Self-regulation of adult thalamocortical neurons

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THALAMOCORTICAL (TC) relay neurons are a critical node of communication for information traveling from the peripheral sensory systems to the cerebral cortex as well as between diverse cortical and subcortical gray matter structures. In addition to the normal physiological signals, pathological signals such as those in central pain and epilepsy also disseminate via these thalamic pathways. The firing pattern of TC neurons is characterized by one of two modes, tonic or bursting, referring to the pattern of sodium action potentials (APs) (Jahnsen and Llinás 1984a; Llinás and Jahnsen 1982). The tonic firing mode consists of APs fired at a continuous and regular rate throughout a depolarizing stimulus, while burst firing mode occurs only from a hyperpolarized membrane potential (V_m) and consists of a brief cluster of APs firing at high frequencies (2–10 APs at 250-1,000 Hz) (Deschenes and Steriade 1982; Jahnsen and Llinás 1984b; Llinás and Jahnsen 1982). Burst firing is enabled by the presence of T-type Ca^{2+} channel currents present in TC neurons that are generated by the α-subunit Ca_{3.1} and that are deactivated when V_m is hyperpolarized beyond resting levels. Ca_{3.1} mediates a low-threshold, slow, but transient depolarizing potential that drives burst firing in TC neurons (Anderson et al. 2005; Kim et al. 2001). Evidence also suggests that Ca^{2+} influx through Ca_{3.1} inhibits tonic firing (Anderson et al. 2005).

Recent extracellular recordings from thalamus found that visceral pain initially increases TC neuron firing rate in ventral posterior lateral (VPL) nucleus but within minutes overall firing rate decreases and firing mode switches to brief intermittent bursts (Kim et al. 2003). The concurrent emergence of burst firing during the inhibition of overall firing rate suggests the possibility that the neurons have become hyperpolarized, as this would de-inactivate the Ca_{3.1} T-type calcium channels necessary for burst firing in TC neurons. Consistent with this notion, genetically deleting Ca_{3.1} abolished the burst firing (Kim et al. 2003). Importantly, deleting Ca_{3.1} only partially (40–50%) prevented the decrease in tonic firing rate. Although V_m of the TC neuron was not assessed, we speculate that the switch to burst firing likely results from an excitation-induced activation of K^+ channels that then hyperpolarized V_m. The shunting effect of this presumed hyperpolarizing K^+ current would also explain the inhibition of overall firing rate. Therefore we explored mechanisms whereby excitation might hyperpolarize and inhibit TC neurons.

In principle, V_m could hyperpolarize in response to a wide variety of known endogenous neuromodulators. Rapid transient hyperpolarization of TC neurons occurs via GABA_A receptor synaptic transmission, from either the thalamic reticular nucleus (nRT) or local GABAergic neurons (Crabtree et al. 2002; Crabtree and Isaac 1998). However, this ligand-gated Cl^- channel current is short-lived and only weakly hyperpolarizes V_m because of the rapid kinetics of GABA_A synaptic transmission and the relatively high Cl^- reversal potential (E_{Cl} ~ −80 mV in TC neurons) (Ulrich and Huguenard 1997a). This mechanism may not be well suited to produce the strong and lasting V_m hyperpolarization needed to achieve a persistent inhibition of tonic firing rate and switch to burst firing mode. GABA_B receptor synaptic transmission produces a stronger and longer-lasting hyperpolarization of V_m, because of the slower kinetics of GABA_B G protein receptor signaling and the activation of K^+ conductances with a more negative equilibrium potential (E_K ~ −100 mV) (Ulrich and Huguenard 1997b). However, hyperpolarizing V_m via GABAergic synaptic transmission requires continuous GABAergic neuron AP firing at high rates. Such firing can fail because of energy depletion and in some circumstances may lead to GABAergic neuron cell death. Extrasynaptic GABA can also inhibit through tonic inhibition mediated by specific GABA_A receptor subunits, providing a way to couple circuit activity to local circuit inhibition (Herd et al. 2013). Yet, inactive surrounding circuits may also be inhibited by this mechanism, and therefore...
it lacks specificity. A cell-autonomous mechanism to activate K⁺ channels would overcome this limitation. Bacci et al. (2004) recently reported that cortical GABAergic interneuron firing triggers calcium-dependent cannabinoid release and autocrine activation of hyperpolarizing G protein-regulated inward rectifier (GIRK) K⁺ channels. However, this autocrine cannabinoid mechanism has not been described in glutamatergic neurons. Furthermore, this protective mechanism for GABAergic neurons would disinhibit glutamatergic neurons, leaving them prone to excitatory cell death. Importantly, TC neurons not only gate sensory signals but also transmit these signals through very high tonic fire rates (Kasten et al. 2007).

