Unique features of action potential initiation in cortical neurons

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Online supplementary information (Part 2 of 3):

Hodgkin-Huxley type models

In this part of the Supplementary Information we describe the Hodgkin-Huxley type conductance based models used in the study and the modifications which were applied to these models, such as changes of sodium channel activation curves and single channel stochasticity. We also describe parameter ranges explored. Finally, we show that rapidness and onset potential variability of AP initiation are strongly antagonistic in the whole class of Hodgkin-Huxley type conductance based models.

Contents

Hodgkin-Huxley type conductance based models

For the simulations, we used two different models (Model A and B), which were constructed to match the subthreshold MP dynamics and the firing statistics of cortical neurons.

Model A (Destexhe et al.)

Model A is a single compartment Hodgkin-Huxley type neuron model (Destexhe et al, 1999). As shown by Destexhe et al.,1999, this model reproduces the statistics of MP fluctuations and spike-trains of neocortical neurons *in vivo*. In this model, the dynamics of the MP is given by,

$$C_m \frac{dV}{dt} = -g_L (V - E_L) - I_{Na} - I_{Kd} - I_M - A^{-1} I_{syn}, \quad (2)$$

with the current densities

$$I_{Na} = \overline{g}_{Na} m^3 h (V - E_{Na}) \quad (3)$$

$$I_{Kd} = \overline{g}_{Kd} n^4 (V - E_K)$$

$$I_M = \overline{g}_M p (V - E_K),$$

where C_m=1 μF/cm^2 is the specific membrane capacity, g_L=0.045 mS/cm^2 is the leak conductance density, and E_L=−80 mV is the leak reversal potential. I_{Na} is the voltage-dependent Na^+ current and I_{Kd} is the 'delayed-rectifier' K^+ current, which underlie the AP repolarisation. I_M is a non-inactivating K^+ current causing spike frequency adaptation and A is the total membrane area, which was 34636 μm^2. m, h, n, p are dynamical activation variables (for details see Destexhe et al., 1999).

The total synaptic current consists of excitatory and inhibitory parts:

(4) $$I_{syn} = g_e(t)(V - E_e) + g_I(t)(V - E_I),$$

with the reversal potentials E_e and E_I. The conductances $g_e(t)$ and $g_I(t)$ are Ornstein-Uhlenbeck processes with correlation times τ_e and τ_I.

To compare the AP onset dynamics between cortical neurons and the model in the presence of a fluctuating input (e.g. Fig. 1c, Fig. 2e,f), we used this model with exactly the same parameters as in the above paper.

Robustness against variations in model parameters and structure

By various modification of the models we tried to achieve a better match of recorded and simulated APs. As described in the subsequent sections, we modified the sodium current activation curve and the peak conductances, included various adaptation currents (Wang et al. 2003), replaced the simple mean channel kinetics with a stochastic dynamics of population of individual sodium channels (Schneidman et al. 1998), assessed the impact of state-dependent instead of voltage-dependent inactivation of sodium channels (Patlak et al. 1991). None of these modifications brought the model behaviour anywhere close to reproducing the two salient features of the recorded APs.

To study the impact of sodium channel activation on the AP initiation we modified the model described above by changing the Na-peak conductance $\bar{g}_{Na}$ (by factors of 2, 5, 10, 20) and the $m(t)$ activation curve, by changing its steepness by factors of 0.2, 0.5, 1.5, 2, 5, 10.

The m^3-term describing the activation of the sodium channels was replaced by the sodium activation curve measured by Huguenard et al. (1989), with the two parameters k and $V_{1/2}$:

(5) $$I_{Na} = \bar{g}_{Na}(1+\exp((-V+V_{1/2}+\Delta)/k)^{-1} n(t)(V-E_{Na})$$

We used combinations of k (changed by factors of 0.2, 0.5, 1.5, 2, 5, 10) and Δ (-10mV, -5mV, 6mV, 12mV, 20mV, 22mV) to change the AP onset rapidness while preserving a stationary firing rate of approximately 10Hz. In addition, the potassium peak conductance was changed by factors of 2 and 5 to assess its impact on the onset potential variability.

Model B: Adaptation currents and AP initiation (Wang et al.)

To assess the impact of adaptation currents on the response variability in Hodgkin-Huxley type models, we also implemented a two-compartmental model including adaptation currents developed by Wang et al. (2003).

