Ionic Mechanisms for Intrinsic Slow Oscillations in Thalamic Relay Neurons

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ABSTRACT  The oscillatory properties of single thalamocortical neurons were investigated by using a Hodgkin-Huxley-like model that included Ca\(^{2+}\) diffusion, the low-threshold Ca\(^{2+}\) current (I\(_{\text{L}}\)) and the hyperpolarization-activated inward current (I\(_{\text{h}}\)). I\(_{\text{L}}\) was modeled by double activation kinetics regulated by intracellular Ca\(^{2+}\). The model exhibited waxing and waning oscillations consisting of 1–25-s bursts of slow oscillations (3.5–4 Hz) separated by long silent periods (4–20 s). During the oscillatory phase, the entry of Ca\(^{2+}\) progressively shifted the activation function of I\(_{\text{L}}\) terminating the oscillations. A similar type of waxing and...
based on voltage-clamp data of $I_T$ and $I_h$. Special emphasis is given to uncovering the role of $I_h$ in organizing the transitions between multiple oscillatory and resting states of the TC cell.

**MATERIALS AND METHODS**

Our single compartment model of a TC cell used a Hodgkin-Huxley-type scheme (Hodgkin and Huxley, 1952) for the ionic currents. The equation describing the derivative of the membrane potential $V$ was:

$$C_m \frac{dV}{dt} = -g_L(V - E_L) - I_1 - I_h - I_{K_2} + I_{ext},$$  \hspace{1cm} (1)

where $C_m = 1 \mu F/cm^2$ is the specific capacity of the membrane, $g_L = 0.05$ mS/cm$^2$, and $E_L = -86$ mV are, respectively, the leakage conductance and the leakage reversal potential. The value of $g_L$ was chosen to obtain a membrane time constant of 20 ms, and $E_L$ was adjusted to match the resting membrane potential to $-60$ mV (Jahr and Rinzel 1984a) when $I_T$

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<td></td>
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<td>$\tau_a(V) = 0.15m_a(V)(1.7 + \exp[-(V + 30.8)/13.5])$</td>
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<td>$K(V) = \sqrt{0.25 + \exp[(V + 39.4)/30]} - 0.5$</td>
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<td>$I_h$</td>
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<td>$\alpha_2(V) = 1/(\tau_2(V)K(V) + 1)$</td>
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<td>$I_{K_2}$</td>
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posed by McCormick (1992), is the regulation of $I_h$ by binding of intracellular $\text{Ca}^{2+}$, as found in whole cell voltage-clamp studies of $I_h$ in sino-atrial node cells (Hagiwara and Iriyama, 1989). Evidence for the control of the voltage-dependent properties of $I_h$ by intracellular $\text{Ca}^{2+}$ were also obtained in cat neocortical neurons (Schwindt et al., 1992). As the $\text{Ca}^{2+}$ dependence of $I_h$ has not yet been studied in TC cells, it was assumed to be similar to that of sino-atrial node cells.

The activation curve of $I_h$ in sino-atrial node cells shifts toward more positive potentials as the intracellular $\text{Ca}^{2+}$ concentration ($[\text{Ca}]_i$) is increased (Hagiwara and Iriyama, 1989). Calmodulin and protein kinase C were not involved in the $\text{Ca}^{2+}$ modulation of $I_h$, suggesting that $\text{Ca}^{2+}$ ions directly affected $I_h$ channels (Hagiwara and Iriyama, 1989). There is also an increase in the conductance of $I_h$ following the binding of $\text{Ca}^{2+}$. We have developed a kinetic model for intracellular calcium ($\text{Ca}^{2+}$) binding to the open channels of $I_h$ that is consistent with these data. The open state gates $S_{\text{open}}$ and $F_{\text{open}}$ were assumed to have $n$ binding sites for $\text{Ca}^{2+}$ which, when occupied, lead to the open forms $S_{\text{bound}}$ and $F_{\text{bound}}$ according to:

