A Frequency Analysis on Calcium T-type Signaling in the Nervous System

Alireza Khodaei
Computer Science and Engineering
University of Nebraska-Lincoln
Lincoln, Nebraska 68588
Email:akhodaei@cse.unl.edu

Hamid Vakilzadian
Electrical and Computer Engineering
University of Nebraska-Lincoln
Lincoln, Nebraska 68588
Email:hvakilzadian1@unl.edu

Massimiliano Pierobon
Computer Science and Engineering
University of Nebraska-Lincoln
Lincoln, Nebraska 68588
Email:pierobon@cse.unl.edu

***Abstract*—We expressed the importance of frequency characteristics of T-type calcium signaling in every level of the nervous system. We also highlighted the need for performing frequency analysis on calcium signals in order to understand their impact on various functions within the nervous system. We provided the necessary analytical and computational frameworks for doing power spectral density (PSD) analysis of calcium T-type signals. For this purpose, we modeled a calcium T-type channel gating mechanism using a Markov scheme and formulated the calcium ionic current with the help of some empirical data. Our analysis shows calcium T-type current fades out rapidly at an exponential rate when frequency is increased but decreases almost linearly with depolarization of the membrane.**

***Keywords*—Frequency Analysis, Power Spectral Density, Neurons, Calcium Signaling, Calcium T-type Channels, Calcium Markov Process, Calcium Monte Carlo Simulation.**

I. INTRODUCTION

Ionized calcium (CAI) is perhaps the most typical signal transduction element in all of biology. In general, calcium signals (i.e., changes in intracellular calcium concentration $\left[Ca^{2+}\right]_i$ over the time) are regarded as universal signals that regulate many critical cellular processes, such as gene expression, proliferation, fertilization, differentiation, contraction, and apoptosis [1] [2]. As for neurons, calcium signals transduce the membrane potential into actions in every level of the nervous system, including cellular and molecular circuits and systems and cognitive and behavioral levels. For instance, a proper calcium signal initiates a signaling pathway that changes synaptic strength through long-term potentiation (LTP) and long-term depression (LTD) processes [3], builds memory circuits (engram) in the brain, and eventually brings about learning and memory functions in mammalian species [3].

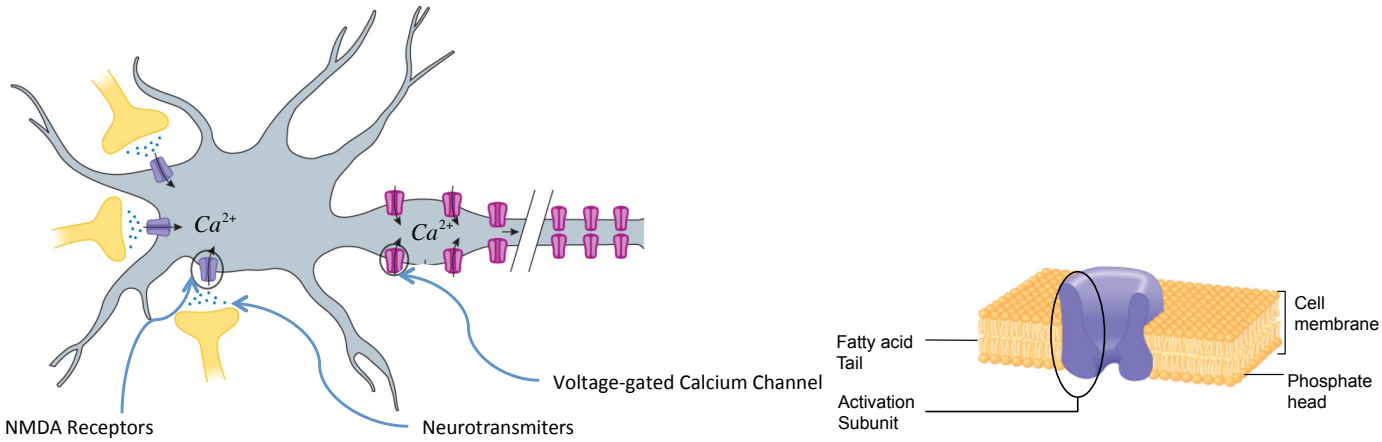
Any irregularity in calcium signaling can cause physiological and behavioral problems, such as epilepsy seizures [4], elevated anxiety, and impaired memory [5]. Interestingly, extracellular calcium signals (i.e., fluctuations of ionized calcium outside the neurons) are known to bear intercellular information [6]. Therefore, calcium signals can be modulated by various types of cellular information to regulate a variety of functions in every levels of the nervous system. The neuronal calcium signals are actually modulated by the mechanisms that control ionized calcium influx into the neurons. These mechanisms comprise special types of protein called calcium ion channels. The calcium ion channels that regulate calcium influx are either ligand-gated N-methyl-D-aspartic acid (NMDA) channels (NMDA receptors) or voltage-gated ion channels. Fig.1a illustrates that receiving extracellular ligands (e.g., neurotransmitters) from the axon terminals of presynaptic neurons causes the ligand-gated NMDA channels in a postsynaptic neuron to regulate calcium influx, while the voltage-gated calcium channels conduct calcium influx based on the neuron's membrane potential. Fig.1b also illustrates that a calcium channel is basically a transmembrane pore that makes a conductance pathway between the two sides of the neuron membrane that is electrically isolated by a layer of fatty acid. Please note that the figure shows a basic structure scheme for both ligand-gated and voltage-gated channels. As we explain later in this section, the voltage-gated T-type calcium channels have a bit more complicated structure.

