

Supplemental Material

Supplemental figure legends

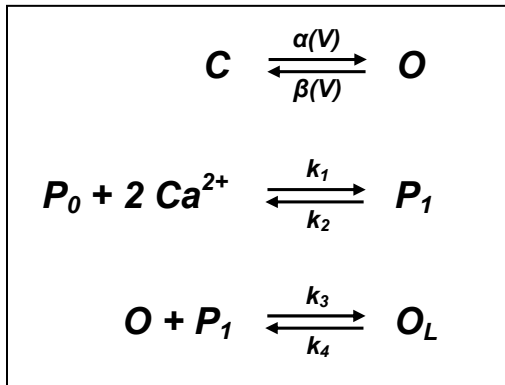
Fig. S1: Voltage-dependent properties of the simulated I_h .

Originally the mathematical description of I_h was derived from data obtained by voltage-clamp recordings with sharp microelectrodes of guinea pig dorsal lateral geniculate relay neurons at 36°C (Huguenard and McCormick, 1992, J. Neurophysiol., 68: 1373-1383).

(A) The activation curve of the modeled I_h had a V_h of -75.0 mV and a slope factor of 5.5 mV⁻¹. I_h does not inactivate, even with prolonged hyperpolarization. (B) The time constants of activation and deactivation of I_h followed single exponential functions and had a bell-shaped dependency on the membrane potential according to

$$\tau_m = 1 / [\exp(-14.59 - 0.086 * V_M) + \exp(-1.87 + 0.0701 * V_M)].$$

The I_h model assumed that the voltage dependency and conductance is influenced by Ca^{2+} indirectly through the binding of Ca^{2+} to a regulating factor (P), which itself binds to the open form of the channel and blocks its transition to the closed form. The full kinetic scheme (Destexhe et al., 1996, J. Neurophysiol., 76: 2049-2070) was



where the first reaction represents the voltage-dependent transitions of HCN channels between closed (C) and open (O) forms, with α and β as transition rates. The second reaction represents the binding of intracellular Ca^{2+} to a regulating factor (P_0 for unbound

and P_1 for bound) with four binding sites for Ca^{2+} and rates of $k_1 = 2.5 * 10^7 \text{ mM}^{-4} \text{ ms}^{-1}$ and $k_2 = 4 * 10^{-4} \text{ ms}^{-1}$. The Ca^{2+} -bound form P_1 associates with the open form of the channel, leading to a *locked* open form O_L , with rates of $k_3 = 0.1 \text{ ms}^{-1}$ and $k_4 = 0.001 \text{ ms}^{-1}$. The current is then proportional to the relative concentration of open channels.

It is reasonable to assume, that the increased availability and faster kinetics of I_h in the computer model in comparison to the measured data presented here leads to higher frequencies of the model network oscillations.

Fig. S2: Contribution of I_h to the low-threshold Ca^{2+} spike (LTS).

All traces were set to an initial potential of -60 mV by DC current injection. Membrane hyperpolarization under current clamp conditions (see current pulse above the traces) induces I_h -dependent anomalous rectification (marked by the asterisk) when I_h is fully present (black voltage trace). After termination of the hyperpolarizing current injection, the membrane potential repolarizes and a LTS is generated (marked by the arrow). With I_h reduced to 20% of the control current anomalous rectification is absent, however a LTS is still generated (red voltage trace). Removal of the T-type Ca^{2+} current (I_T) and the action potential generating Hodgkin-Huxley mechanism (HH) from the model with I_h reduced to 20%, results in a nearly passive behavior of the cell membrane (green voltage trace). Increasing I_h under these conditions back to control levels reinstates anomalous rectification (about 14 mV) and the depolarizing afterpotential (about 6 mV; blue voltage trace). These findings indicate that I_h has only little influence on amplitude and duration of the LTS.

Fig. S3: Dependency of I_h activation curves on HCN isoform composition.

To probe whether the developmentally-regulated HCN expression profile was congruent to the only slightly changed activation curves, we analyzed the steady-state voltage-

dependent I_h properties using a mathematical model. Based on literature given activation curves for the major I_h subcomponents in TC neurons, namely HCN1 (Chen et al., 2001, J. Gen. Physiol., 117: 491-504), HCN2 (Ulens & Tytgat, 2001, J. Biol. Chem., 276: 6069-6072) and HCN4 (Seifert et al., 1999, PNAS, 96: 9391-9396) on the one hand and the corresponding protein levels determined in the present study at three different developmental stages (P7, P30 and P90) on the other hand, a weighted and normalized sum of the activation curves was calculated according to the following equation:

$$result_j = \frac{\sum_i hcn_act_i * hcn_lvl_{ij}}{\sum_i hcn_lvl_{ij}} \quad \begin{matrix} i = \{HCN1, HCN2, HCN4\}, \\ j = \{P7, P30, P90\}, \end{matrix}$$

hcn_act: activation characteristic,
hcn_lvl: protein level.

(A) The model included the activation curves of cloned HCN channels recorded in expression systems taken from literature (HCN1, open squares; HCN2, open circles; HCN4, closed triangles). **(B-D)** Assuming that each HCN isoform contributes to the compound I_h according to its protein expression level, activation curves were constructed by using different fractions of HCN1, HCN2, and HCN4 for different stages of postnatal development. Pie charts depict the applied weighting. Calculated (closed circles) vs. a global control (mean over all control activation curves of the present study) under control conditions (open circles) for P7 **(B)**, P30 **(C)**, and P90 **(D)** demonstrated good agreement at P7 ($\Delta V_h = 4.7$ mV; Fig. 5B), P30 ($\Delta V_h = 0.5$ mV; Fig. 5C), and P90 ($\Delta V_h = 0.3$ mV; Fig. 5D). It should be noted that this approach involves a number of uncertainties (comparison of results from expression system and native cells; considerable differences between activation curves of one and the same cloned isoform in different studies; formation of heteromers with new properties). Still the experimental data suggest that the relative stability of I_h activation curves throughout development at

least partially results from a coordinated developmental expression of the three HCN channel isoforms.

Supplemental table legend

Tab. S1: Passive and active parameters of the TC neuron and RE neuron model cells.

In order to have an autonomous oscillator and a cell with stable membrane resting potential, respectively, TC cell parameters were adjusted (highlighted in bold) in variation to the non-pacemaking TC neuron template (Desthèxe et al., 1996, J. Neurophysiol., 76: 2049-2070). TC = thalamocortical relay neuron; RE = reticular thalamic neuron.