$$S_{\text{open}} + n \text{Ca}^{2+} \rightleftharpoons S_{\text{bound}}$$

$$F_{\text{open}} + n \text{Ca}^{2+} \rightleftharpoons F_{\text{bound}}$$

Efflux due to $\text{Ca}^{2+}$ ATPase pump activity and diffusion to neighboring shells. Only the $\text{Ca}^{2+}$ pump was modeled here. We adopted the following kinetic scheme:

$$\text{Ca}^{2+} + P \rightleftharpoons \text{CaP} \rightarrow P + \text{Ca}^{2+}.$$  \hspace{1cm} (8)

where $P$ represents the $\text{Ca}^{2+}$ pump, $\text{CaP}$ is an intermediate state, $\text{Ca}^{2+}$ is the extracellular $\text{Ca}^{2+}$ concentration, and $e_1$, $e_2$, and $e_3$ are rate constants. $\text{Ca}^{2+}$ ions have a high affinity for the pump $P$, whereas extrusion of $\text{Ca}^{2+}$ follows a slower process (Blaustein, 1988). Therefore, $e_3$ is low compared to $e_1$ and $e_2$, and the Michaelis-Menten approximation can be used for describing the kinetics of the pump. According to such a scheme, the kinetic equation for the $\text{Ca}^{2+}$ pump is:

$$[\text{Ca}] = - \frac{K_p [\text{Ca}]}{[\text{Ca}] + K_p}.$$  \hspace{1cm} (9)

where $K_p = 10^{-4} \text{mM } \text{ms}^{-1}$ is the product of $c_3$ with the total concentration of $P$, and $K_p = c_2/c_1 = 10^{-4} \text{mM}$ is the dissociation constant, which can be interpreted here as the value of $[\text{Ca}]$, at which the pump is half activated (if $[\text{Ca}] \ll K_p$ then the efflux is negligible).

The parameters of the pump were adjusted in order to have a half-activation.

\begin{equation}
\end{equation}
Estimation of the values of parameters

Conductances values and reversal potentials for the above currents were estimated from published values provided by measurements in vitro. However, these data only provide approximate values for these parameters. Also, the complex dendritic geometry of the cell was not taken into account, which would affect these values. For each of the currents considered here, the value of the maximal conductance and the reversal potential are interrelated. For example, if $E_h$ is increased, $g_h$ must be decreased to reproduce similar results. We tested a broad range of maximal conductances and similar results were obtained.

Methods for solving the equations

Estimation of the behavior of the model...
The shift of the $I_h$ activation logarithmic in [Ca]i and a shift of 13 mV is obtained for $C = 6.4$.

The shift should be negligible ($C < 1$) at the resting level, $[Ca]_i \sim 2 \times 10^{-4}$ mM, which gives a lower bound: $Ca_{crit} > 2 \times 10^{-4}$ mM. During activation of $I_T$, the value of [Ca]i just beneath the membrane increases to about $10^{-2}$-$10^{-3}$ mM and $\tau_T(V)$, was fit by a bell-shaped function from measurements of the deactivation time constants (see Fig. 2 C and Table 1).