There are two subtypes of voltage-dependent calcium channels known to be mainstreams in neural activities, namely, T-type and L-type channels. The T-type calcium channels are activated with a small amount of membrane depolarization as we will discuss in more details in Section.II. In contrast, L-type calcium channels are activated by the high voltage of action potentials and have a longer time of activation. The T-type calcium oscillatory signals—the subject of discussion in this paper—are known to regulate thalamocortical rhythms of sleep [7] and performing network synchrony to coordinate burst-firing among the neurons [4].

Although many studies have been dedicated to identifying the roles that calcium signals play in the nervous system, few attempts have been made to understand the important attributes of calcium signaling such as how calcium signals are modulated by the neuronal intracellular information, and what are the underlying mechanisms for the calcium signal modulation. In order to answer such important questions about calcium signaling, it is necessary to perform frequency analysis to acquire knowledge about the frequency characteristics of calcium signals.

Unfortunately, there is a significant gap in the literature in providing the analytical and computational foundations of calcium signal frequency analysis, even though the literature has already recognized the important role of frequency char-

978-1-5090-4767-3/17/$31.00 ©2017 IEEE



(a) Different Types of Calcium Channels

(b) Calcium Channel Basic Structure

Fig. 1. Calcium Channel Different Types and Basic Structure

acteristics in calcium signaling. For instance, [8] discusses the ability of calcium signals to operate through frequency modulation (FM) to piggyback information across a cell. However, no frequency analysis of calcium signal is presented and hence, valuable information, such as the proper frequency range that is used used by the cell to modulate the calcium signal, is missing. In [9], an empirical framework is proposed to obtain the power spectral density (PSD) of calcium signals from single-cell-recorded data. Many benefits of analyzing calcium signals using PSD can be imagined. For example in [10], PSD analysis is used to investigate the heart cells spontaneous activities happening due to calcium signaling in cerebellar Purkinje cells. However, obtaining a calcium signal using PSD with in vitro approaches like the one presented in [9] is a rather tedious job with the results varying from trial to trial.

In this paper, we formulate an analytical framework to obtain neuronal calcium T-type signals using PSD. We also perform PSD analysis of calcium T-type signals by performing a Monte Carlo simulation. We focus our study on the behaviors and properties of the mechanisms that modulate calcium T-type signals in the cellular and molecular levels of the nervous system (i.e., the T-type calcium ion channels in the neurons). The dynamics of these mechanisms determine the frequency characteristics of the calcium signal and synthesize the effects on operations and functions within the upper levels of the nervous system. Hence, performing a PSD frequency analysis at the cellular and molecular levels helps to identify the aspects that impact functions and behaviors within the upper levels of the nervous system. For example, knowing the frequency at which calcium T-type is highly modulated in a neuron helps to make hypotheses about the spiking frequency that most likely modifies synaptic strength and modulate learning and memory.

