The membrane resting potential of thalamocortical relay neurons is shaped by the interaction among TASK3 and HCN2 channels

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Running title: Interaction of TASK and HCN channels

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Abstract

By combining molecular biological, electrophysiological, immunological and computer modeling techniques, we here demonstrate a counterbalancing contribution of TASK channels, underlying hyperpolarizing K⁺ leak currents, and HCN channels, underlying depolarizing Iₜₙ, to the resting membrane potential of thalamocortical relay (TC) neurons. RT-PCR experiments revealed the expression of TASK1, TASK3 as well as HCN1-4. Quantitative determination of mRNA expression levels and immunocytochemical staining demonstrated that TASK3 and HCN2 channels represent the dominant thalamic isoforms and are co-expressed in TC neurons. Extracellular acidification, a standard procedure to inhibit TASK channels, blocked a TASK current masked by additional action on HCN channels. Only in the presence of the HCN blocker ZD7288 the pH-sensitive component was typical for a TASK current, i.e., outward rectification and current reversal at the K⁺ equilibrium potential. In a similar way extracellular acidification was only able to shift the activity pattern of TC neurons from burst to tonic firing during block of Iₜₙ or genetic knock out of HCN channels. A single compartmental computer model of TC neurons simulated the counterbalancing influence of TASK and HCN on the resting membrane potential. It is concluded that TASK3 and HCN2 channels stabilize the membrane potential by a mutual functional interaction, that the most efficient way to regulate the membrane potential of TC neurons is the converse modulation of TASK and HCN channels, and that TC neurons are potentially more resistant to insults accompanied by extracellular pH shifts in comparison to other CNS regions.

Key words: thalamus, resting membrane potential, leak current, pH, computer modeling
Introduction

Despite its fundamental importance, rather little is known about the ionic conductances underlying the resting membrane potential of central neurons. In general, the resting potential is assumed to be determined by channels active below firing threshold, with $I_h$ (Pape 1996) and $I_{K-leak}$ (Jones 1989) playing major roles. Modulation of currents active below threshold is of particular interest since neuronal excitability is regulated in this manner. Recently the molecular nature of $I_h$ and $I_{K-leak}$ was illuminated by the cloning of four members of the HCN channel family giving rise to native $I_h$ currents in neurons and heart cells (Craven and Zagotta 2006) and five members of the TASK channel family, typically giving rise to highly regulated time- and voltage-independent $K^+$ background currents (Patel and Lazdunski 2004). Depending on their sensitivity to changes in extracellular pH and based on sequence homologies TASK1, TASK3, and TASK5 channels constitute one subclass (Lesage 2003). Despite this progress in understanding, the molecular constituents and functional interaction of $I_{K-leak}$ and $I_h$ in specific cell types are yet not well understood.

TC neurons offer a model system to gain our understanding of membrane currents that constitute the resting membrane potential for the following reasons: (1) TC neurons display large state-dependent shifts in membrane potential which are associated with a change from rhythmic burst firing at hyperpolarized potentials during slow-wave sleep to tonic single spike activity at depolarized potentials during wakefulness (Steriade et al. 1997). Most importantly, the depolarization-induced cessation of burst activity depends on the down-regulation of $I_{K-leak}$ and the up-regulation of $I_h$ by a number of transmitters of the ascending arousal system of the brainstem (McCormick 1992). Due to their important function as targets of multiple regulatory pathways, $I_{K-leak}$ and $I_h$ are in the main focus of the present study. (2)
Previous studies have begun to unravel the functional roles of HCN and TASK channels in TC neurons. While it was shown that inhibition of TASK1 and TASK3 channels depolarize TC neurons, thereby preferring tonic single spike activity (Meuth et al. 2006; Meuth et al. 2003), the genetic knock out or block of the HCN2 channels hyperpolarize TC neurons, thereby preferring burst firing (Ludwig et al. 2003). (3) The pH-dependency of HCN (Zong et al. 2001) and TASK (Rajan et al. 2000) channels and their corresponding membrane currents (Meuth et al. 2003; Munsch and Pape 1999) offer an experimental tool to probe this mutual functional interaction. Therefore we used extracellular acidification, molecular biological, electrophysiological, and computer modeling techniques, to demonstrate the contribution of HCN2 and TASK3 / TASK1 channels to the regulation of the resting membrane potential in TC neurons.
Methods

Preparation

Rats and mice (postnatal days 12 - 29) were anaesthetized with halothane, decapitated and used for electrophysiological, immunohistochemical, and molecular biological analysis. A block of tissue containing the thalamus was removed and placed in ice cold saline, containing (mM): Sucrose, 200; PIPES, 20; KCl, 2.5; NaH$_2$PO$_4$, 1.25; MgSO$_4$, 10; CaCl$_2$, 0.5; dextrose, 10; pH 7.35 with NaOH. Thalamic slices were prepared as coronal sections on a vibratome. Prior to recording, slices were kept submerged in standard artificial cerebrospinal fluid (ACSF; mM): NaCl, 125; KCl, 2.5; NaH$_2$PO$_4$, 1.25; NaHCO$_3$, 24; MgSO$_4$, 2; CaCl$_2$, 2; dextrose, 10; pH adjusted to 7.35 by bubbling with a mixture of 95% O$_2$ and 5% CO$_2$.

Whole cell patch-clamp

Recordings were performed on visually identified TC neurons of the dorsal lateral geniculate nucleus (dLGN) at room temperature. Slices were recorded in a solution containing: NaCl, 120; KCl, 2.5; NaH$_2$PO$_4$, 1.25; HEPES, 30; MgSO$_4$, 2; CaCl$_2$, 2; dextrose, 10; pH 7.3 or 6.3 was adjusted with HCl. Electrical activity was measured with pipettes pulled from borosilicate glass (GC150T-10, Clark Electromedical Instruments, Pangbourne, UK), connected to an EPC-10 amplifier (HEKA Elektronik, Lamprecht, Germany), and filled with (in mM): K-gluconate, 95; K$_3$-citrate, 20; NaCl, 10; HEPES, 10; MgCl$_2$, 1; Ca Cl$_2$, 0.5; BAPTA, 3; Mg-ATP, 3; Na-GTP, 0.5. The internal solution was set to a pH of 7.25 with KOH and an osmolality of 295 mOsm / kg. Typical electrode resistance was 2-3 M$\Omega$, with an access resistance in the range of 5 -15 M$\Omega$. Series resistance compensation of more than 40% was routinely used.
Electrophysiological experiments were governed by Pulse software (HEKA Elektronik) operating on an IBM-compatible PC. A liquid junction potential of $8 \pm 1$ mV ($n = 6$) was taken into account.

$I_h$ was activated using hyperpolarizing voltage steps from a holding potential of -43 mV to -133 mV in 10 mV increments. In order to increase stability of whole-cell recordings the pulse length was decreased by 1500 ms with increasing depth of the hyperpolarization (3.5 s pulse length at -130 mV). Steady-state activation of $I_h$ activation, $p(V)$, was estimated by normalizing the tail current amplitudes ($I$), 50 ms after stepping to a constant potential from a variable amplitude step using the following equation:

$$p(V) = (I - I_{\text{min}}) / (I_{\text{max}} - I_{\text{min}})$$

with $I_{\text{max}}$ being the tail current amplitude for the voltage step from -133 mV to -98 mV and $I_{\text{min}}$ for the voltage step from -43 mV to -98 mV, respectively. $I_h$ activation was usually well accounted for a Boltzmann distribution of the following form:

$$p(V) = 1 / (1 + \exp((V - V_h) / k))$$

where $V_h$ is the voltage of half-maximal activation and $k$ the slope factor.

During current-clamp recordings the instantaneous frequency ($f$) of action potential generation was determined by analyzing the first two action potentials elicited by a depolarizing current pulse.

All results were presented as mean $\pm$ SEM. Substance effects were tested for statistical significance using the non-parametric Mann-Whitney test. Where applicable (Gaussian distribution of measured values), a parametric t-test modified for small samples was used. Differences were considered statistically significant if $p < 0.05$. 
Drugs

ZD7288 (BIOTREND, Cologne, Germany) was directly dissolved in the external recording solution.