Therefore we hypothesized that TC neurons might possess a specialized autoinhibitory mechanism to prevent pathological signal transmission and glutamatergic neuron cell death in thalamus. This intrinsic mechanism might hyperpolarize Vₘ to inhibit tonic firing rate and promote burst firing as recently shown in vivo (Kim et al. 2003). We report that such mechanisms exist in TC neurons and that this function is independent of GABAergic and glutamatergic synaptic transmission, low-threshold T-type Ca²⁺ currents, Na⁺-K⁺-ATPases, and even GIRK K⁺ channels and intracellular Ca²⁺ signaling (Bacci et al. 2004). We also report evidence that the TC glutamatergic neuron autoinhibition occurs in part through ATP-regulated K⁺ channels, as recently reported in cholinergic (Allen and Brown 2004) and dopaminergic (Avshalumov et al. 2005) neurons. The results define a novel thalamic mechanism for robust, Ca²⁺-independent, and cell-autonomous inhibition of fast-spiking glutamatergic neurons in the central nervous system.

MATERIALS AND METHODS

All protocols were approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee and comply with the policies and regulations of The Journal of Physiology’s animal ethics standards as outlined by Drummond (2009). Adult (2–6 mo old) C57BL/6 mice, as well as Ca₃.1-knockout mice in a C57BL/6 genetic background, were genotyped as previously described and utilized in this study (Anderson et al. 2005). Slice preparations were as previously described (Kasten et al. 2007). Mice (80 total) were deeply anesthetized with isoflurane (5%, inhaled) and decapitated, and the brain was quickly removed and placed in ice-cold, oxygenated (95% O₂-5% CO₂) sucrose cutting solution containing (in mM) 243 sucrose, 5 KCl, 5 MgSO₄, 1 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 glucose. Coronal brain slices (230–280 μm) were cut and transferred to oxygenated (5% CO₂) artificial cerebrospinal fluid (ACSF) for 40–60 min at 37°C and then stored at room temperature for 1–9 h before recording in ACSF containing (in mM) 124 NaCl, 2.5 KCl, 1 MgCl₂, 2 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 25 glucose.

TC relay neurons were visually identified for recording at ×400 magnification with infrared (IR) DIC optics on an upright Olympus BX-51WI microscope (Olympus, Tokyo, Japan). More than 90% of the neurons within these thalamic nuclei are glutamatergic (see the Allen Brain Atlas, Mouse Connectivity, Transgenic Characterization, comparing the glutamatergic neuron Scl1a7a6-IRES-Cre reporter and GABAergic neuron Scl32a1-IRES-Cre reporter; http://www.brain-map.org). GABAergic neurons are extremely rare outside the reticular nucleus within the thalamus. These rare, typically smaller, bipolar neurons were avoided with the use of IR-guided microscopy. GABA neurons are also outliers in their electrophysiological properties, including a higher input resistance. Such a rare cell was excluded from the analysis. Recording pipettes were pulled from 1.5-mm-OD capillary tubing (A-M Systems, Carlsborg, WA) with a Flaming/Brown P-97 pipette puller (Sutter Instruments, Novato, CA) and had tip resistances of 3–5 MΩ when filled with internal solution. Internal solution contained (in mM) 135 potassium methanesulfonate, 10 HEPES, 4 KCl, 2 NaCl, 4 MgATP, 0.3 Tris-GTP, 7 phosphocreatine, 1 EGTA, 0.1 CaCl₂ (pH = 7.25, osmolality ~ 290 mosM). In studies utilizing the calcium chelator BAPTA in the patch pipette, potassium methanesulfonate concentration was reduced to maintain osmolality. Multiple attempts to perform perforated patch recordings in these adult TC neurons were unsuccessful.