The rest of this paper is organized as follows. In Section II,we discuss the structure of calcium T-type channels as well as the membrane voltage range in which they operate. The gating mechanisms and behaviors of T-type calcium channels are explained and modeled in Section III. In Section IV, we provide and interpret the results of our Monte Carlo simulation of T-type calcium channel. We conclude our work in Section V.

II. Calcium T-type Channel

The calcium T-type channels are known as low-voltage gating channels. It means that they conduct calcium inux when the neurons membrane is at depolarized subthreshold potentials. The threshold here is considered to be a certain membrane potential at which the neuron spikes action potential(s). Although neurons do not spike action potential in response to subthreshold depolarizing oscillations in their membrane, they generate oscillatory graded potentials. These oscillatory graded potentials conserve frequency components of the original membrane oscillations and attenuate while they propagate through the neuron membrane as if they are going through a frequency-selective load impedance [11] [12].

In fact, individual neurons in complex neuronal assemblies (e.g., the brain) receive oscillatory subthreshold waves all of the time due to the properties of both neural circuits and single neuron cells [13]. The calcium T-type channels transduce these subthreshold oscillations of the membrane into action by modulating calcium inux according to the frequency of the oscillations and the local membrane potential. Therefore, the frequency and amplitude of calcium T-type signals is correlated to the frequency and magnitude of subthreshold oscillations in the nervous system.

There are several different structural configurations suggested in the literature for the number of activation and inactivation gates from which we have adopted the configuration proposed in [14] and [15]. This configuration suggests that the structure of a calcium T-type ion channel to be consisted of the assembly of two activation subunits as Fig.1b illustrates. These activation subunits sometimes are called α subunits as they are made of α proteins. A calcium T-type channel also has an inactivation subunit as we explain later in Section III. These subunits are sometimes called gates due to the role they play in regulating the ion ux in or out of a cell. An increase in the membrane depolarization can cause an activation gate to open and may make an inactivation gate close. Fig. 2 is a schematic diagram of the structure of a calcium T-type channel. The activation subunits can be imagined as the side walls of the channel as it was illustrated in Fig. 1b. The inactivation subunit is located at the bottom of the channel's conduit and is represented by a ball-shaped object in the figure.

In Fig. 2a, the channel is shown in its closed state in which the both activation gates are closed and consequently, no ion flux occurs. Most calcium channels are closed in the resting state of a neuron in the absence of depolarization stimuli. Fig. 2b illustrates the state in which the activation gates are opened and the inactivation gate is not yet closed. This occurs when the membrane voltage is depolarized but not big enough to trigger the inactivation gate. In this case, the channel conducts a calcium ion inux. This is the case when the membrane receives some depolarizing stimuli which triggers activation gates, but it is not enough to stimulate the inactivation gate. Fig. 2a shows the channel when the inactivation gate is engaged. As the figure illustrates, even though the activation gates are open, the channel does not conduct ion flux as it is blocked by the operation of the inactivation gate.

III. Modeling Calcium T-type Channels

In this section, we model the way that the gating behaviors of the calcium T-type channels affect the modulation of calcium T-type signal. More specifically, these gating behaviors determine how the frequency and magnitude of changes in the membrane voltage are translated into calcium influx current. It is noteworthy to mention that in general, three ion types are known to be more effective in neuronal activities, namely, sodium, potassium, and calcium.

The first two ion types affect the generation and propagation of action potentials. Their gating behavior is illustrated by the widely recognized Hodgkin-Huxley (HH) model [16]. However, the HH model doesn't account for a number of biophysical facts, such as firing burst(s) of action potentials in response to a long-lasting input stimuli [15]. This is because the HH model does not take into account the calcium ion effect activities—and it was not intended to do so as it is based on the squad giant axon which has no calcium channels.

