Abstract

Voltage-gated sodium channels play an important role in action potentials. If enough channels open during a change in the cell's membrane potential, a small but significant number of sodium ($Na^+$) ions will move into the cell reducing its electrochemical gradient and further depolarizing the cell. Voltage-gated $Na^+$ channels play a fundamental role in the excitability of nerve and muscle cells. $Na^+$ channels both open and close more quickly than potassium ($K^+$) channels, producing an influx of positive charge ($Na^+$) toward the beginning of the action potential and an efflux ($K^+$) toward the end. The study of $K^+$ channels is essential as they appear to be more diverse in structure and function than any other types of ion channel. $K^+$ channels shape the action potential, set the membrane potential, and determine firing rates. There already are some drugs in clinical use that target $K^+$ channels which improve our ability to regulate excitability.

In this research, we study the influence of voltage dependence on channel activation and inactivation by simulating different channel subtypes as well as the effect of different kinetic parameters on membrane excitability.

Keywords: brain information processing; cell membrane potential; channel activation

Introduction

Information processing in the brain results from the spread and interaction of electrical and chemical signals among neurons. The equations that describe brain dynamics generally do not have analytical solutions. The use of spiking neuron models, rather than the traditional rate-based models is largely motivated by the growing inspiration from experimental neuroscience and the design of new neural models aimed to use the knowledge gained from simulations and theoretical analysis of the models in order to better understand the brain dynamics (Maas, 1998; Gerstner, 2001; Dayan, P. and Abbott, L., 2001).

Voltage-gated sodium and potassium channels play an important role in the initiation and propagation of action potentials in neurons, as well as in shaping the membrane potential and cell firing rates (Schreiber et al., 2009). Potassium channels, originally identified as the molecular entities mediating flows of potassium ions across nerve membranes in action potential generation, are now known in virtually all types of cells in all organisms, where they are involved in a large number of physiological functions. They are integral membrane proteins forming transmembrane pores through which $K^+$ specifically permeates. All $K^+$ channels carry out a single basic function: the formation of a transmembrane leak extremely specific for $K^+$ ions. Since cells almost universally maintain cytoplasmic $K^+$ concentrations much higher than those extracellularly, the opening of a $K^+$ channel automatically implies a negative-going change in electrical voltage across the cell membrane known as membrane hyperpolarization. Disruption in $Na^+$ and $K^+$ channels leads to several human genetic diseases as cardiac arrhythmias, deafness, epilepsy, diabetes, and misregulation of blood pressure (Li-Smerin Y, Hackos DH, Swartz KJ, 2000; Yu F.H., Catterall W. A., 2003).

The goal of this research is to simulate and study the influence of voltage dependence on sodium and potassium channel activation and inactivation by simulating different channel subtypes as well as the effect of different kinetic parameters on membrane excitability.
excitability. The temporal activities of both channels can be modeled by two equivalent approaches: Hodgkin-Huxley-type (HH) formalism or by Markovian scheme. Our simulations are based on HH model (Hodgkin AL, Huxley AF, 1952) which accepts that each channel occupies a distinct state with differential equations describing transitions between these states and treats each channel as a population that is affected by three independent gating variables.

Computations in Spiking Neurons Network

Neurons are highly specialized in generating electrical signals in response to chemical and other inputs, and transmitting them to other cells. A typical biological neuron consists of the following components (Gerstner and Kistler, 2002): a) Dendrites: input device; b) Axon: output device; c) Soma: central processing unit receiving input through its dendrites and producing spikes as an output through its axon to other neurons. It performs an important non-linear processing step: if the total input exceeds a certain threshold, then an output signal, the action potential, is generated; d) Synapse: coupling between two neurons via dendrites and axons (Fricker, D. and Miles, R., 2001).

The membrane potential is the potential difference between the interior and exterior of the cell. In non-excitable cells, and in excitable cells in their baseline states, the membrane potential is held at a relatively stable value, called the resting potential. For neurons, typical values of the resting potential range from –70 to –80 mV; that is, the interior of a cell has a negative baseline voltage of a bit less than one tenth of a volt. In this state, the membrane is modeled by the following equation:

\[ \frac{dV}{dt} = -g_L(V - E_L) + S(t) + G(t) \]  

where \( C_m = 1 \ \mu F/\text{cm}^2 \) is the specific capacitance, \( V \) is the membrane potential, \( g_L = 5 \times 10^{-5} \ \text{S/cm}^2 \) is the leak conductance density and \( E_L = -60 \ \text{mV} \) is the leak reversal potential. The function \( S(t) \) represents the spiking mechanism and \( G(t) \) stands for synaptic interactions.