Computer simulations with NEURON®

For computer simulations, a previously described single-compartment TC neuron model (Huguenard and McCormick 1992; McCormick and Huguenard 1992) was adapted to NEURON (Hines and Carnevale 2001; Meuth et al. 2005). The model is based on the mathematical description of $I_A$, $I_{K2}$, $I_C$, $I_L$, $I_T$, $I_{NaP}$, and $I_h$ and displays the two typical modes of action potential generation in thalamic cells: burst firing with two to six action potentials riding on a low-threshold $Ca^{2+}$ spike and single spike activity with tonic trains of action potentials. This well established model was extended by incorporating the inward rectifying current $I_{Kir}$ of the Hodgkin-Huxley form (Williams et al. 1997) and the background potassium current $I_{TASK}$. The general equation describing the membrane potential over the time is:

$$C_m \frac{dV}{dt} = - (I_{leak} + I_{TASK} + I_{Kir} + I_h) + I_{inject},$$

where $C_m$ is the membrane capacitance and $I_{inject}$ is the injection current. The non-inactivating current $I_{Kir}$ was modeled as described before (Williams et al. 1997):

$$I_{Kir} = g_{Kir} \cdot m^a (V - E_{Kir}),$$

where $g_{Kir}$ is the maximal conductance of the current $I_{Kir}$, while $m$ is the activation variable, and $a$ is its exponent and is equal to 3. $E_{Kir}$ is the reversal potential of the current $I_{Kir}$. The outwardly rectifying pH-sensitive leak current $I_{TASK}$ was modeled as a mathematical fit through the currents measured in the current publication:
\[ I_{\text{TASK}} = g_{\text{TASK}} m (V - E_{\text{TASK}}), \]

where \( g_{\text{TASK}} \) is the maximal conductance of the current \( I_{\text{TASK}} \), while the driving force is defined as reversal potential \( E_{\text{TASK}} \) subtracted from the membrane potential \( V \). The activation variable \( m \) is defined as:

\[ m = y_0 + A_1 e^{(x/t_1)} / (V - E_{\text{TASK}}). \]

All values were assumed for maximal conductances and fixed reversal potentials and were systematically varied in some simulations. To demonstrate the contribution of the leak potassium current \( I_{\text{TASK}} \) to the total leak of thalamocortical relay neurons, we calculated a pH-insensitive (\( g_{\text{leak}} \)) and a pH-sensitive leak (\( g_{\text{TASK}} \)) conductance, respectively. The proportion of both leaks was changed systematically in order to achieve a model that simulates the pH effect in rat TC neurons. All computer modeling was carried out at 35°C.

The magnitude of pH-dependent effects on TASK (90% reduction) and HCN (25% reduction) channels used in computer modeling were estimated from previously published data. The reduction of \( I_h \) resulting from an intracellular shift of 0.8 pH units is around 25% (Munsch and Pape 1999). Both, TASK1 and TASK3 channels are inhibited by acidification, although over different pH ranges (pK ~7.5 and ~6.7 for TASK1 and TASK3, respectively) (Duprat et al. 1997; Kim et al. 2000; Rajan et al. 2000). Tandem-linked heterodimeric TASK channel constructs displayed pH sensitivity (pK ~7.3) closer to that of TASK1 than TASK3 (Berg et al. 2004; Czirjak and Enyedi 2002). Therefore TASK1 and TASK3 channels reveal 95% and 75% inhibition at pH 6.4, respectively, and an intermediate value of 90% was used for modeling.
Preparation of dissociated cell cultures from the dorsal thalamus

Dorsal thalami were prepared from embryos (Long-Evans rats) at stage E19 and subsequently transferred into ice-cold Hanks Balanced Salt Solution (HBSS, without Ca/Mg). After triple washing with 5 ml HBSS each, 2.0 ml HBSS, containing 0.5% trypsin, were added to the tissue, followed by incubation at 37°C for 20 minutes. Tissue was washed again 5 times with 5 ml HBSS each and finally transferred into 2 ml tubes with HBSS, containing 0.01% DNAseI. In order to dissociate thalamic tissue, it was 3 times pressed slowly through a 0.9 mm-gauged needle followed by 3 passages through a 0.45 mm-gauged needle. The remaining cell suspension was poured through a Nylon-tissue (mesh aperture 125 µm) into a 50 ml tube and filled up with 18 ml Dulbecco’s modified Eagle Medium (DMEM; Gibco, Eggenstein, Germany). After estimating cell quantity, the suspension was diluted with DMEM in accordance to the required density of 16,000 cells/ml. 500 µl of this suspension were placed on each well of a 24-well plate, containing defatted, baked, and poly-D-lysine coated cover slips. The cell cultures were incubated at 37.0°C and 5% CO₂ up to the appropriate time points and finally fixed with 4% PFA for 10 minutes.

Immunochemistry

After 21 day in vitro (DIV 21) PFA-fixed cells were washed three times with 10 mM PBS and subsequently pre-incubated at 4°C in blocking solution (10 mM PBS, 10% horse normal serum (NHS), 2% bovine serum albumin (BSA), 5% sucrose, 0.3% Triton X100). After 1h, primary antibodies (rabbit anti-HCN2, 1:500, Alomone Labs, Israel; goat anti-TASK3, 1:300, Santa Cruz Biotechnology, Germany; rabbit anti-parvalbumin, 1:500, Swant, Switzerland; mouse anti-MAP2 1:1000, Sigma, Deisenhofen, Germany) were added to the blocking solution and incubated over
night. Thereafter cultures were washed with 10 mM PBS including 0.3% Triton X100 and incubated with secondary antibodies (Cy5-conjugated rabbit-anti-mouse IgG, 1:1000, Sigma; Cy3-conjugated goat-anti-rabbit IgG, 1:1000; Dianova, Hamburg; Germany, Alexa-Fluor-488 conjugated donkey-anti-goat, 1:1000; Molecular Probes; in blocking solution) for 2 h, washed and cover slipped with Moviol. Omission of primary and secondary antibodies resulted in lack fluorescent signals.

Reverse transcription RT-PCR assays

Poly(A) mRNA was prepared from freshly dissected tissue by extraction with Trizol reagent according to the manufacturer’s instructions (Oligotex, Qiagen, Germany). First-strand cDNA was primed with oligo(dT) from 0.5-1 µg of mRNA and synthesized using the SuperScript II enzyme (Invitrogen Life Technologies) at 42°C for 50 min. PCR was performed in 30 µl reaction mixture using 0.75 U Taq polymerase (Qiagen) for HCN templates or 0.75 U HotStarTaq polymerase (Qiagen) for TASK templates amplification; mixture in both cases contained 1.5 mM MgCl₂, 0.2 mM of each dNTP, and 50 pmol of each primer. Cycling protocols were: 3 min at 94°C, 35 cycles: 30 sec at 94°C, 1 min at 58°C, 1 min at 72°C, 7 min at 72°C for HCNs and 15 min at 95°C, 35 cycles: 30 sec at 94°C, 1 min at 58°C, 1 min at 72°C, 10 min at 72°C for TASK amplification. The following primers were used:

**HCN1 (nucleotides 1462-1750) accession No. AF247450**

for: CTC TCT TTG CTA ACG CGG AT

rev: TTG AAA TTG TCC ACC GAA

**HCN2 (nucleotides 1059-1428) accession No. AF247451**

for: GTG GAG CGA ACT CTA TTC GT
rev: GTT CAC AAT CTC CTC ACG CA

*HCN3 (nucleotides 1713-1945) accession No. AF247452*

for: GCA GCA TTT GGT ACA ACA CG
rev: AGC GTC TAG CAG ATC GAG C

*HCN4 (nucleotides 1871-2042) accession No. AF247453*

for: GCA GCG CAT CCA CGA CTA C
rev: CGT CAC AAA GTT GGG GTC TGC

*TASK1 (nucleotides 220-735) accession No. AB048823*

for: CAC CGT CAT CAC CAC AAT CG
rev: TGC TCT GCA TCA CGC TTC TC

*TASK2 (nucleotides 330-959) accession No. AF259395*

for: TGG GCG CCT CTT CTG TGT CTT CTA
rev: TCC CCT CCC CCA CTT GTT TTC ATT

*TASK3 (nucleotides 188-602) accession No. AF192366*

for: ATG AGA TGC GCG AGG AGG AGA AAC
rev: ACG AGG CCC ATG CAA GAA AAG AAG

*TASK5 (nucleotides 137-700) accession No. AF294353*

for: GAG CCT GGG CGA GCG TCT GAA C
rev: CGG GCC CGG AGT CTG TCT GG
TREK1 (nucleotides 447-1119) accession No. NM_172041
for: ACA GAA CTT CAT AGC CCA GCA T
rev: TCC CAC CTC TTC CTT CGT CT

TREK2 (nucleotides 1192-1639) accession No. NM_023096
for: CAG TGG GCT TTG GTG ATT TTG T
rev: AGG CGT AGG TTA TTG GGT CTG TT

TRAAK (nucleotides 766-1143) accession No. NM_053804
for: AAC TGG TTG CGA GCG GTG TC
rev: GGG CTT CTT CGT TGG GTT GG

THIK1 (nucleotides 404-805) accession No. NM_022293
for: CGT GGG CAC AGT GGT AAC TA
rev: GCT CCA CAG GAG ATG GCT AC

THIK2 (nucleotides 764-1013) accession No. NM_022292
for: CCT TCC TCC GGC ACT ACG AG
rev: ATG AAG GCC AGC AGC GAG AT

Multiplex and nested PCR in isolated neurons

mRNAs from 10 identified TC neurons were collected using the Dynabeads mRNA direct micro kit (Dynal, Norway). First cDNA was primed with oligo(dT)$_{25}$, immobilized on the beads, and synthesized using the Sensiscript reverse transcriptase (Qiagen) at 37°C for 1h.
Following reverse transcription, the cDNAs for TASK3 and HCN2 were amplified simultaneously as a multiplex PCR. For the amplification of TASK3 the primers described above were used, and for HCN2 (nucleotides 688-1652) the multiplex primers were as follows: forward, TAC CTG CGT ACG TGG TTC GT, reverse, AAA TAG GAG CCA TCT GAC A. First multiplex amplification was performed in 50 µl containing 50 pmole of each primer, 5 U Platinum Taq polymerase (Invitrogen) by using the following cycling program: 4 min at 95°C, 2 cycles: 30 sec at 94°C, 1 min at 58°C, 5 min at 72°C, then cDNA library was removed and amplification proceeded with additional 35 cycles: 30 sec at 94°C, 1 min at 58°C, 1 min at 72°C, 7 min at 72°C. Nested amplification was carried out individually for each target in 50 µl reaction mix using 5 µl from the first amplification product, 10 pmole of correspondent primers and 2.5 U Platinum Taq polymerase. Cycling protocol was: 4 min at 95°C, 30 cycles: 30 sec at 94°C, 1 min at 58°C, 1 min at 72°C, 7 min at 72°C.

The efficiency of cDNA synthesis was controlled by single PCR amplification of GAPDH form the cDNA libraries using primers mentioned above. PCR amplification was performed in 50 µl containing 10 pmole of each primer, 2.5 U Platinum Taq polymerase, cycling protocol was: 3 min at 95°C, 2 cycles: 30 sec at 94°C, 1 min at 58°C, 5 min at 72°C, then cDNA library was removed and amplification proceeded with another 37 cycles: 30 sec at 94°C, 1 min at 58°C, 1 min at 72°C, 7 min at 72°C.

Quantitative real-time PCR

The hybridization primer/probe assays for real-time PCR detection were purchased from Applied Biosystems. Following assay-on-demand probes were used: GAPDH: P/N 4308313, β2 microglobulin: Rn00560865_m1, TASK1: Rn00583727_m1, HCN1: Rn00584498_m1, HCN3: Rn00586666_m1, HCN4: Rn00572232_m1. The designed
probes were: TASK3 - for: TCC TTC TAC TTC GCT ATC ACT GTC A, rev: TTG CCA
GCA TCG GTT CCA, reporter: CAT GTC CAT ATC CGA TAG TTG; HCN2 - for: ACA
AGG AGA TGA AGC TGT CAG ATG, rev: TGT CAG CCC GCA CAC T, reporter:
CAG ATC TCC CCA AAA TAG. Real time PCR was performed using the ABI Prism
7000 Sequence Detection System (Applied Biosystems, Darmstadt, Germany); PCR
program was: 2 min at 50°C, 10 min at 95°C, 40 cycles: 15 s at 95°C and 1 min at
60°C. Results were analyzed with the ABI Prism 7000 SDS software. The efficiency
of real time primers was assessed by plotting Ct values versus corresponding dilution
factor of total thalamic cDNA. Linear regression revealed slope factors that were
maximally 8% different between β2-microglobulin, TASK1, TASK3, and HCN2.
Results

Expression of HCN and TASK channel isoforms in rat dLGN

In a first experimental step, the expression of different HCN and TASK channel isoforms was determined through RT-PCR analysis on dLGN tissue. Strong signals for TASK1 and TASK3 were found, while TASK2 was less expressed and TASK5 was not detectable (Fig. 1A, left panel). In order to determine the expression of TASK isoforms on a more quantitative level, we subjected TASK1 and TASK3 mRNAs to a real time PCR approach. Expression levels of TASK3 were found to be $4.4 \pm 0.2 (n = 3)$ times higher compared to TASK1 (Fig. 1D, left panel) after normalization to the constitutively expressed house keeping gene $\beta_2$-microglobulin. Similar expression ratios were found for cortical ($3.6 \pm 0.1; n = 3$) and hippocampal ($3.5 \pm 0.1; n = 3$) tissues which were used for comparison (data not shown). When cerebellar tissue, known to express high levels of TASK1 was tested, the TASK3 / TASK1 expression ratio was found to be $0.7 \pm 0.1 (n = 3)$. Expression of HCN channels was assessed in a similar way. Standard PCR protocols revealed the expression of all four HCN isoforms (Fig. 1A, right panel). Real time PCR was used to get more quantitative results of isoform expression. The expression level of HCN2 could be assessed to be $7.5 \pm 0.1$ times higher compared to HCN3 and HCN4, and $12 \pm 0.1 (n = 3)$ times higher compared to HCN1 (Fig. 1D, right panel). Together with earlier results obtained from HCN2 deficient mice (Ludwig et al. 2003), TASK1 deficient mice (Meuth et al. 2006), and Long Evans rats (Meuth et al. 2003) we concluded that HCN2 and TASK3 are the dominant isoforms in dLGN.

The expression of other members of the $K_{2P}$ family of ion channels (Patel and Lazdunski 2004) was probed by RT-PCR on tissue level. The use of specific primer
pairs revealed the presence of TREK-1, TREK-2, TRAAK, THIK-1, and THIK-2 mRNA in dLGN tissue (Fig. 1B).