We adopted nomenclature that the HH model uses to model the gating behavior of a sodium channel. We know from Section II that the calcium channel model is comprised of two activation gates and one inactivation gate. We define m as an activation variable and h as an inactivation variable. These variables encode the state of their related gates between close and open states. Therefore, all conformational states of the calcium channel subunits can be encoded as m^2h in which two m variables (i.e., $m*m$) encode the combination of four states of the two activation gates; and one h variable encodes two states of the inactivation gate. This makes a total of eight states. However, two states—in which one of the activation gates is open and the other on is closed—are essentially the same. Therefore, the total number of states for the calcium channel reduces to six states.

A Markov chain can represent these six states along with the transition rules between each pair of states. Such a Markov chain is illustrated in Fig. 3. In this Markov chain, the subscription of each variable denotes the number of open gates in its related gate category. For example, $[m_0h_1]$ denotes a state in which none of the two activation gates is open; but the inactivation gate is open. The Markov chain shown in Fig. 3 takes into account all of the possible states of activation and inactivation gates, as well as the transition rates between the states. In Fig. 3, $[m_2h_1]$ is the only state in which the channel is open and conducts ionic current. The Markov chain in Fig. 3 is organized from left to right to demonstrate an order in which two activation gates are closed, one activation gate is closed, and no activation gate is closed, respectively. In addition, the upper row presents the states in which the inactivation gate is open (Fig. 2a and Fig. 2b), and the lower row represents the states in which the inactivation gate is closed (i.e., Fig.2c).

There are also four rate constants presented in the above Markov chain. The $\alpha_m(v)$ is the rate of activation gates transiting from a closed to an open state. On the other hand, $\beta_m(v)$ is the rate of activation gates transiting from an open to a closed state as a function of membrane voltage v. Similar rate constants can be defined for the inactivation gate, (i.e., $\alpha_h(v)$ and $\beta_m(v)$). As the notations show, all rates are voltage dependent hence, the rates change based on the local membrane potential where the calcium ion channel resides. There are two ways imaginable to find these rate constants. One way is to use thermodynamic models, as explained in [14]. Another way is to carry out single channel recordings and fit a function to the resulting empirical data. We've selected the latter approach to have our analysis be closer to reality. Therefore, we used the experimental data provided in [14]. Given $\alpha_m(v)$ and $\beta_m(v)$, we are able to calculate the *steady-state activation constant* m_∞ and *activation time constant* τ_m as follows:

$$m_\infty(v) = \frac{\alpha_m(v)}{\alpha_m(v)+\beta_m(v)}$$
$$\tau_m(v) = \frac{1}{\alpha_m(v)+\beta_m(v)} \quad (1)$$

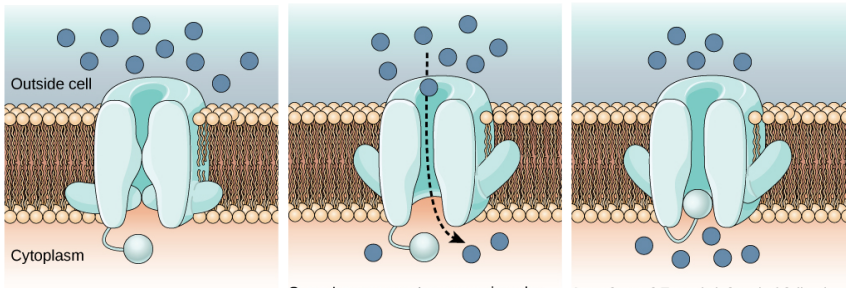
Similarly, we can write the equations of *steady-state inactivation constant* h_∞ and *inactivation time constant* τ_h as the followings:

$$h_\infty(v) = \frac{\alpha_h(v)}{\alpha_h(v)+\beta_h(v)}$$
$$\tau_h(v) = \frac{1}{\alpha_h(v)+\beta_h(v)} \quad (2)$$