The two types of postsynaptic potentials are: EPSP – excitatory and IPSP – inhibitory. When excitatory neurotransmitter binds to its receptor on postsynaptic membrane it causes partial depolarization by opening Na\(^+\) channels and allowing Na\(^+\) to enter the cell very rapidly. If membrane potential raises enough to threshold level, action potential will develop and excite the neuron (Chen et al., 2005). Neurotransmitter binding to a receptor at inhibitory synapses leaves the charge on the inner surface more negative (flow of K\(^+\) out of the cytosol makes the interior more negative relative to the exterior of the membrane and reduces the postsynaptic neuron’s ability to produce an action potential). Thus IPSPs increase negative charge on membrane causing hyperpolarization (Somogyi, 2005; Chen et al., 2006; Schreiber et al., 2009; Silberberg et al., 2005).

To maintain a potential difference a current has to flow. This is the activity of the ion pumps located in the cell membrane which transport ions to maintain ionic concentration. Predominantly sodium, potassium, calcium and chloride are the ionic species involved. For example, the Na\(^+\) concentration is higher outside than inside a neuron while, on the contrary, K\(^+\) is more concentrated inside the cell than in the extracellular medium. Ions flow according to their concentration gradient through a variety of ionic channels which open and close in response to voltage changes as well as to internal or external signals (Hille, 2001). The depolarization caused by opening of voltage-sensitive sodium channels allows sodium ions to flow into the cell. The sodium channels only open in response to a partial depolarization, such that a threshold voltage is exceeded. As sodium floods in, the membrane potential reverses, such that the interior is now positive relative to the outside. This positive potential causes voltage-sensitive potassium channels to open, allowing K\(^+\) ions to flow out what cause the potential to become more negative than the resting potential. The fall in potential triggers the sodium channels to close, setting the stage for restoration of the resting potential by sodium pumps. After the pulse, the membrane potential does not directly return to the resting potential, but passes through a phase of hyperpolarization below the resting value (Stuart and Palmer, 2006).

Most of the spiking neural simulators using excitatory and inhibitory neurons are based on two different approaches: a) with current-based synaptic interactions; b) with conductance-based synaptic interactions. Hodgkin and Huxley (HH) type of models use the second approach. There is a third model consisting of IF neurons interacting through voltage deflections or voltage-jump synapses (Vogels and Abbott, 2005).

For conductance-based synaptic interactions, the membrane equation of neuron \( i \) is given by:

\[ C_m \frac{dV_i}{dt} = -g_L(V_i - E_L) + S(t) \cdot \sum g_j(t)(V_i - E_j) \]  

where \( V_i \) is the membrane potential of neuron \( i \), \( g_j(t) \) is the synaptic conductance of the synapse from neuron \( j \) to neuron \( i \), and \( E_j \) is the reversal potential of that synapse. \( E_j \) is 0 mV for excitatory synapses, or -80 mV for inhibitory synapses. Synaptic interactions are implemented as follows: when a spike occurred in neuron \( j \), the synaptic conductance \( g_j(t) \) is instantaneously incremented by a quantum value (6 nS and 67 nS for excitatory and inhibitory synapses, respectively) and decayed exponentially with a time constant of 5 ms and 10 ms for excitation and inhibition, respectively.

Current-based synaptic interactions are modeled with the following equation:
\[ C_m \frac{dV}{dt} = -g_L(V - E_L) + S(t) - \sum_j g_j(t)(\bar{V} - E_j) \]  

(3)

where \( \bar{V} = 60 \text{ mV} \) is the mean membrane potential. The conductance quanta were of 0.27 \( nS \) and 4.5 \( nS \) for excitatory and inhibitory synapses, respectively. The other parameters are the same as for conductance-based interactions. Voltage-jump type of synaptic interactions requires abrupt increase of membrane potential by a value of 0.25 \( \text{mV} \) for each excitatory event, and respectively a decrease by 2.25 \( \text{mV} \) for each inhibitory event.

As the range of computational problems related to spiking neurons is very large it requires in some cases to use detailed biophysical representations of the neurons (Destexhe and Sejnowski, 2003) or conductance-based models, such as HH. In other cases, one does not need to realistically capture the spike generating mechanisms, and simpler models, such as the integrate-and-fire (IF) model is sufficient.