Next, the cellular localization of HCN2 and TASK3 channels was determined. Neurons were acutely isolated from the dLGN, TC neurons were identified in populations of neurons using established criteria (Pape et al. 1994), and groups of 10 TC neurons were harvested for RT-PCR analysis. The use of HCN2- and TASK3-specific primer pairs revealed detectable PCR signals for the two ion channels (Fig. 1C). Furthermore, TASK3- and HCN2-specific antibodies revealed a dense population of HCN2 (716 ± 17 cells / mm²; Fig. 1G, middle image) and TASK3 607 ± 29 cells / mm²; Fig. 1G, left image) expressing cells in dLGN slices (five independent preparations). Furthermore the overlap of images revealed 604 ± 26 cells / mm² (Fig. 1G, right image) with co-localization of both channels. The average cell density in Nissl-staining was 808 ± 31 cells / mm² (n = 6; coronal sections of 14 μm thickness), indicating that 89 %, 75 %, and 75 % of the cells express HCN2, TASK3, and HCN2/TASK3, respectively, thereby providing evidence for largely overlapping expression. In thalamic cell cultures (DIV 21), 100 % of TC neurons (a total of 30 TC neurons were identified in 10 different cultures from 3 independent preparations) were positive for TASK3 (Fig. 1E, left image) and HCN2 (Fig. 1E, middle image). Their localization in TC neurons (a total of 24 cells were identified in 8 different cultures from 3 independent preparations) was further demonstrated by the finding that parvalbumin (Fig. 1F, lower left image), a specific marker protein of TC neurons (Jones and Hendry 1989; Meuth et al. 2005; Sieg et al. 1998), was expressed in
Characterization of pH-sensitive ramp currents

In the following extracellular pH changes from a physiological value of 7.3 (control) to 6.3, a value that is reached during ischemic insults (Siemkowicz and Hansen 1981) were used to demonstrate the functional interaction of HCN and TASK channels in TC neurons. Since currents through HCN and TASK channels are sensitive to extracellular acidification (Malcolm et al. 2003; Meuth et al. 2003; Stevens et al. 2001), this experimental paradigm results in a concomitant down-regulation of both, $I_{K\text{-leak}}$, and $I_h$. Currents through TASK channels were evoked by holding TC neurons at -30 mV and ramping the potential in 800 ms to -120 mV once every 20s (Fig. 2A, inset). The rate of hyperpolarization $0.11 \text{ mV / ms}$ is sufficiently slow to allow the membrane current to reach steady-state at each potential and it is expected that only constitutively open channels can follow the ramp (Millar et al. 2000; Watkins and Mathie 1996). The current-voltage ($I$-$V$) relationship of the pH-sensitive current was obtained by subtracting currents recorded at pH 6.3 from control currents (i.e., pH 7.3 - pH 6.3). The $I$-$V$ relationship of the pH-sensitive currents (about 10 min after extracellular acidification) was characterized by outward rectification (Fig. 2A, gray trace) and a reversal potential of $-90 \pm 2 \text{ mV}$ ($n = 10$; Fig. 2B, grey circle), i.e. some 14 mV positive to the expected $K^+$ equilibrium potential ($E_K = -104 \text{ mV}$). To demonstrate that pH-dependent regulation of $I_h$ contributed to the deviation ZD7288, a HCN channel blocker, was used. Incubation of TC neurons with 100 µM ZD7288 before extracellular acidification was performed, resulted in an $I$-$V$ relationship of pH-sensitive ramps that revealed the typical features of a current carried by TASK.
channels (Meuth et al. 2003), including pronounced outward rectification (Fig. 2A, black trace) and a significantly (p < 0.0001) more hyperpolarized reversal potential of $-103 \pm 2$ mV ($n = 8$; Fig. 2B black square), i.e. close to the expected $K^+$ equilibrium potential.

To directly prove the modulation of HCN channels by extracellular acidification, we activated $I_h$ from a holding potential of -43 mV by using hyperpolarizing voltage steps of increasing ($\Delta V = -10$ mV) amplitude and decreasing ($\Delta t = -1500$ ms) duration (15.5 s at -53 mV to 3.5 s at -133 mV) followed by a constant step to -98 mV (Fig. 2C, inset). Recordings were performed in the presence of 150 µM Ba$^{2+}$ in order to block TASK and inward rectifier channels (Meuth et al. 2003). An analysis of deactivating currents revealed a half-maximal value of $I_h$ activation at a membrane potential of $-87 \pm 2$ mV ($n = 7$) at pH 7.3 (Fig. 2D, black squares). Ten minutes after switching the extracellular solution from pH 7.3 to pH 6.3, half-maximal activation of $I_h$ was significantly (p = 0.00001) shifted to $-100 \pm 1$ mV ($n = 7$; Fig. 2D gray circles). To ensure that this effect was not due to run-down phenomena, $I_h$ protocols were delivered in 10 min intervals under control conditions in a different set of experiments. No significant (p = 0.153) differences could be found under these recording conditions ($1^{st}$ protocol: $-86 \pm 3$ mV; $2^{nd}$ protocol: $-84 \pm 3$ mV; $n = 4$; data not shown).

In conclusion these findings indicated a contribution of both, HCN and TASK channels to the pH-sensitive current component in dLGN TC neurons and point to an opposing functional influence on membrane excitability.

Effect of extracellular acidification on thalamic activity modes
The functional consequence of concomitant modulation of HCN and TASK channels by acidification was probed under current-clamp conditions. To ensure robust burst responses, recordings were obtained at slightly hyperpolarized values ($V_H = -73 \pm 1$ mV, $n = 12$; Fig. 3A) of the membrane potential with respect to the resting value ($V_R$) of $-71 \pm 1$ mV ($n = 25$; Fig. 3A) using DC injection. Under these conditions, depolarizing current steps elicited high-frequency ($f_H = 134 \pm 10$ Hz, $n = 6$; Fig. 3B) bursts with 2-5 action potentials riding on top of a low threshold Ca$^{2+}$ spike (Fig. 3C). Changing the extracellular pH from 7.3 to 6.3 resulted in a non-significant depolarization of the membrane potential to $V_{pH6.3} = -68 \pm 1$ mV (Fig. 3A), during which burst firing ($f_{pH6.3} = 113 \pm 1$ Hz; Fig. 3B) was preserved (Fig. 3D; $n = 5$).

Different results were obtained in the presence of ZD7288. Application of ZD7288 resulted in a significant ($p < 0.01$) hyperpolarization of the resting membrane potential ($V_{ZD}$) to $-79 \pm 2$ mV ($n = 7$; Fig. 3A). After bringing the membrane potential back to the control level of about -72 mV using DC current injection, a step depolarization revealed that burst firing ($f_{ZD/H} = 136 \pm 11$ Hz; $n = 6$; Fig. 3B) persisted (Fig. 3E). Subsequent extracellular acidification resulted in a strong depolarization of the membrane potential ($V_{ZD/pH6.3}$) to $-52 \pm 3$ mV ($n = 6$; Fig. 3A) accompanied by a change in firing mode from burst to tonic ($f_{ZD/pH6.3} = 32 \pm Hz$, $n = 6$; Fig. 3B, 3F).

These findings show that extracellular acidification results in a net depolarization of TC neurons. The magnitude and in consequence the functional relevance of this depolarization is controlled by an interplay between TASK and HCN channels. The main characteristics of the two thalamic activity modes with high-frequency burst firing at hyperpolarized potentials and low-frequency tonic firing at depolarized potentials were unchanged.
Next the role of the HCN2 isoform was assessed through the use of a mouse strain deficient of HCN2 channels (HCN2⁻/⁻) (Ludwig et al. 2003). The resting membrane potentials of TC neurons in wild type (HCN2⁺/⁺) mice (VR/HCN2⁺/⁺ = -69 ± 1 mV, n = 22; Fig. 4A) were significantly (p < 0.01) more positive compared to HCN2⁻/⁻ mice (VR/HCN2⁻/⁻ = -81 ± 1 mV, n = 21; Fig. 4A), confirming previous findings (Ludwig et al. 2003). A change of extracellular pH from 7.3 to 6.3 in cells held at a potential (VH) of -73 ± 1 mV using DC current injection (n = 8; Fig. 4A, 4C) resulted in a non significant hyperpolarization of the membrane potential (VpH6.3/HCN2⁺/⁺) to -75 ± 1 mV in HCN2⁺/⁺ mice (n = 6; Fig. 4A, 4D). At pH 7.3 and pH 6.3 cells fired high-frequency bursts of action potentials with intra-burst frequencies of fHCN2⁺/⁺ = 114 ± 2 Hz and fPH6.3/HCN2⁺/⁺ = 111 ± 6 Hz, respectively (n = 6; Fig. 4B). In HCN2⁻/⁻ mice held at -73 mV under control conditions the intra-burst frequency was fHCN2⁻/⁻ = 111 ± 2 Hz (n = 5; Fig. 4B, 4E). At pH 6.3 TC neurons were significantly (p < 0.01) more depolarized with VpH6.3/HCN⁻/⁻ = -58 ± 2 mV (n = 5; Fig. 4A, 4F) and revealed tonic firing (fPH6.3/HCN2⁻/⁻ = 33 ± 7 Hz, n = 5; Fig. 4B, 4F). These data indicate that HCN2 channels carry a major part of Ih function in TC neurons.