In the above equations, steady-state activation/inactivation constants and activation/inactivation time constants have the same meaning as in the Hodgkin-Huxley model [16]. Due to the extreme imbalance between extracellular and intracellular calcium ion concentration, the calcium ionic current does not follow the Ohm rule (i.e., it is not a linear function of channel conductance). In this case, we must use the general theoretical framework laid by Goldman, Hodgkin, and Katz [17] [18] that determines the magnitude of an arbitrary ionic flux given the different ion types that move across the membrane independent of each other (i.e., they dont affect each others ux). We've already respected the above assumption as our Markov model includes activation and inactivation variables of the only calcium ion type. The GHK equation for calcium T-type ionic current can be written as follows:

$$I_T(v,t) = m^2(v,t)h(v,t)\bar{P}_{Ca^{2+}}g(v,[Ca_o],[Ca_i]) \quad (3)$$

In (3), $m(v,t)$ and $h(v,t)$ are functions of the voltage membrane and time. These functions reflect the ratio of open activation gates to all activation gates and the ratio of open inactivation



(a) Closed Channel (b) Open Channel (c) Inactivated Channel

Fig. 2. Ion Channel Structure

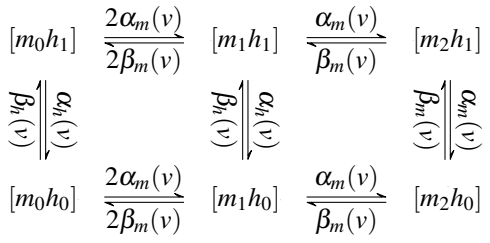


Fig. 3. T-type Calcium Channel Markov Chain

gates to all inactivation gates over the area of an isopotential patch of membrane, respectively. How to obtain these functions will be discussed later in this section. $\bar{P}_{Ca^{2+}}$ is the maximum permeability of the membrane to calcium mass. The value of $\bar{P}_{Ca^{2+}}$ is listed in Table I. One important element of (3) is the membrane calcium ion conductance which is represented by $g(v,[Ca_o],[Ca_i])$ and can be obtained from (4):

$$g(v,[Ca^{2+}]_o,[Ca^{2+}]_i) = \frac{z^2F^2v}{RT([Ca^{2+}]_o - [Ca^{2+}]_i)} \frac{e^{-zvF/RT}}{1-e^{-zvF/RT}} \tag{4}$$

The above equation represents the membrane calcium conductance as a nonlinear function of the membrane voltage v, calcium ion's extracellular concentration $[Ca_o]$ and calcium ion's intracellular concentrations $[Ca_i]$. Both values are listed in Table I. T is temperature in Kelvin and z is the number of valence electrons of calcium ion. The rest of (3) is the known physical constants; and their values are listed in Table I.

	Value	Unit
$\bar{P}_{Ca^{2+}}$	3	$\mathrm{cm\,s^{-1}}$
z	2	—
Ca_o	3	mmol
Ca_i	10	nmol
R	8.3144598	$\mathrm{m^2kg^2/s^2Kmol}$
F	96 485.3329	$\mathrm{Cmol^{-1}}$

TABLE I
THE VALUES OF GHK EQUATION'S CONSTANTS

The first step to obtain $m(v,t)$ is to determine the differential equation governing the kinetic scheme of an activation gate illustrated in Fig. 4 Therefore, we can write the differential equation of the activation gate as the follows:

$$\frac{dm}{dt} = \alpha_m(v)(1-m) - \beta_m(v)m \tag{5}$$

Fig. 4. The Activation Subunit Markov Chain

The solution of (5) gives $m(v,t)$ as the follows:

$$m(v,t) = m_\infty + (m_0 - m_\infty(v))e^{-t/\tau_m(v)} \tag{6}$$

Using a similar approach, we can obtain $h(v,t)$ as:

$$h(v,t) = h_\infty + (h_0 - h_\infty(v))e^{-t/\tau_h(v)} \tag{7}$$

We assume the initial values in (6) and (7) (i.e., m_0 and h_0) to be zero. Now we have to obtain the steady-state activation constant $m_\infty(v)$ and activation time constant $\tau_m(v)$. By using experimental data in [14] and the set of equations in (1), we obtain the following:

$$m_\infty(v) = \frac{1}{1+e^{(-v+57)/6.2}} \tag{8}$$

$$\tau_m(v) = 0.612 + \frac{1}{e^{-(v+132)/16.7}} + e^{(v+16.8)/18.2} \tag{9}$$

Similarly, by using the empirical data in [14] and (2) for steady-state inactivation constant $h_\infty(v)$ and inactivation time constant $\tau_m(v)$, we obtain the followings:

$$h_\infty(v) = \frac{1}{1+e^{(v+81)/4}} \tag{10}$$

$$\tau_h(v) = \begin{cases} 28 + \frac{1}{e^{-(v+22)/10.5}} + e^{(v+16.8)/18.2}, & \text{if } v \geq -81 \\ e^{(v+467)/66.6}, & \text{if } v < -81 \end{cases} \tag{11}$$

