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研究課題名（和文）プルキンエ細胞樹状突起の情報処理におけるイオンチャネル分布のはたす機能的役割

研究課題名（英文）The role of the ion channel distribution in dendritic computation of the cerebellar Purkinje cell

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研究成果の概要（和文）：小脳プルキンエ細胞を主モデルに、ニューロンの樹状突起でのイオンチャネルの確率的な相互作用を研究するためのコンピュテーショナル方法、プログラム、シミュレーションを開発。 局所的電流刺激に対する電位反応を利用して、樹状突起を機能的ユニットに区画化するアルゴリズムを開発し、樹状突起形態構造と局所的電位信号伝達の関係を研究。進化的アルゴリズムを用いたイオンチャネルモデル最適化プログラムの開発。並列STEPSシミュレーションエンジンを開発。これをスーパーコンピュータで使用し、樹状突起全体とその中での確率的プロセスを含むプルキンエ細胞のモデルをシミュレートして、細胞の局所的、全体的確率的挙動を観察。

研究成果の概要（英文）：We developed computational tools, methods, and simulation models to study the effect of stochastic ion channel interactions in dendrites, using the cerebellar Purkinje cell as a primary model. We developed a novel algorithm to discover “functional subunits” in a dendritic tree, based on the extent of depolarization by a synaptic input within each subunit. This demonstrated that neuronal morphology impacts how synaptic inputs and ion channels interact with each other via membrane potential, leading to nonlinear computation. We also developed a program to build a model of an ion channel from experimental data, by using the evolutionary algorithm. Finally, we developed a parallelized version of the STEPS simulator that can simulate stochastic processes in a neuron with complex 3D geometry, by using a cluster supercomputer. Using this, we ran a simulation of a Purkinje cell with a full dendritic tree, which vividly showed regional details of how stochasticity impacts cellular dynamics.

研究分野：　神経科学

キーワード：　計算論的神経科学　分子細胞神経科学　プルキンエ細胞　樹状突起　神経情報処理　イオンチャネル

１．研究開始当初の背景

Ion channels are fundamental building blocks of the active membrane and computational function of a neuron. Their dynamical activeness originates from the voltage-dependent and/or ligand-dependent gating.

In early days, it was controversial how many active ion channels are present in a dendritic tree, a major part of a neuron that is crucial for integrating synaptic inputs. Patch clamp studies revealed that many different types of active ion channels are abundant in dendrites (Llinas and Sugimori, J Physiol, 1980), contributing to active processing of synaptic inputs before reaching a site of action potential initiation, so-called, “dendritic computation” (Hofman et al, Nature, 1997; Mel, Neural Comput, 1994).

Crucially, ion channels often cooperate with each other to synergistically define certain computational functions. For example, voltage-dependent channels are affected by the membrane potential that are contributed by the current introduced by other channels. Calcium-activated potassium (KCa) channels are activated by calcium ions flowing through nearby calcium-permeating channels (Womack et al, J Neurosci, 2004). Also, some voltage-dependent potassium (Kv) channels have the Kv channel-interacting proteins (KChIP) that enable modulation of the activation/inactivation of the channels by binding of calcium ions from other channels (An et al, Nature, 2000).

These different types of interactions should lead to different characteristics. As an electric signal spreads widely and rapidly, the voltage-mediated interaction is relatively global, and therefore can be important in an extended domain. In contrast, the calcium-mediated interaction crucially depends on how calcium ions diffuse and react with calcium buffers, which sets how many calcium ions can effectively reach calcium-binding domains of a channel, and therefore becomes more local.

Another important aspect of such local interactions is that they can be quite stochastic. This is because the number of calcium ions that mediate the interaction can be low in a small domain in dendrites, and also because a single interaction is stochastic by itself (Anwar et al., J Neurosci, 2013).

However, there have not been many studies that comprehensively investigated how different ion channels interact locally and globally in dendrites. First, measuring activation certain ion channels in experiments is still very challenging in dendrites due to their size. Second, computational modeling has been also difficult since there has not been any tool to simulate ion channel activation and interaction with spatial dendritic geometry and also with full stochastic effects.

２．研究の目的

Our research aimed to develop computational methods and tools to investigate the interactions among active ion channels in neuronal dendrites, with emphases on elucidating the effects of dendritic geometry and stochasticity.

