96:1517–29
53. Nayak TK, Harinath S, Nama S, Somasundaram K, Sikdar SK: Inhibition of human two-pore domain K^+ channel TREK1 by

local anesthetic lidocaine: Negative cooperativity and half-of-sites saturation kinetics. Mol Pharmacol 2009; 76:903–17

54. Mulle C, Steriade M, Deschênes M: The effects of QX314 on thalamic neurons. Brain Res 1985; 333:350–4
55. Akasu T, Shoji S, Hasuo H: Inward rectifier and low-threshold calcium currents contribute to the spontaneous firing mechanism in neurons of the rat suprachiasmatic nucleus. Pflügers Arch 1993; 425:109–16
56. Hughes SW, Cope DW, Crunelli V: Dynamic clamp study of I_h modulation of burst firing and delta oscillations in thalamocortical neurons in vitro. Neuroscience 1998; 87: 541–50
57. Ying SW, Abbas SY, Harrison NL, Goldstein PA: Propofol block of I_h contributes to the suppression of neuronal excitability and rhythmic burst firing in thalamocortical neurons. Eur J Neurosci 2006; 23:465–80
58. Budde T, Caputi L, Kanyshkova T, Staak R, Abrahamczik C, Munsch T, Pape HC: Impaired regulation of thalamic pacemaker channels through an imbalance of subunit expression in absence epilepsy. J Neurosci 2005; 25:9871–82
59. Blas-Valdivia V, Cano-Europa E, Hernández-Garcia A, Ortiz-Butrón R: Hippocampus and amygdala neurotoxicity produced by systemic lidocaine in adult rats. Life Sci 2007; 81:691–4
60. Lopez-Galindo GE, Cano-Europa E, Ortiz-Butron R: Ketamine prevents lidocaine-caused neurotoxicity in the CA3 hippocampal and basolateral amygdala regions of the brain in adult rats. J Anesth 2008; 22:471–4
61. Du C, Yu M, Volkow ND, Koretsky AP, Fowler JS, Benveniste H: Cocaine increases the intracellular calcium concentration in brain independently of its cerebrovascular effects. J Neurosci 2006; 26:11522–31
62. Ferrante FM, Paggioli J, Cherukuri S, Arthur GR: The analgesic response to intravenous lidocaine in the treatment of neuropathic pain. Anesth Analg 1996; 82:91–7
63. Finnerup NB, Biering-Sørensen F, Johannesen IL, Terkelsen AJ, Juhl GI, Kristensen AD, Sindrup SH, Bach FW, Jensen TS: Intravenous lidocaine relieves spinal cord injury pain: A randomized controlled trial. ANESTHESIOLOGY 2005; 102: 1023–30
64. Puig S, Sorkin LS: Formalin-evoked activity in identified primary afferent fibers: Systemic lidocaine suppresses phase-2 activity. Pain 1996; 64:345–55
65. Chen X, Shu S, Bayliss DA: HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. J Neurosci 2009; 29:600–9
66. Chen X, Sirois JE, Lei Q, Talley EM, Lynch C III, Bayliss DA: HCN subunit-specific and cAMP-modulated effects of anesthetics on neuronal pacemaker currents. J Neurosci 2005; 25:5803–14
67. Chen X, Shu S, Kennedy DP, Willcox SC, Bayliss DA: Subunit-specific effects of isoflurane on neuronal I_h in HCN1 knockout mice. J Neurophysiol 2009; 101:129–40
68. Lewis AS, Chetkovich DM: HCN channels in behavior and neurological disease: Too hyper or not active enough? Mol Cell Neurosci 2011; 46:357–67