Recordings were made with a HEKA triple EPC-10 patch-clamp amplifier utilizing PatchMaster 2 software (HEKA Instruments, Southboro, MA) at room temperature (~22°C) or 35°C in oxygenated (5% O₂-95% CO₂) ACSF. Current-clamp recordings were obtained from thalamic relay neurons in the centromedial (CM) nucleus and other nuclei as described in the figures. Cells were recorded only if series resistance was >15 MΩ; most demonstrated a series resistance of 7–12 MΩ. Series resistance was 80% compensated, and junction potentials were uncorrected. Data were unfiltered and sampled at 2–20 kHz. Burst firing is defined as two or more APs occurring at the beginning of a current pulse with an interspike interval of <10 ms (>100 Hz). Firing rate (spikes/s across the full 500-ms pulse) and Vₘ were measured from the first and last traces of the high-frequency stimulation protocol described below. To fit inset traces into limited space, APs are truncated. Statistics were assessed by Student’s paired t-test for single measurements and ANOVA for repeated measurements.

Drugs were bath applied with gravity perfusion in oxygenated (95% O₂-5% CO₂) ACSF. All recordings were performed in the presence of AMPA/kainate antagonist DNQX (10 μM; Tocris, Ellisville, MO) and GABAₐ receptor antagonist picrotoxin (100 μM; Sigma, St. Louis, MO). Tetrodotoxin (TTX), ouabain, BaCl₂, glibenclamide, and tolbutamide were obtained from Sigma-Aldrich.

RESULTS

Repeated firing causes thalamocortical neurons to hyperpolarize Vₘ and self-inhibit. The autonomous response of individual TC neurons to prolonged AP firing was assessed with direct intracellular depolarizing current injections during whole cell patch-clamp recordings in coronal brain slices from adult mice. Current injection amplitudes were adjusted such that a 500-ms pulse elicited 5–20 spikes. These pulses were initially repeated once every 10 s, revealing a consistent AP firing rate with each stimulus. The pulses were then repeated at a higher rate of once every second for 4 min (240 depolarizations). This protocol was selected because it allowed strong stimulation of the TC neuron while allowing measurements of Vₘ between stimuli. We initially focused on CM neurons because of their relatively high baseline input resistance and their potential as a target of cortical seizures. Figure 1A reveals that repeated current injections cause tonic AP firing. Interestingly, when the same pulse stimulus was given at a higher rate (every 1 s), the Vₘ between injections gradually hyperpolarized, reaching a steady state after ~1 min (Fig. 1A). The pattern of APs evoked also changed: early pulses caused a continuous “tonic” AP firing response (10–40 Hz), but as Vₘ hyperpolarized tonic firing rate decreased, eventually changing to a single AP (Fig. 1A). In CM, a low-threshold calcium spike was observed, but burst firing was most often absent, even during the rebound from a hyperpolarizing current injection firing only single spikes (Kasten and Anderson, data not shown). This contrasts with the change from tonic to burst firing (initial couple spikes.
firing at ≥100 Hz) observed in neurons of other thalamic nuclei [VPL and medial dorsal, central division (MDc) in Fig. 2, C and D, and Fig. 4D]. After the first firing trial 1, upon switching back to the low firing rate stimulus, \( V_m \) returned to resting (Fig. 1A, left) or even slightly depolarized values (Fig. 1A, right), labeled “Early”, \( n = 8 \). These results indicate that the firing history of TC neurons determines the magnitude of the self-inhibition and \( V_m \) hyperpolarization. CM thalamic neurons challenged with this firing protocol hyperpolarized \( V_m \) by 13.6 ± 0.9 mV \( (P < 0.001, n = 16; \text{Fig. 1C}) \), and firing rate was inhibited by 62 ± 5% of baseline \( (P < 0.001, n = 16; \text{Fig. 1D}) \).