We aimed ultimately at building and running a neuron model with a full dendritic tree, reconstructed from imaging data, in a fully stochastic way, and compare the results with deterministic simulations, by using the cerebellar Purkinje cell (PC) as a model system. PC has a uniquely complex dendritic tree with spines, with an extensive distribution of calcium and KCa channels.

For this, we aimed to develop several computational tools for building models of active ion channels and a powerful computer simulation platform to run a stochastic simulation with morphological details that can make use of currently available supercomputers.

３．研究の方法

We developed the following computational tools:

1. ChannelTune is a computer software for building a computer model of an ion channel based on electrophysiological recording data. We built this by using the parallelized and multi-object evolutionary optimization algorithm by using a Python package *inspyred* (https://pythonhosted.org/inspyred) as the program is written in Python.
2. Parallel STEPS is an improved version of the Stochastic Engine for Pathway Simulation (STEPS; Hepburn et al, BMC Syst Biol, 2012), which can simulate stochastic cellular processes in a complex geometrical domain by using a parallel computational backend such as the MPI in a cluster supercomputer.
3. We developed and used the following computational models/simulations:
   a. Deterministic passive model: We ran

deterministic simulations of neuron models with various reconstructed morphologies and the passive membrane, and analyzed the data to characterize quantitatively how the voltage-mediated signaling between two points, evoked by a synaptic input, is affected by neuronal morphology.

b. Deterministic active model: We ran the same version of simulations with deterministically active neuron models, including the PC models (Anwar et al., J Neurosci, 2013; Zang et al., https://doi.org/10.1101/284026) and CA pyramidal neuron (Kim Y et al. 2015. Elife 4: e06414), and investigated how the results from the passive model can help the active model simulation.

c. Stochastic active model: We ran the stochastic simulation of the PC model (Anwar et al., J Neurosci, 2013), extended to the full dendritic tree, in the parallel STEPS simulator.

4．研究成果

1) Novel algorithm to characterize functional subunits in a dendritic tree:

We made two observations from the simulations of deterministic passive neuron models with various morphologies. First, a branching structure in a dendritic tree often puts a sharp limit on dendrite-to-dendrite current transfer. Second, if the transfer resistance from a point a to b is denoted by R_{ba} and a passive spatial impact of a current input at a is $\mathbf{R}_a = [R_{1a}, R_{2a}, \ldots]$, a non-passive contribution in the leading order is proportional to the overlap between two, $\mathbf{R}_b \cdot \mathbf{R}_a$, which can be used as a similarity measure to cluster and classify the input locations.

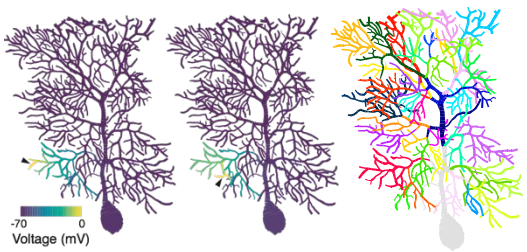

Figure 1. Left, Middle: Depolarization of dendrites with a local synaptic input (arrow). Note that the inputs with different locations evoke similar responses. **Right:** Functional subunits found by classifying the responses.

In this way, we found that the dendritic segments form distinctive "functional subunits." We also found dendritic morphology affects shapes of the functional subunits. In cortical pyramidal cells, most subunits were single branches, but PCs had many branched bundles as subunits.

How synaptic inputs are distributed with respect to functional subunits was a predictor of whether the inputs will be summed linearly or not. In the passive membrane case, the inputs within one subunit summed sublinearly, even though they were distributed to different branches. Simulation of the same model with active dendrites showed that synaptic inputs within one subunits better triggered a dendritic spike, leading to supralinear summation, compared to when the inputs were distributed to different subunits.