The model has two compartments, one representing the soma and the initial segment (V_s) and the other representing the dendritic tree (V_d). The AP generating currents are located in the soma. High threshold Ca^{2+}, as well as Ca^{2+}-dependent K^+ currents are present in both compartments. In addition, the somatic compartment incorporates a slow Na^+-activated K^+ current.

The dynamics of the model is defined by the following differential equations:

(1) $C_m \frac{dV_s}{dt} = -I_L - I_{Na} - I_{Ca,s} - I_{KCa,s} - I_{KCa} - (g_c / p)(V_s - V_d) + I_{ext}(t)$

(2) $\frac{dh}{dt} = \phi_h(\alpha_h(V_s)(1-h) - \beta_h(V_s)h)$

(3) $\frac{dn}{dt} = \phi_n(\alpha_n(V_s)(1-n) - \beta_n(V_s)n)$

(4) $\frac{d[Ca^{2+}]_s}{dt} = -\alpha_{Ca,s} I_{Ca,s} - [Ca^{2+}]_s / \tau_{Ca,s}$

(5) $\frac{d[Na^+]}{dt} = -\alpha_{Na} I_{Na} - 3R_{pump}(\varphi_{Na}([Na^+]_{eq}))$

(6) $C_m \frac{dV_d}{dt} = -I_L - I_{Ca,d} - I_{KCa,d} - (g_c /(1-p))(V_d - V_s)$

(7) $\frac{d[Ca^{2+}]_d}{dt} = -\alpha_{Ca,d} I_{Ca,d} - [Ca^{2+}]_d / \tau_{Ca,d}$

The membrane capacity is denoted by $C_m = 1\mu F / cm^2$, the external current is $I_{ext}(t)$ and the leak current is $I_L = g_L(V - V_L)$. The coupling current between the soma and the dendrite is proportional to $V_d - V_s$, with the coupling conductance $g_c = 2\,\text{mS/cm}^2$. The parameter p=(somatic area/total area)=0.5. The maximum conductances are given by $g_L = 0.1$, $g_{Na} = 45$, $g_K = 18$, $g_{Ca,s} = g_{Ca,d} = 1$, $g_{KCa,s} = g_{KCa,d} = 5$, $g_{KNa} = 5\,\text{mS/cm}^2$. The reversal potentials are given by $V_L = -65$, $V_{Na} = 55$, $V_K = -80$, $V_{Ca} = 120\,\text{mV}$.

The sodium current in the somatic compartment is given by $I_{Na} = g_{Na} m_\infty{}^3(V_s) h (V_s - V_{Na})$ with $m_\infty(V_s) = \alpha_m /(\alpha_m + \beta_m)$, $\alpha_m(V) = -0.1(V+33)/(\exp(-0.1(V+33)))$ and $\beta_m(V) = 4\exp(-(V+58)/12)$. The inactivation variable h is described by $\alpha_h(V) = 0.07(\exp(-(V+50)/10))$ and $\beta_h(V) = 1/(\exp(-0.1(V+20))+1)$. The delayed rectifier is given by $I_K = g_K n^4 (V_s - V_K)$ with the activation variable n given by $\alpha_n(V) = -0.01(V+34)/(\exp(-0.1(V+34))-1)$ and

$\beta_n(V) = 0.125\exp(-(V+44)/25)$. The temperature factors are $\phi_h = \phi_n = 4$. The time constants are $\tau_x(V) = 1/(\alpha_x + \beta_x)$.

The high-threshold calcium current is given by $I_{Ca} = g_{Ca} v_\infty^{\,2}(V)(V - V_{Ca})$ with the activation variable $v_\infty(V) = 1/(1+\exp(-(V+20)/9))$. The voltage-independent, calcium activated potassium current is given by $I_{KCa}(V) = g_{KCa}([Ca^{2+}]_i/([Ca^{2+}]_i + K_D))(V - V_K)$, with $K_D = 30\,\mu$M. The intracellular calcium concentration $[Ca^{2+}]_i$ is assumed to be governed by a linear equation with α_{Ca} proportional to the ratio of the membrane area and the volume immediately beneath the membrane, $\alpha_{Ca} = 0.002\,\mu\text{M}(\text{ms}\mu\text{A})^{-1}\text{cm}^2$ in the dendritic compartment and $\alpha_{Ca} = 0.00067\,\mu\text{M}(\text{ms}\mu\text{A})^{-1}\text{cm}^2$ in the somatic compartment. The extrusion and buffering processes are described collectively by a first-order decay process with a time constant $\tau_{Ca} = 80\,\text{ms}$ in the dendrite and $\tau_{Ca} = 240\,\text{ms}$ in the soma.