Please note that $\tau_h(v)$ is a conditional function based on the membrane voltage. Now that we have all the elements of E(3), we are able to calculate calcium T-type ionic current. In the next section, we present our frequency analysis results.

IV. Numerical Results

In this section, we present the numerical results of our Monte Carlo simulation of calcium T-type current based on the Markov chain in Fig.3. We found our simulation result compatible with the analytical framework that we provided in Section III. According to (3), two factors affect the calcium current, namely, membrane voltage and the time. This equation can be transformed to a Fourier domain to show the frequency characteristics of a calcium T-type signal as well as its dependence on local membrane potential. Fig. 5 visualizes our Monte Carlo result. It illustrates the behavior of calcium T-type current in response to the changes in membrane voltage in a range from a hyperpolarized state ($-90\,\mathrm{mV}$) to a depolarized

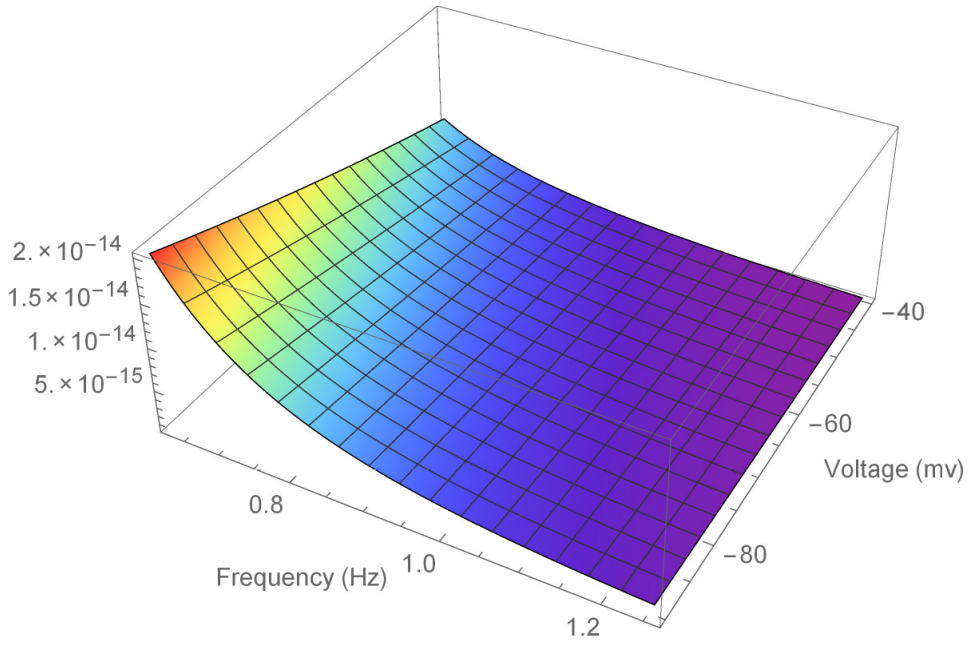


Fig. 5. The Analysis Result Of Calcium T-type Ionic Current

state (−40 mV). The figure also illustrates that the calcium T-type channel conductance decreases exponentially with the increase in frequency so that the channel becomes almost non-conductive around 1.5 Hz. As the figure demonstrates, the magnitude of the T-type calcium ionic current does not exceed 0.02 pA, which is a very small number. Please note that this number is just for a single calcium channel and there are many channels on the neurons' membrane.