Steady-state current components in TC neurons

Ion channels suitable to determine VR should be able to sustain a steady-state current. To determine the contribution of different current components, cells were
kept at a holding potential of –68 mV. Under these recording conditions TC neurons displayed a standing outward current (ISO) with an amplitude of 71 ± 4 pA (n = 10). In a first experimental step, a pharmacological profile of this current was obtained by cumulative application of different ion channel modulators. Figure 5 shows the time course of a typical experiment (Fig. 5A, open squares). Wash in of TTX (1 µM) had no effect, application of the I\textsubscript{h} channel blocker ZD 7288 (100 µM) significantly increased, and lowering the extracellular pH from 7.3 to 6.3 significantly decreased ISO. Addition of Ba\textsuperscript{2+} (150 µM) and TEA (20 mM) / 4-AP (6 mM) resulted in a further significant reduction of the outward current.

In order to increase outward current amplitudes, recordings were obtained at more depolarized values of the membrane potential. At –28 mV (Fig. 5A, closed squares), ISO averaged 343 ± 15 pA (n = 51). TTX (1 µM) resulted in an increase in current amplitude of 7 ± 1 % (n = 9), and additional application of ZD7288 (100 µM) decreased the current (6 ± 2 %, n = 9). Changing the external pH from 7.3 to 6.3 in the continuous presence of TTX and ZD7288 induced a further decrease by 42 ± 3 % (n = 5). Addition of Ba\textsuperscript{2+} (150 µM) and TEA (20 mM) / 4-AP (6 mM) lead to a further decrease by 49 ± 6 % and 82 ± 5 % (n = 5), respectively. All drug effects were statistically significant with respect to current amplitudes under control conditions. In the presence of all blocking agents, the current was almost completely blocked (9 ± 5 pA residual current; n = 5). These results are consistent with the hypothesis that Na\textsuperscript{+} channels, I\textsubscript{h} channels, pH-sensitive TASK channels, Ba\textsuperscript{2+}-sensitive Kir and leak channels as well as TEA / 4AP-sensitive voltage-dependent K\textsuperscript{+} channels constitute
ISO in TC neurons with the degree of their contribution depending on the value of the holding potential.

Computer modeling of steady-state current in TC neurons

We modified an existing TC neuron model (Huguenard and McCormick 1992; McCormick and Huguenard 1992) by adding an inward rectifier K⁺ current (Williams et al. 1997) and substituting the linear characteristic of I_{K-leak} by a Goldman-Hodgkin-Katz formalism (Meuth et al. 2005). Furthermore we made 49% of I_{K-leak} (default value = 10 nS) sensitive to changes in pH and designated this component as the outwardly rectifying TASK current (I_{TASK}). This assumption was based on previous and above findings indicating that the current through TASK channels makes up about 38-59% of ISO in rodent TC neurons (Meuth et al. 2006; Meuth et al. 2003; Musset et al. 2006).

Next, the consecutive pharmacological manipulation of ISO was analyzed using the model cell. Current changes at a holding potentials of -68 mV (Fig. 5B) and -28 mV (Fig. 5C) were compared to averaged experimental data (black lines, n = 5). While a 90% reduction of I_{NaP} (TTX effect) resulted in a 16% increase in ISO at -28 mV with no effect at -68 mV, 90% block of I_{h} (ZD7288 effect) had no effect at -28 mV but increased ISO by 22% at -68 mV. Reduction (the remaining current is stated in %) of I_{TASK}, I_{Kir}, I_{K-leak}, and the delayed rectifier K⁺ current (I_{DR}) was used to simulate extracellular acidification (I_{TASK}: 25%), block by Ba²⁺ (I_{TASK}: 0%, I_{Kir}: 10%; I_{K-leak}: 90%), and block by TEA / 4-AP (I_{Kir}: 0%; I_{K-leak}: 70%; I_{DR}: 55%). In addition the transient K⁺ outward current (I_a) was assumed to be completely 4-AP-sensitive. As is shown in Fig. 5B and 5C, the model cell (grey lines) reliably describes the qualitative changes of ISO in rats (black lines) with a mean deviation between modeled and
measured current amplitudes of $12 \pm 2\%$ ($n = 10$; averaged over all experimental conditions at two holding potentials).

Next we used computer modeling techniques to assess the relative contribution of HCN and TASK channels to the pH effects. The resting membrane potential of the model cell was $V_1 = -72$ mV (Fig. 6A). From this potential a step depolarization evoked a low-threshold Ca$^{2+}$ spike and a high-frequency burst ($f_1 = 102$ Hz; Fig. 6B) of action potentials (Fig. 6C). The effect of extracellular acidification was simulated by simultaneously reducing the maximal conductance of $I_h$ and $I_{\text{TASK}}$ by 25% (Munsch and Pape 1999) and 90% (Meuth et al. 2003) of their initial values, respectively. As a result the membrane potential of the model cell depolarized to $V_2 = -68$ mV (Fig. 6A) with burst firing ($f_2 = 112$ Hz; Fig. 6B) being preserved (Fig. 6D). Next, the block of HCN channels by ZD7288 was simulated by removing $I_h$ from the computer model, resulting in membrane hyperpolarization to $V_3 = -82$ mV (Fig. 6A). Using DC current injection the membrane potential of the model cell was reset to $V_{3/\text{DC}} = -72$ mV (Fig. 6A) and a subsequent step depolarization elicited a burst response ($f_{3/\text{DC}} = 105$ Hz; Fig. 6B, 6E). Next, extracellular acidification was simulated by removing 90% of $I_{\text{TASK}}$, thereby leading to a depolarization of the membrane potential to $-58$ mV (Fig. 6A) accompanied by tonic firing ($f_{4/\text{DC}} = 19$ Hz; Fig. 6B, 6F). In an additional set of simulations the up-regulation of $I_h$ (e.g. by cAMP) was modeled by increasing the default value of $I_h$ conductance by 25% (data not shown). As a consequence the resting membrane potential of the model cell was depolarized to $V_1 = -70$ mV. Simulation of extracellular acidification depolarized the membrane potential to $V_2 = -66$ mV thereby shifting the model to an intermediate firing mode (LTS crowned by a single action potential and followed by tonic firing of 3 action potentials).
Taken together these findings indicate an opposing interaction of TASK3 / TASK1 and HCN2 channels to stabilize the resting membrane potential of TC neurons.