The intracellular $[Na^+]_i$ concentration is incremented by Na^+ influx through I_{Na}. The Na^+-dependent K^+-current is determined by $I_{KNa} = g_{KNa}\omega_\infty([Na^+]_i)(V_s - V_K)$, with the activation function

(8) $$\omega_\infty([Na^+]_i) = \frac{P_{\max}}{1 + (EC_{50}/([Na^+]_i)^{n_H}}$$

$P_{\max} = 0.37$ defines the maximum opening probability of the channels, $EC_{50} = 38.7\text{mM}$ is the $[Na^+]_i$ for half activation and $n_H = 3.5$ is the Hill coefficient. The influx of $[Na^+]_i$ is controlled by $-\alpha_{Na} I_{Na}$ with $\alpha_{Na} = 3\cdot 10^{-4}\,\text{mM}$.

The extrusion of $[Na^+]_i$ by the ion pump was modelled as $-3R_{pump}(\varphi_{Na}([Na^+]_i) - \varphi([Na^+]_{eq})$, where $\varphi_{Na}(x) = x^3/(x^3 - K_p^{\,3})$, $K_p = 15\,\text{mM}$ and

$R_{pump} = 6 \cdot 10^{-4}$ mM/ms . The sodium concentration at the resting state is given by $[Na^+]_{eq} = 8\,\text{mM}$.

This model was driven by a fluctuating synaptic input current using the same point conductance model as in (Destexhe et al., 1999) and described above. The model was subsequently modified in the same way as the first model, i.e. the steepness of voltage-dependence of the activation was increased by factors of 0.2, 0.5, 1.5, 2, 5, 10 while preserving a stationary firing rate of approximately 10 Hz, by either changing the mean input current or shifting the activation curve as in Model A.
In both models, we simulated 5s long periods of neuronal activity, whereby in the second model, care was taken that the adaptation currents where statistically stationary. The simulated data were then analyzed using the same procedures as the experimentally obtained data.

All numerical simulations were performed in C++, all analyses were done using Matlab V6.5 (R13). For the numerical integration of the differential equation we used an Euler integration with an integration step of 10^{-3} ms .

Channel noise and AP initiation

In Hodgkin-Huxley type models, the ensemble dynamics of voltage-gated ion channels is described by products of continuous activation and inactivation variables which, in turn, are modelled as first order kinetics. Since natural membranes have a limited number of channels one expects MP fluctuations reflecting discrete channel opening and closing events (Chow & White 1996; Schneidman et al., 1998). Can this discrete switching explain the AP initiation observed in cortical neurons? To answer this question, it is instructive to first assess how many channels on average are involved in the generation of an AP. The conductance of a single voltage-gated

Na-channel is estimated to be on the order of $\gamma = 20$ pS (Neumcke & Stämpfli, 1982). From the total sodium current flowing through a patch of the membrane, the number of channels in a membrane area of size 200 µm² in a pyramidal cell has been estimated as approximately N = 12000, yielding a total conductance of 240 nS (Schneidman et al, 1998).

To assess if intrinsic channel noise can account for the AP initiation in cortical neurons, we implemented discrete Markov models, in which the opening and closing of individual sodium and potassium channels was simulated (Schneidman et al., 1998). The kinetic model for a population of sodium channels that reproduces the behaviour of a Hodgkin-Huxley type model for high channel densities is given by the following scheme (Patlak, 1991):

$$\begin{array}{ccccccc} & 3\alpha_m & & 2\alpha_m & & \alpha_m & \\ m_0h_1 & \rightleftharpoons & m_1h_1 & \rightleftharpoons & m_2h_1 & \rightleftharpoons & m_3h_1 \\ & \beta_m & & 2\beta_m & & 3\beta_m & \\ \alpha_h \updownarrow \beta_h & & \alpha_h \updownarrow \beta_h & & \alpha_h \updownarrow \beta_h & & \alpha_h \updownarrow \beta_h \\ & 3\alpha_m & & 2\alpha_m & & \alpha_m & \\ m_0h_0 & \rightleftharpoons & m_1h_0 & \rightleftharpoons & m_2h_0 & \rightleftharpoons & m_3h_0 \\ & \beta_m & & 2\beta_m & & 3\beta_m & \end{array}$$

In this description, each channel has 8 possible states and $\left[m_i h_j\right]$ is the number of channels that are in state $m_i h_j$. An individual channel is open, when it is in the state $m_3 h_1$, but closed in all other states. Thus, the total sodium membrane conductance is given by $g_{Na} = \gamma_{Na}[m_3 h_1]$.