Self-regulated firing exists in multiple thalamic nuclei. To determine whether this self-regulatory response to firing exists in the glutamatergic neurons of other major thalamic relay nuclei, we repeated the protocol in neurons from multiple thalamic nuclei. A lateral dorsal (LD) TC neuron with 80-pA depolarizing current injection hyperpolarized \( V_m \) from −70 mV to −81 mV, inhibited firing rate from 20 Hz to 4 Hz, and switched from tonic to burst firing (Fig. 2A). A CM TC neuron with 75-pA current injection hyperpolarized \( V_m \) from −65 mV to −82 mV, inhibited firing rate from 24 Hz to 6 Hz, but failed to burst (Fig. 2B). A VPL TC neuron with 140-pA current injection hyperpolarized \( V_m \) from −70 to −84 mV, inhibited firing rate from 42 Hz to 16 Hz, and developed an initial burst response to the pulse (Fig. 2C). A MDc TC neuron with 400-pA current injections hyperpolarized \( V_m \) from −66 mV to −82 mV, inhibited firing rate from 62 Hz to 4 Hz, and fired an initial burst (Fig. 2D). A subset of neurons developed a persistent hyperpolarization of \( V_m \) after the stimulus trial shown (Fig. 2A). These studies reveal that self-regulated firing is pervasive among thalamic nuclei.

**Firing-induced hyperpolarization requires Na\(^+\) but not Ca\(^{2+}\) entry.** T-type Ca\(^{2+}\) channels are necessary for thalamic neuron burst firing. While the function of thalamic burst firing is largely unknown, one study has found it necessary to stabilize sleep (Anderson et al. 2005). To test whether T-type Ca\(^{2+}\) channels contribute to this autoinhibitory response in vitro, we recorded thalamic neurons in mice with genetic deletion of exons 11–13 in CACN1G, which encodes the major T-type Ca\(^{2+}\) channel subunit, Ca\(_{\text{v}3.1}\) (Anderson et al. 2005). This targeted gene knockout abolishes T-type Ca\(^{2+}\) currents and burst firing in TC neurons (Anderson et al. 2005; Kasten et al. 2007). Despite the absence of burst firing, firing-induced hyperpolarization of \( V_m \) was preserved \([11.2 \pm 2.5 \text{ mV} (n = 8)] \) vs. \([13.6 \pm 0.9 \text{ mV} \text{ in control (ctrl)} (n = 16), P > 0.2; \text{Fig. 3, A and C}) \). Consistent with the studies in vivo (Kim et al. 2003), loss of Ca\(_{\text{v}3.1}\) did partially attenuate firing rate inhibition \([45.5 \pm 4.1 \% (n = 8)] \) vs. \([61.7 \pm 5.1 \% \text{ in ctrl (n = 16), P < 0.05; Fig. 3D}) \).

TC neurons possess an assortment of high-threshold Ca\(^{2+}\) channels that can regulate firing (Budde et al. 1998; Coultet et al. 1989; Crunelli et al. 1989; Kasten et al. 2007). To test whether increases of intracellular Ca\(^{2+}\) are necessary for firing-induced hyperpolarization and inhibition, we included the rapid Ca\(^{2+}\) chelator BAPTA (15 mM) in the pipette solution to...
block increases of intracellular Ca\(^{2+}\) triggered by repetitive firing. BAPTA not only failed to block firing-induced hyperpolarization \([12.6 \pm 1.5 \text{ mV} (n = 9)]\) vs. \([13.6 \pm 0.9 \text{ in ctrl} (n = 16), P > 0.2; \text{Fig. 3, B and C}]\) but also failed to block autoinhibition \([22.6 \pm 2.6 \text{ Hz before vs. } 6.2 \pm 2.0 \text{ Hz after} (n = 9) \text{ inhibition of } 73.0 \pm 9.1\% \text{ vs. } 61.7 \pm 5.1\% \text{ in ctrl} (n = 9), P > 0.2; \text{Fig. 3, B and C}]. Thus Ca\(^{2+}\)-mediated pathways are unnecessary for firing-induced \(V_m\) hyperpolarization or autoinhibition.

Bacci et al. (2004) found that depolarization alone, independent of Na\(^+\) APs, hyperpolarizes \(V_m\) in cortical GABAergic interneurons via Ca\(^{2+}\)-induced cannabinoid signaling. However, the Ca\(^{2+}\)-independence of TC neuron autoinhibition suggested a different mechanism. Consistent with this suggestion, unlike these cortical GABAergic interneurons, blocking voltage-gated Na\(^+\) channels and APs with TTX (500 nM) abolished the depolarization-induced hyperpolarizing of TC glutamatergic neurons (Fig. 4A; \(n = 3\)).