As a number of components contribute to $I_{SO}$ we expanded our modeling approach. Different combinations of hyperpolarizing ($I_{Kir}$, $I_{K-leak}$, $I_{TASK}$) and depolarizing ($I_{h}$, $I_{NaP}$) currents were modulated and compared to the effect seen in rats (Fig. 7). The following experimental conditions were analyzed: (1) Control conditions represent $V_R$ of the model cell (-72 mV) and mean $V_R$ of rat TC neurons ($-71 \pm 1$ mV, $n = 25$) recorded in slices. (2) During extracellular acidification (pH 6.4) a 25% and 90% reduction of $I_{h}$ and $I_{TASK}$ was assumed in native cells, respectively. Therefore in the model cell the indicated depolarizing and hyperpolarizing current was reduced by 25% and 90%, respectively. The resulting changes in $V_R$ are shown. (3) The block of $I_{h}$ hyperpolarizes native TC neurons and the model cell. In order to reach the control level of $V_m$ (around -73 mV) a positive DC current of 100 – 200 pA and 150 pA was injected to native TC neurons and the model cell, respectively. Furthermore, the indicated depolarizing current was removed in the model cell. (4) The last experimental condition simulates the effect of reducing the indicated hyperpolarizing current by 90% while the indicated depolarizing current was removed from the computer model and a positive current of +150 pA was injected. As is shown in Fig. 7, only the combined modulation of $I_{TASK} / I_{h}$ and $I_{K-leak} / I_{h}$ closely matched the experimental data from rats. It is interesting to note that the use of a linear $I_{K-leak}$ component was less effective in reproducing whole cell patch-clamp recordings (data not shown).
Taken together, these data show that the joined modulation of $I_{\text{TASK}}$ and $I_h$ is the most likely cellular action that account for the pH effect seen in native TC neurons.
Discussion

The main results of the present paper can be summarized as follows. (1) All known pacemaker channels (HCN1 – 4), TASK1 – 3 channels, and at least five other members of the K_{2p} channel family (TREK1, TREK2, TRAAK, THIK1, THIK2) are expressed in rat dLGN. (2) The dominant isoforms, namely HCN2 and TASK3, are co-expressed in TC neurons. (3) Current components carried by HCN and TASK channels contribute to the pH-sensitive component elicited by hyperpolarizing ramp protocols in TC neurons. (4) Extracellular acidification leads to depolarization of TC neurons the magnitude of which critically depends on the availability of HCN and TASK channels. Current clamp recordings in rats, HCN2^{-/-} mice, and computer modeling studies demonstrate that the counterbalancing effects of HCN2 and TASK3 / TASK1 channels play an important role in setting the resting membrane potential of TC neurons. (5) While modulation of TASK and / or HCN channels can effectively shift TC neurons between firing modes, the overall characteristics of the two forms of activity are rather unchanged.

\textit{pH-sensitive membrane currents in TC neurons}

TASK1 and TASK3 channels which are sensitive to changes in extracellular pH (Duprat et al. 1997; Kim et al. 2000; Rajan et al. 2000) are expressed in TC neurons (Meuth et al. 2003). Thus pH-sensitive currents evoked by hyperpolarizing voltage ramps revealed typical features of a current carried by TASK channels. However, the reversal potential deviated from that of a pure K^{+} current. Even more surprising, in contrast to the inhibition of TASK channels by bupivacaine and muscarine (Meuth et al. 2003), the closure of TASK channels by external H^{+} ions did not result in a strong depolarization of the membrane potential. Only after blocking I_{h}, pH-sensitive ramp
currents reversed at the expected $K^+$ reversal potential and extracellular acidification induced a strong depolarization, thereby proving the contribution of both, HCN and TASK channels, to the pH-sensitive component.

The counterbalancing modulation of TASK and HCN channels restricts the net effect of acidification on the resting membrane potential. The experimental paradigm used to demonstrate this interaction included the use of DC current injection to achieve similar control values of the membrane potential (~-72 mV). This was done to exemplify the different effects of acidification with (no shift in activity mode) and without $I_h$ (shift in activity mode), rather then to mimic a sequence of events in the brain. The scenario of interacting TASK and HCN channels is strengthened by the fact that $V_R$ of TASK1 deficient mice are significantly more depolarized compared to wild type animals under control conditions and in the presence of ZD7288 (Meuth et al. 2006).

Based on results obtained from rat (Meuth et al. 2003; Musset et al. 2006), TASK1-KO mice (Meuth et al. 2006), and computer modeling (this study) it can be concluded that the pH-sensitive component (acidification to pH 6.3) makes up about 40% of $I_{SO}$ (at -28 mV). However at extracellular pH values exceeding the modulation range of TASK channels (i.e, pH < 6.0) $I_{SO}$ is further reduced indicating the presence of additional pH-sensitive components. To assess whether other ion currents may mediate the pH effect seen in the present study, we used computer modeling. None of the combinations including $I_{Kir}$ or $I_{NaP}$ were able to account for the result obtained from TC neurons. Nevertheless the contribution of more components can not be fully excluded since changes in extracellular pH can modulate the activity of a variety of ion channels and receptors (for review see: Kaila and Ransom 1998). Furthermore, subtle differences between cellular properties may account for differences in the pH effect under control conditions seen in rat (depolarization) and mice
(hyperpolarization). This view is corroborated for example by the finding that TASK3 is clearly the dominant subtype in rat but TASK1 and TASK3 reveal roughly equal mRNA levels in mouse dLGN (Meuth et al. 2006). Similar considerations may apply to HCN channels which show functional expression of HCN1 in rat (Budde et al. 2005) but not in mouse TC neurons (Franz et al. 2000).

Constituents of the resting membrane potential in TC neurons

The resting membrane potentials of TC neurons in different species and thalamic nuclei are reportedly in the range of -60 to -75 mV (McCormick and Pape 1990; Meuth et al. 2003; Porcello et al. 2003; Williams et al. 1997; Zhan et al. 1999), the evolution of which is ascribed to leak currents (Ileak, INa-leak), pacemaker currents (Ih), inwardly rectifying K+ currents (IKIR), voltage-dependent currents active below threshold (IA, IT), and any DC current experimentally injected to the cell (Iinj) (Williams et al. 1997; Zhan et al. 1999). In agreement with this assumption the standing outward current of TC neurons (Meuth et al. 2003) is composed of Ih, IKir, ITASK, a persistent Na+ current, and voltage-dependent K+ currents. The results of the present study allow the assignment of most of the Ih component to HCN2 channels.

The evidence to support the hypothesis that Ih is active at the resting membrane potential of TC neurons under the present recording conditions (-71 mV) is as follows. Ih is a slow inward current activating at potentials negative to -55 mV (see Fig. 2D), shows no inactivation (McCormick and Pape 1990), and has a calculated reversal potential of about -35 mV (Budde et al. 1997). According to the approximation of a Boltzmann distribution to the data points a fraction of 18% of Ih is activated at -71 mV (see Fig. 2D) carrying an inward current of -34 ± 2 pA (n = 28). Block of Ih shifted the resting membrane potential of TC neurons by -8 mV, a value
being in close agreement with previously reported hyperpolarization (-5 to -9 mV) in a number of different neuronal cell types (Day et al. 2005; Doan and Kunze 1999; Lupica et al. 2001; Maccaferri and McBain 1996).

Based on our results on rodent TC neurons (Meuth et al. 2006; Meuth et al. 2003; Musset et al. 2006) and computer modeling (this study) it can be assumed that the classical K⁺ leak current is roughly equally composed of pH-sensitive current through TASK3 / TASK1 channels (I_{TASK}) and other pH-insensitive leak channels (I_{K-leak}). While quantitative PCR experiments, subtype-specific modulation, and gene knockout point to a domination of TASK3 over TASK1, the I_{K-leak} component may be carried by current through other members of the K₂P family. This conclusion is corroborated by the following findings: (1) I_{K-leak} revealing Goldman-Hodgkin-Katz (GHK) rectification (Goldstein et al. 2001) is necessary to give closely matching modeling results. (2) THIK, TRAAK, and TREK channels are expressed in dLGN, although a consignment to defined cell types is still missing. The functional expression of TREK and TRAAK (Patel and Lazdunski 2004) channels in TC neurons is in agreement with the presence of a leak current inhibited by cAMP and Ba²⁺ (Budde et al. 1997; Budde et al. 2005) and an I_{SO} component enhanced by arachidonic acid (Meuth et al. 2006). (3) Modeling of the pH effect gives very similar results when I_{TASK} as well as I_{K-leak} is assumed to be pH-sensitive.

It is noticeable that HCN2^-/- mice show no plastic compensation for the loss of HCN2 channels. It has been noted before that for example cerebellar granule cells show a greater degree of plasticity in comparison to TC neurons in response to TASK-1 deletion (Meuth et al. 2006). The reason for this difference is unknown.