The Hodgkin-Huxley Model

The Hodgkin and Huxley model captures the basic mechanism of generating action potentials in the giant squid axon. This mechanism is essentially preserved in higher organisms. HH model defines the three key features of sodium channels: voltage-dependent activation, rapid inactivation, and selective ion conductance. It is described by the following equation:

\[ C \frac{dV}{dt} = \sum_k g_k(E_k - V_m) + I(t) \]  

(4)

where \( C \) is the capacitance of the semipermeable cell membrane which separates the interior of the cell from the extracellular liquid allowing active ion transport through the membrane. \( \sum_k g_k(E_k - V_m) \) is the sum of all participating ionic currents that pass through the cell membrane (sodium ions \( I_{Na} \), potassium ions \( I_K \) and a leakage current \( I_L \) mainly due to chloride ions) and \( I(t) \) is the applied current. Subscripts \( Na, K \) and \( L \) used to denote specific currents or conductances. Each individual ionic component \( I_k \) has an associated conductance value \( g_k \) and an reversal (equilibrium) potential \( E_k \). The latter represents the potential for which the net ionic current passing through the membrane is zero. Each member \( g_k(E_i - V_m) \) describes the ionic current resulting from the potential difference \( (E_i - V_m) \) which is also known as driving force on particular channels. The conductance \( g_i \) is constant and \( g_{Na} \) and \( g_K \) are time and voltage dependent.

Therefore the squid giant axon Hodgkin-Huxley mathematical model includes the following three types of currents: sodium current \( I_{Na} \), potassium current \( I_K \) and leakage current \( I_L \), i.e.

\[ I_{ion} = I_{Na} + I_K + I_L = g_l(E_l - V_m) + g_{Na}(E_{Na} - V_m) + g_K(E_K - V_m) \]  

(5)

where \( I_{ion} \) is the sum of all participating ionic currents that pass through the cell membrane. The central concept of Hodgkin-Huxley model introduces three state variables that describe the behavior of \( g_{Na} \) and \( g_K \) and control the opening and closing of ion channels (\( m \) controls Na channel opening, \( h \) controls Na channel closing, \( n \) controls K channel opening) is represented by

\[ \sum_k I_k = g_{Na}m^3h(E_{Na} - V_{Na}) + g_{K}n^4(E_K - V_{K}) + g_{L}(E_L - V_L) \]  

(6)

where \( 0 \leq m, n, h \leq 1 \). \( m \), \( n \) and \( h \) are the activation variables which time evolution depends on the voltage-dependent rate constants \( a_m \), \( \beta_m \), \( a_n \), \( \beta_n \), \( a_h \) and \( \beta_h \). The empirical differential equations modeling the behavior of gates \( g_{Na} \) and \( g_K \) are

\[ \frac{dn}{dt} = a_n(v)(1 - n) - \beta_n(v)n; \quad a_n(v) = \frac{0.1(v + 25)}{e^{(v+25)/10} - 1}; \quad \beta_n(v) = 0.125 e^{v/80}; \]  

(7)

\[ \frac{dh}{dt} = a_h(v)(1 - h) - \beta_h(v)h; \quad a_h(v) = 0.07 e^{v/26}; \quad \beta_h(v) = \frac{1}{e^{(v+30)/10} + 1}; \]
where \( v \) is the voltage across the membrane at a given time. The maximal conductances of the sodium and potassium current are respectively \( g_{Na} = 100 \text{ mS/cm}^2 \) and \( g_K = 30 \text{ mS/cm}^2 \) with reversal potential of \( E_{Na} = 50 \text{ mV} \) and \( E_K = -90 \text{ mV} \).

Simulations and Discussions

In our research we use NEURON because of its ability to accommodate the complex geometry and nonlinearities of biologically realistic models, without interfering with its ability to handle more specific details that involve a high degree of abstraction and specific anatomical and biophysical properties. It is capable of modeling and performing hybrid simulations on nets whose elements include both artificial neurons and neuron models with membrane currents governed by voltage-gated ionic conductance. NEURON’s computational engine uses algorithms that are tailored to the equations of the system model (Hines and Carnevale 2004). The package fully supports models containing any combination of conductance-based neurons and analytically computable artificial spiking cells. Simulations of networks that contain conductance-based neurons are second order correct if adaptive integration is used (Lytton and Hines, 2005). NEURON simulation environment is capable of efficient discrete event simulations of networks of spiking neurons and allows extension of its functionality in order to complete specific goals (www.neuron.yale.edu/neuron).

The goal of the experiments is to simulate the affect of manipulation (activation and inactivation) of \( Na \) and \( K \) channels kinetics on membrane excitability. We use the HH model as well as the Moore-Cox (MC) model of the \( Na^+ \) and \( K^+ \) channels. Hodgkin and Huxley differential equations contain the probability variables \( m, n, \) and \( h \) which depend on both voltage and time. The variables \( m \) and \( h \) represent activation and inactivation of the \( Na \) channels, and \( n \) represents \( K^+ \) channels activation. The three relations describing the probabilities that the \( Na \) and \( K^+ \) channels, respectively, will be activated at a given voltage if that voltage is maintained until these variables reach a steady state. The model interface allows change of parameters as shown in Fig. 1. The plotted graph on the same figure displays three curves showing the three probabilities as a function of voltage for \( Na \) channel activation (\( m_{inf} \)), \( Na \) channel inactivation (\( h_{inf} \)) and \( K \) channel activation (\( n_{inf} \)). The action potential and its underlying currents for normal HH kinetics are shown in Fig. 2.