The population of voltage-gated potassium channels is modelled by the following kinetic scheme:

$$\begin{array}{ccccccccc} & 4\alpha_n & & 3\alpha_n & & 2\alpha_n & & \alpha_n & \\ n_0 & \rightleftharpoons & n_1 & \rightleftharpoons & n_2 & \rightleftharpoons & n_3 & \rightleftharpoons & n_4 \\ & \beta_n & & 2\beta_n & & 3\beta_n & & 4\beta_n & \end{array}$$

Here, each channel has 5 possible states. A channel is open when it is in the state n_4.

The total potassium conductance is then given by $g_K = \gamma_K [n_4]$.

The rates α_x and β_x are the standard Hodgkin-Huxley transition rates (Hille, 2001).

In each time step Δt of the simulation, the number of channels Δn_{AB} that switch between states A and B with rate r is determined by choosing a random number from a binomial distribution:

$$(9)\ \mathrm{Prob}(\Delta n_{AB}) = \begin{pmatrix} n_A \\ \Delta n_{AB} \end{pmatrix} p^{\Delta n_{AB}} (1-p)^{(n_A - \Delta n_{AB})}$$

where n_A denotes the number of channels in state A and $p=r\Delta t$.

In Fig. 2SI, results of a simulation of 3 conductance-based models with the number of sodium channels equal to (12000), half (6000) and 10 times lower (1200) than the experimental estimate, and a fixed number of potassium channels (3600) are used. The same fluctuating current was injected into the three models. With a decreasing number of channels, the phase plot trajectories became noisier and the apparent variability of the onset potential larger, (B,D,F), but the steepness of the AP onsets did not change. With none of the three models, the AP onset dynamics matched the values for the onset rapidness and onset potential variability, observed in *in vivo* recordings.

The activation scheme described above is the kinetics that corresponds to the classical Hodgkin-Huxley model in the limit of high channel density and negligible stochastic fluctuations. This scheme, however, is known to incorrectly represent the inactivation of sodium channels, which is state-dependent rather than voltage-dependent (Hille, 2001). To test, the impact of state-dependent inactivation, we also implemented a scheme which includes more dynamical steps for activation and

inactivation (Patlak, 1991, Model 7). With none of the schemes tested, the AP onset rapidness substantially changed. Channel noise is thus not a plausible candidate to explain the AP onset dynamics observed *in vivo* (e.g. Fig. 2 in the paper).