Comparability between data obtained in vitro and in silico
The quantitative aspects of the complex relationship between current amplitudes in vitro, current amplitudes in the computer model, and their effect on the resting membrane potential depend on pharmacological tools, the large parameter space of the computer model, and experimental variations. Therefore it can not be expected to achieve full match between experimental recordings and computer simulations. Still the following considerations reveal a reasonable degree of similarity between experiments and modeling. (i) Injection of small depolarizing and hyperpolarizing current pulses (from resting membrane potential) to the model cell under current clamp conditions resulted in 1-3 mV voltage deflections and allowed the calculation of the input resistance under control conditions (43 M\(\Omega\)) and after the block of \(I_h\) (90 M\(\Omega\)). Multiplying the difference in input resistance (47 M\(\Omega\)) by the amplitude of \(I_h\) at the resting membrane potential of the model cell under control conditions (-130 pA) results in a voltage deflection of -6 mV. In whole-cell recordings which typically reveal higher membrane resistances (some hundred M\(\Omega\)) smaller currents (some tens of pA) are able to induce similar voltage shifts. (ii) Absolute current values of \(I_h\) and \(I_{\text{TASK}}\) (amplitude of the pH-sensitive current in ZD7288) at -71 mV reveal very similar proportions in vitro (\(I_h = 34 \pm 2\) pA, \(n = 28\); \(I_{\text{TASK}} = 31 \pm 2\) pA, \(n = 25\)) and in the computer model (\(I_h = 131\) pA; \(I_{\text{TASK}} = 137\) pA). Thus voltage changes induced by blocking / knocking out \(I_h\) in rats, mice and the computer model (\(V_{\text{ZD}} - V_R = -8\) mV; \(V_{R/\text{HCN2}+/+} - V_{R/\text{HCN2}±/±} = -12\) mV; \(V_3 - V_1 = -10\) mV; median = -10 mV) are in a range that has been described in several neuronal cell types (see above) and are comparable to the effects induced by blocking \(I_{\text{TASK}}\) (\(V_R - V_{\text{ZDpH6.3}} = 19\) mV; \(V_{R/\text{HCN2}+/+} - V_{\text{pH6.3/HCN}±/±} = 11\) mV; \(V_1 - V_{4/\text{DC}} = 13\) mV; median = 14 mV). (iii) The relationship between amplitudes measured in vitro (recording temperature ~21°C) and the computer model (simulation temperature 35°C) is given by the \(Q_{10}\) value. Many enzyme reactions have a \(Q_{10}\) near 3, as does the gating of many ion channels,
including \( I_h \) (Hille 2001). Although values for absolute conductance of \( I_h \) seem to be smaller than 3 (Pena et al. 2006), the \( Q_{10} \) for \( I_h \) current amplitude in intracardiac neurons could be determined as 2.2 (Cuevas et al. 1997). Thus the amplitude of \( I_h \) at the resting membrane potential at 35°C can be expected to be \(-34 \text{ pA} \times 3.1 \) (\( Q_{\Delta T} \) for a temperature difference of 14°C) = \(-105 \text{ pA} \) which is close to the \(-130 \text{ pA} \) generated by the computer model. The temperature dependency of TASK channels is less clear since some \( K_{2p} \) channels reveal a sevenfold increase in current amplitude for a 10°C increment in temperature (Maingret et al. 2000).

Functional implications

The dorsal thalamus has a key role in regulating the flow of sensory information from the periphery to the primary sensory cortical areas and participates in the generation of thalamocortical oscillations associated with different states of consciousness and the status of absence epilepsy (Steriade et al. 1997). TC neurons are depolarized by neurotransmitters of the ascending brainstem system, including noradrenaline, serotonin, and acetylcholine (McCormick 1992). While noradrenaline exerts this effect by the convergent modulation of \( I_{K\text{-leak}} \) (i.e. closure) and \( I_h \) (i.e. depolarizing shift in activation), acetylcholine depolarizes TC neurons by closing TASK and \( I_{KIR} \) channels (Meuth et al. 2003). This depolarization is responsible for the transition of sleep-related rhythmic burst activity to tonic activity associated with periods of wakefulness and REM sleep.

The view that the counterbalancing actions of TASK and HCN on the resting membrane potential is a more general motive in the CNS is in agreement with the broad expression of HCN (Monteggia et al. 2000) and TASK (Talley et al. 2001) channels in the brain, the reciprocal modulation of \( I_h \) and \( I_{\text{TASK}} \) by serotonin and
halothane on hypoglossal motoneurons (Sirois et al. 2002), and the analysis of
dendritic excitability in mouse frontal cortex pyramidal cells (Day et al. 2005).

Pathophysiological implications

Neuronal activity leads to transient extracellular alkalinization followed by a persistent
extracellular acidification (Chesler and Kaila 1992). In dLGN synchronous afferent
activation, tonic activity, and rhythmic burst discharges induce extracellular and
intracellular increases in H⁺ concentrations (Meyer et al. 2000; Tong and Chesler
1999). The data presented here suggest that pH shifts induced by different forms of
activity in dLGN should have rather small effects on the overall firing pattern and
resting membrane potential. This is of special interest for periods of generalized
absence epilepsy where the highly synchronous burst pattern of large populations of
TC neurons is not expected to be altered by pH changes due to rhythmic activity.

Periods of brain ischemia are characterized by a decrease in extracellular pH to
values as low as 6.0 (Siemkowicz and Hansen 1981; Simon et al. 1985). CNS
neurons reveal extremely different sensitivity to ischemic insults (Centonze et al.
2001). The reason for this differential vulnerability is still largely unknown. Vulnerable
neurons respond to ischemia with prolonged and strong membrane depolarization
and subsequent cellular damage. Due to the joined modulation of TASK and HCN
described here, it can be assumed that TC neurons show little depolarization in
response to acidification during ischemic insults and thus a selective non-
vulnerability. It seems however that other influences dominate the reaction of TC
neurons to acute hypoxia (Erdemli and Crunelli 2000, 1998; Steinke et al. 1992;
Szelies et al. 1991). Under these conditions there is an enhanced release of
monoamines and nitric oxide, substances known to strongly activate Iₘ, in the
thalamus. Therefore acute hypoxia leads to membrane depolarization and altered
electrical properties of TC neurons, and makes the dLGN a part of a system-
preferential, topographically organized brain injury following ischemia (Erdemli and
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Figure legends

**Fig. 1**: RT-PCR and immunological analysis of rat thalamic tissue. (A) Expression of TASK1-5 and HCN1-4 channels in dLGN revealed by standard RT-PCR. (B) Expression of K_{2P} channels in dLGN revealed by standard RT-PCR. (C) Co-expression of HCN2 and TASK3 in identified TC neurons revealed by standard RT-PCR. (D) Quantitative real time RT-PCR analysis of TASK1 and TASK3 and HCN1-4 expression in comparison to β_{2}-microglobulin (β_{2}-MG) in dLGN tissue. The number of cycles is plotted versus normalized and baseline corrected fluorescence (ΔRn). (E, F) Immunohistochemical localization of TASK3 (green fluorescence) and HCN2 (red fluorescence) in cell culture (E) and brain slices (F). The overlay (right panel) reveals co-expression of both ion channel proteins. (G) Immunohistochemical localization of microtubule associated protein 2 (MAP2; blue fluorescence), a neuron-specific marker, parvalbumin (green fluorescence), a TC neuron-specific marker, and TASK3 (red fluorescence) in thalamic neurons in cell culture. The overlay (right panel) reveals the co-expression of all three proteins.

**Fig. 2**: Current components sensitive to extracellular acidification in TC neurons. (A) Current to voltage relationship (I/V) of the pH-sensitive current obtained by graphical subtraction (pH 7.3 – pH 6.3) in presence (black trace) and absence (gray trace) of ZD7288 (100 µM). Currents were evoked by ramping the membrane from −30 mV to −120 mV over 800 ms (see inset). (B) Mean values of the reversal potential of pH-sensitive ramp current in the presence (black square) and absence (open circle) of ZD7288. (C) Superimposed families of current traces recorded at pH 7.3 (black traces) and 6.3 (gray traces) in a rat TC neuron. Current traces at -53, -73, -93, -113
and -133 mV are shown. The inset clarifies the voltage protocol. (D) Mean steady-state activation curves of $I_h$ at pH 7.3 (black squares; n = 7) and pH 6.3 (gray circles; n = 7). Continuous lines represent best fits of a Boltzmann equation to the data points.