V. Conclusion

We brought out the importance of investigating the gating behavior of neurobiological ionic channels to enhance both neuroscience and computer science. We explained how this behavior characterizes ionic currents. We focused on modeling the calcium T-type ionic current and provided an approach for modeling calcium channel gating by starting with the channel's Markov model and further formulations. We used our model to analyze the response of the ionic current to the changes in the membrane voltage from hyperpolarization to depolarization and to a range of frequencies. Our analysis showed that calcium T-type ionic current decreases with an increase in the stimulation frequency and membrane depolarization. The calcium ionic current also decreases almost linearly when the membrane is depolarized. This observation is compatible with biological facts as a high intracellular calcium concentration creates a force that stops more calcium influx even when calcium T-type channels are open.

References

[1] M. J. Berridge, M. D. Bootman, and H. L. Roderick, "Calcium signalling: dynamics, homeostasis and remodelling," *Nat Rev Mol Cell Biol*, vol. 4, no. 7, pp. 517–529, 2003.

[2] D. E. Clapham, "Calcium signaling," *Cell*, vol. 80, no. 2, pp. 259 – 268, 1995.

[3] S. M. Breedlove, N. V. Watson, and M. R. Rosenzweig, *Biological psychology: An introduction to behavioral, cognitive, and clinical neuroscience.* Sunderland, MA: Sinauer Associates, Inc. Publishers., 2010.

[4] S. M. Cain and T. P. Snutch, "T-type calcium channels in burst-firing, network synchrony, and epilepsy," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1828, no. 7, pp. 1572 – 1578, 2013.

[5] G. Gangarossa, S. Laffray, E. Bourinet, and E. Valjent, "T-type calcium channel cav3.2 deficient mice show elevated anxiety, impaired memory and reduced sensitivity to psychostimulants," *Frontiers in Behavioral Neuroscience*, vol. 8, p. 92, 2014.

[6] D. M. Egelman and P. R. Montague, "Computational properties of peridendritic calcium fluctuations," *Journal of Neuroscience*, vol. 18, no. 21, pp. 8580–8589, 1998.

[7] J. Lee and H. S. Shin, "T-type calcium channels and thalamocortical rhythms in sleep: a perspective from studies of t-type calcium channel knockout mice," *CNS Neurol Disord Drug Targets*, vol. 6, no. 1, pp. 63–9, 2007.

[8] M. J. Berridge, "The am and fm of calcium signalling," *Nature*, vol. 386, no. 6627, pp. 759–60, Apr 24 1997.

[9] P. Uhlén, "Spectral analysis of calcium oscillations," *Sci STKE*, vol. 2004, no. 258, p. pl15, 2004.

[10] "Fluctuations in membrane current driven by intracellular calcium in cardiac purkinje fibers," *Biophysical Journal*, vol. 38, no. 3, pp. 259 – 269, 1982.

[11] A. Khodaei and M. Pierobon, "An intra-body linear channel model based on neuronal subthreshold stimulation," in *2016 IEEE International Conference on Communications (ICC)*, 2016, pp. 1–7.

[12] ——, "Subthreshold linear modeling of dendritic trees: A computational approach," in *2016 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, Aug 2016, pp. 235–238.

[13] L. Ge and X.-d. Liu, "Electrical resonance with voltage-gated ion channels: perspectives from biophysical mechanisms and neural electrophysiology," *Acta Pharmacologica Sinica*, vol. 37, no. 1, pp. 67–74, 2016.

[14] G. Lemasson and R. Maex, *Computational neuroscience: Realistic modeling for experimentalists*. CRC Press, 2001, ch. Which Formalism to Use For Modeling Voltage-Dependent Conductances, pp. 149–178.

[15] C. Koch, *Biophysics of Computation: Information Processing in Single Neurons (Computational Neuroscience Series)*. New York, NY, USA: Oxford University Press, Inc., 2004.

[16] A. L. Hodgkin and A. F. Huxley, "A quantitative description of membrane current and its application to conduction and excitation in nerve," *J Physiol*, vol. 117, no. 4, pp. 500–44, 1952.

[17] D. E. Goldman, "Potential, impedance, and rectification in membranes," *The Journal of General Physiology*, vol. 27, no. 1, pp. 37–60, 1943.

[18] A. L. Hodgkin and B. Katz, "The effect of sodium ions on the electrical activity of the giant axon of the squid," *The Journal of Physiology*, vol. 108, no. 1, pp. 37–77, 1949.