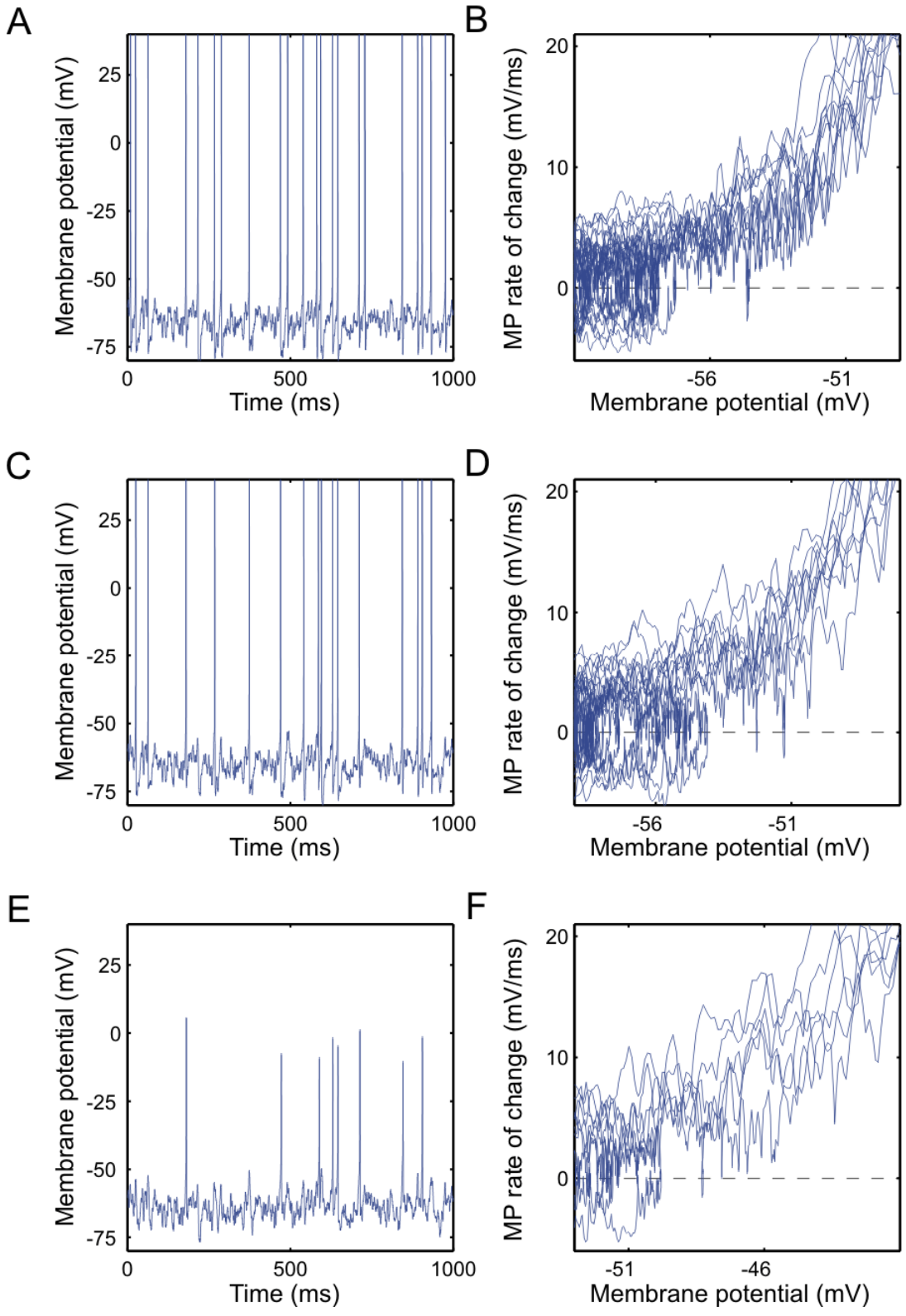

Fig 2SI: Impact of channel noise on the onset dynamics of APs in a conductance based neuron model. (A,C,E) Voltage trace of a model neuron with the same fluctuating synaptic background input and a different number of voltage-gated sodium channels (A: 12000, C: 6000, E: 1200) and 3600 potassium channels. (B,D,F): Corresponding phase plots. With a decreasing number of sodium channels the AP onset dynamics becomes noisier. Both, the AP onset span and the onset rapidness remain unaffected.

AP onset variability and steepness are antagonistic in Hodgkin-Huxley type models

In conductance based models, the dynamics of the MP $V(t)$ is given by:

$$(10) \qquad CdV(t)/dt = g_{Na}h(t)m(t)^3(V_{Na} - V(t)) + I_K + \cdots$$

Here C denotes the membrane capacity, g_{Na} the sodium channel peak conductance, $m(t)$ the sodium channel activation and $h(t)$ the inactivation. The activation and inactivation follow a first order kinetics, where the time constant of the activation $\tau_m(V)$ is typically on the order of 0.2ms and the inactivation time constant $\tau_h(V)$, as well as the time constants of all other channels, are typically much larger.

During the initial AP phase, the maximum rate of change of the MP can thus be estimated by replacing $m(t)$ by its steady state value $m_\infty(V)$ and replacing the inactivation variable by a constant h_0:

$$(11) \qquad dV(t)/dt \le I_0 + g_{Na}h_0 m_\infty{}^3(V)(V_{Na} - V(t))$$

Right at the AP onset, the sodium activation curve starts exponentially:

$$(12) \qquad \begin{aligned} m^3(V) &= (1+\exp(-(V-V_{1/2})/k))^{-1} \\ &\simeq \exp((V-V_{1/2})/k) \end{aligned}$$

A multiplication of $m^3(V)$ by a factor G is therefore equivalent to a shift of the whole curve by $k \log G$. For APs with a gradual onset ($k = 6$), a shift of 10mV would therefore require a 5-fold change in the effective sodium peak conductance, while for more rapid onsets ($k = 1$) it would require a 22000-fold change.

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