**Fig. 3**: Effect of extracellular acidification on thalamic activity modes in rat TC neurons recorded under current clamp conditions. (A) Mean values of resting membrane potentials under different recording conditions. $V_R$ = resting potential under control conditions; $V_{ZD}$ = resting potential in the presence of 100 µM ZD7288; $V_H$ = holding level of the membrane potential achieved by DC current injection; $V_{pH6.3}$ = resting potential at pH 6.3; $V_{ZD/pH6.3}$ = resting potential at pH 6.3 in the presence of ZD7288. (B) Mean firing frequencies under different recording conditions. The firing frequency was determined for the first two action potentials elicited by the depolarizing pulse. $f_H$ = firing frequency at $V_H$; $f_{ZD/H}$ = firing frequency at $V_H$ in the presence of ZD7288; $f_{pH6.3}$ = firing frequency at pH 6.3; $f_{ZD/pH6.3}$ = firing frequency at pH 6.3 in the presence of ZD7288. (C-F) Depolarizing current pulses (300 ms duration, 100 – 200 pA) from a control potential ($V_H$) of about –73 mV elicited robust burst responses in the absence (C) and presence (E) of ZD7288. Extracellular acidification results in a depolarizing shift of the membrane potential and generation of tonic trains of action potentials in response to the same depolarizing current pulse in the presence (F), but not in the absence (D) of ZD7288.

**Fig. 4**: Effect of extracellular acidification on thalamic activity modes in mice. (A) Mean values of resting membrane potentials under different recording conditions. $V_{R/HCN2+/+}$ = resting potential under control conditions in HCN2$^{+/+}$ mice; $V_{R/HCN2/-}$ = resting potential under control conditions in HCN2$^{-/-}$ mice; $V_H$ = holding level of the
membrane potential achieved by DC current injection; $V_{pH6.3/HCN2^{+/+}}$ = resting potential at pH 6.3 in HCN2$^{+/+}$ mice; $V_{pH6.3/HCN2^{-/-}}$ = resting potential at pH 6.3 in HCN2$^{-/-}$ mice. 

(B) Mean firing frequencies under different recording conditions. The firing frequency was determined for the first two action potentials elicited by the depolarizing pulse. $f_{HCN2^{+/+}}$ = firing frequency at $V_H$ in HCN2$^{+/+}$ mice; $f_{HCN2^{-/-}}$ = firing frequency at $V_H$ in HCN2$^{-/-}$ mice; $f_{pH6.3/HCN2^{+/+}}$ = firing frequency at pH 6.3 in HCN2$^{+/+}$ mice; $f_{pH6.3/HCN2^{-/-}}$ = firing frequency at pH 6.3 in HCN2$^{-/-}$ mice. 

(C, D) Depolarizing current pulses (400 ms duration, 100 – 200 pA) were applied from a holding potential of about –73 mV. In TC neurons from HCN2$^{+/+}$ mice pulse depolarization elicited a burst response at pH 7.3 (C) and pH 6.3 (D). (E, F) In TC neurons from HCN2$^{-/-}$ mice pulse depolarization elicited a burst response at pH 7.3 (E). Extracellular acidification resulted in a depolarizing shift of the membrane potential and generation of tonic trains of action potentials in response to the same depolarizing current pulse (F).

**Fig. 5**: Pharmacological profile of the standing outward current in thalamocortical relay neurons at different holding potentials. (A) Amplitude of the net outward current plotted against time in a relay neuron recorded under voltage-clamp conditions at –28 mV (black data points) and –68 mV (open data points) under control conditions (1), and during cumulative application of TTX (1 µM; 2), ZD 7288 (100 µM; 3), H$^+$ (pH 7.2 to pH 6.4; 4), Ba$^{2+}$ (150 µM; 5), and TEA / 4-AP (20 mM / 6 mM; 6), as indicated by horizontal lines. The holding current was recorded every 20 s for a duration of 800 ms. (B, C) Comparison of averaged data from recordings in five neurons (black traces) and computer modeling (grey traces) at –68 mV (B) and –28 mV (C). Normalized $I_{SO}$ amplitudes are plotted vs. time. Recording conditions as in A. For reduction of currents in the model cell see text.
Fig. 6: Computer simulation of activity modes in rat TC neurons. (A, B) Resting membrane potentials and firing frequencies of the model cell under different conditions: Control conditions ($V_1$, $f_1$); with $I_h$ (the current through HCN channels) and $I_{TASK}$ (the current through TASK channels) reduced by 25% and 90%, respectively ($V_2$, $f_2$); without $I_h$ ($V_3$); without $I_h$ and the membrane potential reset to the control level ($V_{3/DC}$, $f_{3/DC}$); without $I_h$, DC current injection, and 90% reduction of $I_{TASK}$ ($V_{4/DC}$, $f_{4/DC}$). (C) Depolarizing current pulses (300 ms duration, 150 pA) were applied from a potential of −72 mV. With $I_h$ and $I_{TASK}$ set to 100%, the membrane resting potential settled at -72 mV. A step depolarization resulted in a burst response. (D) Setting $I_h$ and $I_{TASK}$ to 75% and 10%, respectively, resulted in a membrane depolarization to -68 mV and a burst of action potentials following a depolarizing current step. (E) With $I_h$ and $I_{TASK}$ set to 0% and 100%, respectively, a DC current injection of 150 pA was necessary to set the resting potential to a value of -72 mV. A step depolarization resulted in a burst of action potentials. (F) Setting $I_h$ and $I_{TASK}$ to 0% and 10%, respectively, resulted in a membrane depolarization to -58 mV and a tonic train of action potentials following a depolarizing current step.

Fig. 7: Modeling the effect of hyperpolarizing ($I_{Kir}$, $I_{K-leak}$, $I_{TASK}$) and depolarizing ($I_h$, $I_{NaP}$) membrane currents on $V_{rest}$. Different pairs of currents (as indicated) were altered in a computer model to reproduce the results found in native rat TC neurons. The experimental conditions were as follows: 1 – control condition: $V_{rest}$ of the model cell and mean $V_R$ of rat TC neurons. 2 – pH 6.4: In native TC neurons a 25% and 90% reduction of $I_h$ and $I_{TASK}$ was assumed, respectively. Therefore in the model cell the indicated depolarizing and hyperpolarizing current was reduced by 25% and
90%, respectively. 3 – Block of $I_h$ and injection of +150 pA DC current in native TC neurons. In the model cell the indicated depolarizing current was eliminated and +150 pA DC current was injected. 4 – pH 6.4 and block of $I_h$ during DC current injection. In the model cell the indicated depolarizing current was eliminated, the indicated hyperpolarizing current was reduced by 90%, and +150 pA DC current was injected. Note that only the combination $I_{TASK} / I_h$ and $I_{K-leak} / I_h$ closely matches the native situation (rat).
Fig. 6

A. Graph showing membrane potential ($V_M$) vs. frequency (Hz).

B. Scatter plot of frequency vs. voltage.

C. Diagram illustrating signal changes with parameters $I_h = 100\%$ and $I_{TASK} = 100\%$.

D. Diagram illustrating signal changes with parameters $I_h = 75\%$ and $I_{TASK} = 10\%$.

E. Diagram illustrating signal changes with parameters $I_h = 0\%$ and $I_{TASK} = 100\%$.

F. Diagram illustrating signal changes with parameters $I_h = 0\%$ and $I_{TASK} = 10\%$. 

Notations:
- $V_1$, $V_2$, $V_3$, $V_{3/DC}$, $V_{4DC}$
- $f_1$, $f_2$, $f_3$, $f_{3/DC}$, $f_{4/DC}$
- 20 mV, 200 ms scale