Simulation of voltage-clamp experiments using these functions produced curves and measurements indistinguishable from those obtained by McCormick and Pane (1990a).
FIGURE 2 Activation and deactivation kinetics of \( I_h \). (A) Simulation of voltage-clamp protocols of activation of \( I_h \). (a) From an initial holding value of \(-55 \text{ mV}\), the voltage was clamped at various levels (from \(-105 \text{ to } -70 \text{ mV}\)) for 4 s, then clamped again to \(-55 \text{ mV}\). (b) Time course of the current compared to the gating variables. During this activation protocol (initial voltage of \(-30 \text{ mV}\), current recorded after clamping to \(-50 \text{ mV} \text{ at } t = 0\)) the current follows the time course of the slow variable \( S_i \). A time constant of about 3 s was estimated from fitting a single exponential to the current trace. (B) Simulation of protocols of deactivation of \( I_h \). (a) The voltage was clamped at \(-105 \text{ mV}\) for 4 s, then clamped to various levels from \(-85 \text{ to } -55 \text{ mV}\). (b) Time course of the membrane potential (mV) vs. time (s).
FIGURE 3  Resting states and slow oscillations in the presence of $I_T$ and Ca$^{2+}$-dependent $I_h$, obtained at four values of the maximal conductance of $I_h$.  (A) Hyperpolarized resting state close to $-84$ mV for $g_h = 0$.  (B) Slow oscillations of about 3.5 Hz for $g_h = 0.01$ mS/cm$^2$.  (C) Waxing and waning oscillations of about 4-8 Hz for $g_h = 0.04$ mS/cm$^2$.  (D) Depolarized resting state around $-58$ mV for $g_h = 0.11$ mS/cm$^2$. The maximum conductance of $I_T$ was kept fixed at $g_{K_C} = 1.75$ mS/cm$^2$.

cation occurred around $g_h = 0.02$ mS/cm$^2$ from slow oscillations to a state where the slow oscillations were interrupted by short silent phases (Fig. 6 B). As $g_h$ increased, the length occurred as the depolarized state was approached, with oscillatory phase reducing to a minimum length before disappearing (sometimes a low amplitude periodic oscillation
consistent with the assumption that the binding of Ca\textsuperscript{2+} is critical for the onset and termination of the oscillatory phase. The silent phase, which depends on the return of \(S_2\) and \(F_2\) during each cycle of the oscillatory phase. The resulting depolarization inactivates \(I_T\) and the oscillations damp. Compared with the Ca\textsuperscript{2+}-dependent waxing and waning
the stable limit cycle coexisted with the stable stationary state (Fig. 10 B). The state of the system within this interval of $S_2$ depended on its previous history.

Thus, in a waxing and waning sequence, $S_2$ oscillates between values which drive the system alternately between stable stationary states and slow oscillations. As shown by Fig. 10 B, the waxing and waning oscillations are driven around a hysteresis loop by the slow oscillations of $S_2$, as depicted by dotted arrows: as $S_2$ decreases during the silent phase, the membrane potential hyperpolarizes slowly and follows the stable stationary state branch (arrow 1). As the critical point is reached, the stationary state loses its stability and the system jumps to the oscillating branch (arrow 2). $S_2$ then starts to increase and follows the oscillating branch, while the amplitude of the oscillations decreases (arrow 3).
FIGURE 10  Singular approximation applied to the Ca^{2+}-dependent model of waxing and waning oscillations. (A) For extreme values of the slow variable $S_2$, treated as a parameter, the system exhibited either slow oscillations ($S_2 = 0.09$) or a stable stationary state ($S_2 = 0.65$). Other parameters are the same as in Fig. 3. (B) Bifurcation diagram of the system as a function of $S_2$. During the slow oscillations of $S_2$, the system alternated between a slow oscillatory state and a resting state, tracing a hysteresis loop as shown in the diagram. The order of events underlying the waxing and waning sequence are indicated by dotted arrows. Dashed lines represent unstable states (USS, unstable stationary state; SSLC, unstable limit cycle), and continuous lines represent stable states (SSS, stable stationary state; SLC, stable limit cycle). (C) Corresponding sequence of events in a single cycle of the waxing and waning oscillations. (D) Trajectories of waxing and waning oscillations in the V/S diagram. Here the full system was simulated without considering $S_2$ as a parameter. Dashed lines represent the presumed position of oscillatory and stationary branches and dotted arrows depict the same sequence of events as in B.

Irisawa, (1989) and neocortical neurons (Schwindt et al., 1992). These data suggest that intracellular Ca^{2+} ions directly affect $I_h$ channels and shift the activation function toward more depolarized potentials. We assumed that the Ca^{2+} dependence of $I_h$ is caused by direct binding of extracellular Ca^{2+} ions on the open form of $I_h$ channels (for a different model of this shift in the context of TC cells, see Toth and Crunelli (1992b)). Our model accounts for the positive shift of the activation function of $I_h$ with increased intracellular Ca^{2+}, but not for the substantial increase of conductance. It should be possible to verify the predicted logarithmic shift (Eq. 12) from whole cell patch-clamp experiments.