Firing hyperpolarizes \(V_m\) via a K\(^+\) conductance and self-inhibits via shunting. Firing not only hyperpolarized \(V_m\) but also markedly decreased input resistance \([931 \pm 96 \text{ M}\Omega \text{ vs. } 547 \pm 90 \text{ M}\Omega, P < 0.01, n = 6]\), suggesting the possible activation of K\(^+\) conductances to hyperpolarize \(V_m\) and inhibit firing rate. However, TC neurons also display a large nonlinear membrane conductance; hyperpolarizing \(V_m\) from rest opens both hyperpolarization-activated, nonselective cation conductances (\(I_h\)) and inwardly rectifying K\(^+\) channels that reduce input resistance (Kasten et al. 2007; Meuth et al. 2006). To establish that firing activates a K\(^+\) conductance to hyperpolarize \(V_m\), we raised extracellular K\(^+\) concentration ([K\(^+\)]) from 3.5 to 12 mM, shifting the reversal potential for K\(^+\) (\(E_K\)) from −102 to −63 mV. Shifting \(E_K\) toward resting \(V_m\) blocked the firing-induced \(V_m\) hyperpolarization but preserved firing-induced inhibition (Fig. 4B; \(n = 3\)), revealing the shunting effects of the firing-activated K\(^+\) conductance. The findings suggest that firing activates a K\(^+\) conductance to hyperpolarize \(V_m\) and inhibit firing. Also consistent with this suggestion, prior hyperpolarization of \(V_m\) below resting values to −85 mV (\(E_K\) is −102 mV) with tonic negative current injections failed to occlude the firing-induced inhibition of evoked firing rate \([71.0 \pm 12.0\% \text{ (} n = 7\) vs. 61.7 \pm 5.1\% \text{ in ctrl} (n = 16), P > 0.2; \text{Fig. 4C}]. In fact, even from this relatively hyperpolarized potential, repeated firing further hyperpolarized \(V_m\) beyond −85 mV (\([\Delta V_m\); 4.3 \pm 1.9 mV (\(n = 7\) vs. 13.6 \pm 0.9 mV in ctrl (\(n = 16\), \(P < 0.01; \text{Fig. 4C}\) toward \(E_K\). To confirm that

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**Fig. 2.** Cell-autonomous regulation of firing rate and mode in multiple thalamic nuclei. Thalamocortical neurons from multiple nuclei exhibit action potential firing-induced \(V_m\) hyperpolarization, firing rate inhibition, and firing mode switching from continuous tonic to transient with or without an initial high-frequency burst of 2 spikes at >100 Hz (see inset, top). A: lateral dorsal thalamocortical neuron (LDn) with 80-pA depolarizing current injections. B: CM thalamocortical neuron with 75-pA current injections. C: ventral posterior lateral (VPL) thalamocortical neuron with 140-pA current injections. D: medial dorsal, central division (MDc) thalamocortical neuron with 400-pA current injections. Examples are representative of 3–5 cells each. Insets: traces, early and late in firing protocol. In all traces, a line marks the baseline resting \(V_m\) prior to and during stimulation.

**Fig. 3.** Ca\(^{2+}\) signaling is not required for firing-induced \(V_m\) hyperpolarization in thalamocortical neurons. A: CM thalamocortical neurons from mice lacking Ca\(_{\text{v}}\) T-type Ca\(^{2+}\) channels (KO). B: CM thalamocortical neuron with chelation of intracellular Ca\(^{2+}\) with pipette BAPTA (15 mM). C and D: effects of chelating intracellular Ca\(^{2+}\) with pipette BAPTA. WT, wild-type control. *\(P < 0.05\). Insets: traces, early and late in firing protocol. In all traces, a line marks the baseline resting \(V_m\) prior to and during stimulation.
this mechanism functions under physiological conditions, we increased bath temperature to $T = 35^\circ C$ and repeated the same firing protocol. Under these conditions, the firing protocol robustly hyperpolarized $V_m$ and inhibited firing rate [Fig. 4D; MDc ($n = 3$) and CM ($n = 4$)]. Additionally, the persistent hyperpolarization of $V_m$ developed within the first stimulus challenge (Fig. 4D) rather than after multiple challenges at room temperature (Fig. 1B).