Our studies include two groups of simulations for the \( Na^+ \) and \( K^+ \) channels. Group one refers to: 1) Effect of the \( m \) variable on depolarizing direction of the voltage. 2) Effect of the \( h \) relation on the hyperpolarizing direction. 3) Effect of the probability variable \( n \) on the activation of \( K^+ \) channels. In the second group of simulations we included the voltage clamp and studied changes in the \( Na^+ \) and \( K^+ \) currents implementing shifts in \( m, n, \) and \( h \) curves.

Fig. 1. Model interface and graph showing HH probability variables controlling \( Na \) and \( K \) channels

Fig. 2. \( Na \) and \( K \) currents underlying the action potential for standard HH kinetics
We simulate the depolarization for the Na channels by moving the activation curve $m$ in positive direction. Shifting $m$ by 1 $mV$ to the right slightly lowers the kinetics of activation of the Na channels (Fig. 3a). This means that at any given voltage, fewer Na channels are likely to open. When the probability curve for Na activation shifts to the right, this means that at any given voltage the probability that channels will open will be lower. Further shifts of the curve $m$ to the right makes the membrane less and less excitable by decreasing the probability that channels would open in response to a given depolarization.

At some certain level of $m$, the action potential can completely fail. Our experiments show that we can restore it by performing stronger depolarization which will open a given fraction of channels or if we increase the amplitude of the stimulus pulse in order to excite the neuron again. To observe the hyperpolarization for the Na channels by moving the activation curve $m$, we increase the total time to 25 $ms$ and shift $m$ in the hyperpolarizing direction by 1 $mV$ at a time ((Fig. 3b). Accordingly, shifts of $n$ in the depolarizing direction increase excitability by decreasing the $K^+$ current that opposes the Na current. With a shift of 3 $mV$ in the depolarizing direction, the membrane will fire impulses spontaneously.

The $h$ relation for the Na channels describes the probability that channels will not be inactivated at a certain voltage. That is, at –50 $mV$ only a small fraction of the Na channels are available for opening. Shifting the $h$ curve in the positive direction increases the number of Na channels which are available for activation at a given voltage (for example -50 $mV$). More Na channels will be inactivated and not available for opening when the current pulse depolarizes the membrane if the $h$ curve moves in the negative direction. The diminished Na current results in an action potential that takes off later from the pulse, has a slower rising phase, and reaches smaller amplitude. Continuous rising of $h$ curve shift in negative direction can lead to smaller values of Na current. At some point it eventually will not overcome the opposing $K^+$ current, so the action potential will fail. During the second group of simulations we change the voltage clamp by using a 4 $ms$ step from –65 $mV$ to 0 $mV$. The inactivation curve for $I_{Na}$ ($h$) 20 $mV$ and gating of $IK^-$ ($n$) 20 $mV$ were shifted in 1 $mV$ steps in both directions. If we shift $IK^-$ ($n$) curve 20 $mV$ to the left, the finite $IK^-$ is at 100 $mV$ and it does have a steeply rising current of large amplitude (Fig. 4). If we shift $IK^-$ ($n$) curve 20 $mV$ to the right, $IK^-$ will rise slowly and peak at a lower amplitude.

Fig. 5 demonstrates the possibility for the neuron to generate action potential after removing inactivation followed by delivering hyperpolarization. The delay in $I_{Clamp}$ is set to 0.5 $ms$, the duration is10 $ms$ and the total time is set to 30 $ms$. The initial amplitude is -0.03 $nA$. The 10 $ms$ hyperpolarization allows time for the probability $h$ to change to 1 so that all of inactivation is removed from the Na channels. Thus, at the end of the pulse all of the Na channels are available to open in response to the sudden depolarization.
Conclusions

The cell biology and physiology of $K^+$ channels presents great challenges for the future. These channels are ubiquitous. They appear to be more diverse in structure and function than any other types of ion channel. $K^+$ channels are instrumental in governing excitable tissue. They shape the action potential, set the membrane potential, and determine firing rates. Potassium channel openers represent a new class of compounds that have some current clinical use. As the biology of voltage-gated channels is better understood, rational targets for further simulations will emerge.

References