**Combinations of currents giving rise to waxing and waning oscillations**

The properties of waxing and waning oscillations (Leresche et al., 1991) were reproduced by our model, which included $I_T$ and Ca^{2+}-dependent $I_h$. The silent phase was many seconds long, during which the membrane potential slowly hyperpolarized. A transition to periodic oscillations could be elicited by application of a depolarizing current step only for some values of the parameter $g_{h}$. Experimental studies report this transition in only two out of 39 cat TC cells (Leresche et al., 1991).

More importantly, the sequence of resting and oscillatory behavior obtained was identical to that determined in vitro (Soltesz et al., 1991). In these experiments, noradrenaline (NE) was used to change $I_h$, but NE also shifts the activation function of $I_h$ by a few millivolts (McCormick and Pape, 1990b). We did not include this shift in our simulations.

We also found intermediate patterns of oscillations which were not reported experimentally. Close to the transition between slow oscillations and waxing and waning oscillations there were long oscillatory phases and short silent phases. TC cells in vitro show a variety of patterns of waxing and waning oscillations with silent and oscillatory phases of different lengths. The range of patterns found in the model for different values of the parameters suggests that the variability observed in vitro might arise from a heterogeneity of the conductance values among neurons.

We also investigated the occurrence of waxing and waning oscillations in a model comprising $I_T$, $I_h$, and the slow K^{+} current $I_{k_2}$ (Destexhe and Bolyoyantz, 1993). The main difference was that the frequency inside the oscillatory phase was significantly higher in $I_{k_2}$-dependent waxing and waning oscillations (10–14 Hz) compared to the same oscillations obtained from the Ca^{2+}-dependent mechanism (3.5–4 Hz). The frequency of oscillations in the Ca^{2+}-dependent model was much closer to the experimental data of Leresche et al. (1991).

In the case of the Ca^{2+}-dependent model, the waxing and waning oscillations were modulated by the kinetics of binding of Ca^{2+}, whereas, in the case of the $I_{k_2}$-dependent model, they appear to be modulated by the slow activation of $I_h$. Although the values of $I_T$ and leakage parameters were the same, 10-fold higher values of $g_{h}$ were needed to observe similar types of behavior for the $I_{k_2}$-dependent model.
tonic firing, slow oscillations, and waxing and waning oscillations in TC cells. Experiments can be designed to test which of the two proposed mechanisms is responsible for the oscillations. The higher frequency of the $I_{K_{a}}$-dependent model makes it less plausible than the $Ca^{2+}$-dependent model. The $I_{K_{a}}$-dependent model predicts that the waxing and waning oscillations should not survive blockage of all voltage-dependent $K^+$ currents (but not the leak $K^+$ currents, needed to maintain the level of membrane potential). The $Ca^{2+}$-dependent model could be tested by altering the intracellular $Ca^{2+}$ levels while monitoring the period of waxing and waning oscillations. The $Ca^{2+}$-dependent model predicts that this period should be sensitive to intracellular $Ca^{2+}$.

The intrinsic oscillating properties of TC cells are difficult to reconcile with the various types of oscillations found in vivo (Nunez et al., 1992). The occurrence of spindling in vivo is thought to be a combination of intrinsic and network properties (Steriade and Llinas, 1988; Steriade et al., 1993b). In particular, single thalamic reticular cells are characterized by 7–12-Hz intrinsic oscillations (Avanzini et al., 1989; Bal and McCormick, 1993), close to the typical frequency of sleep spindles. Spindle rhythmicity was also found in the isolated reticular thalamus in vivo (Steriade et al., 1987). On the other hand, TC cells have a clear tendency to oscillate at a lower geniculate nucleus. J. Physiol. (Lond.), 413:543–561.