The GIRK inhibitor barium (100 $\mu$M; Fig. 5A), which blocks firing-induced hyperpolarization of GABAergic neurons in cortex (Bacci et al. 2004), failed to block firing-induced $V_m$ hyperpolarization in TC neurons.

The Na$^+$.K$^+$.ATPase inhibitor ouabain, previously shown to inhibit the hyperpolarizing response of hippocampal neurons to glutamate and calyx of Held terminals to firing (Kim et al. 2007; Thompson and Prince 1986), actually enhanced firing-induced $V_m$ hyperpolarization and firing rate autoinhibition (Fig. 5B). The results indicate that TC neurons autoregulate firing via mechanisms distinct from cortical [low threshold spike (LTS)] GABAergic neurons and the calyx of Held synaptic terminus.

Firing autoregulation in thalamocortical neurons is inhibited by $K_{ATP}$ channel blockers. Activating $K_{ATP}$ channels with diazoxide inhibited tonic firing in TC neurons, but blockers of these channels had minimal effects during a brief (1 s) excitatory stimulus (Kasten et al. 2007). We speculated that $K_{ATP}$ channels might instead contribute during periods of prolonged AP firing.

Consistent with this notion, the $K_{ATP}$ channel blocker tolbutamide (100 $\mu$M) markedly decreased the firing-induced $V_m$ hyperpolarization and self-inhibition (Fig. 6, A–C). Tolbutamide reduced the $\Delta V_m$ hyperpolarization to $6.7 \pm 1.2$ mV [vs. $13.6 \pm 0.9$ mV in ctrl ($n = 9$ each), $P < 0.001$] and diminished autoinhibition to $23.8 \pm 3.2\%$ [vs. $61.7 \pm 5.1\%$ in ctrl ($n = 9$ each), $P < 0.001$].

Another $K_{ATP}$ channel inhibitor (glibenclamide, 30 $\mu$M, $n = 3$ of 3, example in Fig. 6D) had similar effects. The small residual hyperpolarization and inhibition (Fig. 6B) could involve compensatory activation of Ca$^{2+}$.activated K$^+$ currents (Kasten et al. 2007), Na$^+$.activated K$^+$ currents (Bhattacharjee et al. 2002, 2003; Kim and McCormick 1989), or the Na$^+$.K$^+$.ATPase (Kim et al. 2007; Thompson and Prince 1986). The results provide evidence that $K_{ATP}$ channels mediate a major component of the firing-induced hyperpolarization and autoinhibition of glutamatergic TC neurons.

**DISCUSSION**

This study reveals that TC glutamatergic neurons self-inhibit. While firing-induced autoinhibition and $V_m$ hyperpolarization have been observed in several other neuronal subtypes, including superior colliculus neurons, GABAergic neurons, the synaptic terminus of the calyx of Held, and others (Bacci et al. 2004; Kim et al. 2007; Kim and McCormick 1998; Kubota and Saito 1991; Llinás and Lopez-Barneo 1988), this study identifies this mechanism in relay neurons of the thalamus. Importantly, self-inhibition could occur even at relatively low firing rates and displayed plasticity. $K_{ATP}$ channels have been functionally identified in cholinergic neurons (Allen and Brown 2004) and were shown to regulate baseline tonic firing in dopaminergic neurons (Avshalumov et al. 2005). However,
this channel subtype had not been previously implicated in the regulation of induced glutamatergic neuron AP firing rate. The finding of plasticity of this mechanism is also novel; the reversible (and a component of slowly reversible or irreversible) homeostatic response to firing was potentiated by prior neuronal activity, revealing a new form of plasticity of membrane excitability. KATP-dependent hyperpolarization provides an important link between energy metabolism and neuronal firing within the thalamus that could mediate homeostatic responses to the excessive discharges that impinge on thalamus in conditions such as epilepsy, peripheral pain syndromes, and possibly also certain psychiatric diseases (reviewed in Jeanmonod et al. 1996). This energy-dependent regulatory mechanism of thalamic sensory transmission may also explain changes in sensory transmission that occur across the sleep-wake cycle as energy is depleted and replenished.

KATP channels play important role in inhibiting thalamic output. Multiple neuromodulators and ion channels are capable of regulating $V_m$ and firing in TC relay neurons. The nonselective cation and two-pore K$^+$ channels shape resting $V_m$ and regulate the firing response of TC neurons (Meuth et al. 2006). Strong GABAAergic input from nRT neurons generates both a GABA$A$ channel-mediated Cl$^-$ conductance and a GABA$B$ receptor-mediated G protein-activated inward-rectifying K$^+$ channel conductance (Cox et al. 1997; Ulrich and Huguenard 1996) to inhibit firing. Finally, cholinergic stimulation is capable of altering firing of TC neurons (Varela and Sherman 2007).

We previously performed a comprehensive analysis of the ion channels that regulate firing in TC neurons and identified specific K$^+$ channels (Kv1 and Kv3.2 voltage-gated, SK Ca$^{2+}$-activated, and K$\alpha_{2,2}$-like leak channels regulate components of tonic firing) and Ca$^{2+}$ channels (largely T type and N type) that regulate elements of the firing response profile (Kasten et al. 2007). The present findings extend this characterization to the domain of prolonged tonic firing. While K$\alpha_{ATP}$ channels are strongly expressed in thalamus (Dunn-Meynell et al. 1998; Gehlert et al. 1991), our initial studies found that K$\alpha_{ATP}$ channel blockers had a relatively small contribution to the firing response during a brief 1-s stimulus (Kasten et al. 2007). By contrast, these channels have a profound inhibitory effect during prolonged tonic firing.

The finding that even very low firing rates (as low as 10 Hz; Fig. 1C) induce autoinhibition indicates that this mechanism
could have effects even during physiological conditions and normal behavioral states. Importantly, $K_{\text{ATP}}$-mediated inhibition of TC neurons is independent of extrinsic synaptic inputs and occurs at the single-cell level, permitting highly focused regulation of neuronal circuit transmission within the thalamus. This would be an ideal mechanism to limit the transmission of signals generated from a limited peripheral somatosensory pain field or a cortical or subcortical seizure focus. Kim et al. (2003) showed that nociceptive stimuli cause an initial increase of tonic firing rate of TC neurons in the somatosensory thalamus that then decreases and switches to Cav3.1-dependent burst firing cannot occur without $V_{\text{m}}$ hyperpolarization to deinactivate the channel. We show that tonic firing alone is sufficient to hyperpolarize $V_{\text{m}}$ and that this response is mediated in large part by the activation of $K_{\text{ATP}}$ channels. As observed in vivo (Kim et al. 2003), we found that a relatively smaller portion of the in vitro autoinhibition was lost when Ca$_{3.1}$ T-type Ca$^{2+}$ channel-dependent burst firing; however, Ca$_{3.1}$ T-type Ca$^{2+}$ channel-dependent burst firing cannot occur without $V_{\text{m}}$ hyperpolarization to deinactivate the channel. We show that tonic firing alone is sufficient to hyperpolarize $V_{\text{m}}$ and that this response is mediated in large part by the activation of $K_{\text{ATP}}$ channels. As observed in vivo (Kim et al. 2003), we found that a relatively smaller portion of the in vitro autoinhibition was lost when Ca$_{3.1}$ T-type Ca$^{2+}$ channels were deleted (~30–40%; Fig. 3D), whereas a much greater portion of this autoinhibition was lost when $K_{\text{ATP}}$ channels were inhibited (~75%; Fig. 6C).

$K_{\text{ATP}}$ channels are adaptable modulators of neuronal function. While $K_{\text{ATP}}$ channels activate primarily in response to low intracellular [ATP], a broad variability in the [ATP] sensitivity of $K_{\text{ATP}}$ channels has been observed in different systems due largely to differences in subunit composition and splice variants (Inagaki et al. 1995, 1996; Sakura et al. 1999). Furthermore, $K_{\text{ATP}}$ channel protein is strongly upregulated in response to repeated neuronal activity caused by picrotoxin seizure kindling (Jiang et al. 2004). In addition to direct regulation via intracellular [ATP] and [ADP], $K_{\text{ATP}}$ channels are also activated by mitochondrial release of peroxide, as demonstrated in heart and vascular tissue and more recently in brain (Avshalumov et al. 2005; Avshalumov and Rice 2003; Bao et al. 2005; Ichinari et al. 1996; Thompson et al. 1998). Peroxide-induced oxidation of $K_{\text{ATP}}$ channels (an irreversible modification requiring protein turnover to clear) increases the [ATP] needed to inhibit channels in excised patches causing activity even at baseline [ATP] (Ichinari et al. 1996). Such mechanisms (peroxide modification, increased protein expression, or increased surface recruitment) could potentially explain the plasticity (including the tonic irreversible $V_{\text{m}}$ hyperpolarization) observed with repeated stimulation (Fig. 1A, right, and Fig. 1B).

Other channels contributing to firing-induced inhibition. Blocking $K_{\text{ATP}}$ channels nearly eliminated firing-induced autoinhibition (~75%) but only partially inhibited firing-induced hyperpolarization (~50%). Several other channels were considered as potential explanations for this residual firing-induced hyperpolarization, including slow afterhyperpolarization (sAHP) K$^+$ channels, Ca$^{2+}$-activated K$^+$ channels, and Na$^+$-activated K$^+$ channels. TC neurons possess a wide range of Ca$^{2+}$ channels, which can regulate firing through small- and large-conductance K$^+$ channels (SK and BK Ca$^{2+}$-activated K$^+$ channels) as well as the sAHP Ca$^{2+}$-activated K$^+$ channel (Kasten et al. 2007; Zhang et al. 2009); however, these channels generally provide feedback inhibition on a much faster timescale (ms to s) to hyperpolarize and inhibit firing. Significantly, complete chelation of intracellular Ca$^{2+}$ with BAPTA produced no alteration in firing-induced $V_{\text{m}}$ hyperpolarization or autoinhibition, suggesting that Ca$^{2+}$-dependent pathways are not required. Na$^+$-activated K$^+$ ($K_{\text{Na}}$) channels are activated in response to high concentrations of intracellular Na$^+$, and activity of these channels can be enhanced by decreases of intracellular [ATP] (Bhattarcharjee et al. 2003; Schwindt et al. 1989; Tamsett et al. 2009; Yang et al. 2007). $K_{\text{Na}}$ channels
have been implicated in firing-induced hyperpolarization, and mRNA for these channels is present in thalamus (Bhattacharjee et al. 2002, 2003; Kim and McCormick 1998; Zhang et al. 2010). Activation of K<sub>ATP</sub> channels could explain the residual hyperpolarization that occurs in the presence of K<sub>ATP</sub> channel blockers and could also explain the paradoxical increase of V<sub>m</sub> hyperpolarization and firing rate inhibition found when the Na<sup>+</sup>-K<sup>+</sup>-ATPase was blocked (Fig. 5B). The enlarged response would be consistent with a greatly enhanced increase of intracellular [Na<sup>+</sup>] and K<sub>ATP</sub> channel activation. An alternative non-ion channel-based mechanism that should also be considered in future studies is feedback inhibitory glioneuronal transmission.

Functional implications. The cell-autonomous hyperpolarization of TC neurons described here will supplement the well-described feedback inhibition induced by GABAergic neurons of the nRT (Cox et al. 1997; Crabtree et al. 2002; Crabtree and Isaac 1998; Destexhe et al. 1998; Ulrich and Huguenard 1997a, 1997b). However, this cell-autonomous, self-regulatory mechanism in thalamus is distinct from GABAergic inhibition, operating on a much slower timescale and demonstrating plasticity following prior bouts of high neuronal activity. This new mechanism could overcome the failure of inhibition that might occur when energy is depleted or death occurs in GABAergic neurons and would permit the regulation of thalamic signal transmission at the single-cell level.

In conclusion, this study demonstrates a novel form of self-regulation in TC neurons that likely occurs through K<sub>ATP</sub> channels. Enhancing K<sub>ATP</sub> channel activity may hold promise for treating several neurological and psychiatric disorders (epilepsy, schizophrenia, Parkinson’s disease, depression, and neurogenic pain) associated with excessive excitatory discharge of thalamus (Jeanmonod et al. 1996).

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AUTHOR CONTRIBUTIONS


REFERENCES