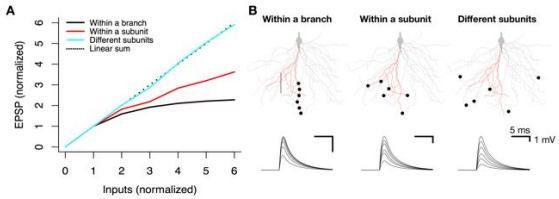

Figure 2. A. Summation EPSPs with distributed synaptic inputs in a passive CA1 pyramidal cell when activated synapses are in a single dendritic branch (black), different branches within a subunit (red), and different subunits (cyan), respectively. **B.** Top: Synapse locations (black dots) in each case. The red part is a subunit. Bottom: EPSPs at the soma.

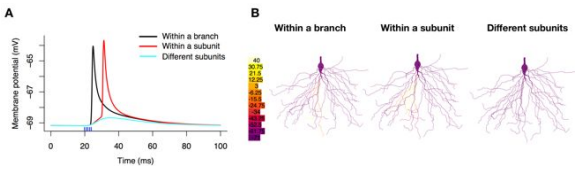

Figure 3. A. Generation of a dendritic spikes with distributed synaptic inputs in an active CA1 pyramidal neuron model. Blue lines on the x-axis represent spike inputs into synapses. Synapses are distributed in the same way as Fig. 2 in each case. **B.** Membrane potential at dendritic spike generation. t = 20.5 ms, 30.5 ms, and 30.5 ms, respectively.

Recent studies suggested that individual dendritic branches are functional subunits (Branco et al., Curr Opin Neurobiol 2010), and single (pyramidal) neurons can act like two-layer neural networks (Poirazi et al., Neuron, 2003). Our results show that a wide variety of neuronal morphology in neural systems can define the functional subunits differently across distinct types of neurons, tailoring the synaptic summation property differently. This work is currently

in preparation for journal publication.

2) Development of ChannelTune: We have written a custom optimization computer program for fitting parameters of an ion channel model to electrophysiology recording data, which we named *ChannelTune*. The optimization process was based on evolutionary strategy with multi-objective optimization, which enabled to deal flexibly with multiple current recordings with different voltage commands.

We tested the program by reconstructing a resurgent sodium channel model (Raman and Bean, J Neurosci, 2001) from simulated synthetic data, which is generated from a typical voltage-clamp step protocol where the membrane voltage was held at a certain voltage, ranging from -90 to 30 mV by an increment of 10 mV. In this test, the original values of the model parameters were successfully found both with a reasonable precision and within limited computational timeframe for all tested parameter sets (up to four free parameters). Multi-objective optimization using all 13 types of step stimulation produced solutions extremely close to the original values within the same number of optimization cycles. Increased number of free parameters posed additional complications for the optimizer and required longer computational time for achieving good precision.

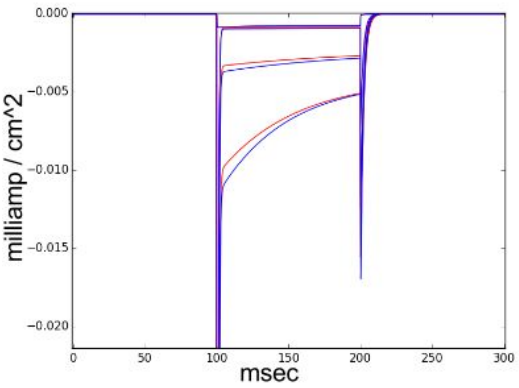

Figure 4. Red: target current traces produced with the original value ζ=0.03. ζ is the voltage-dependent rate for exiting from the open/blocked state, entered by the second mechanism of inactivation. 4 out of 13 traces are shown (middle part from top to bottom): -60, 30, 10, -10 mV. **Blue:** corresponding traces produced using the value of ζ=0.0341, optimized for -60 mV objective. The discrepancy for other traces is noticeably higher.

This software is publicly released under an open-source license (GPL v3.0) (https://github.com/CNS-OIST/channeltune).

3) Parallel STEPS: STEPS performs an exact stochastic simulation of reaction-diffusion systems in arbitrarily complex 3D geometries. The core simulation algorithm is an implementation of Gillespie's SSA, extended to deal with diffusion of molecules over the elements of a 3D tetrahedral mesh (TetOpSplit solver). It also supports accurate and efficient computation of local membrane potentials on tetrahedral meshes, with the addition of voltage-gated channels and currents (EField solver). Tight integration between the reaction-diffusion calculations and the tetrahedral mesh potentials allows detailed coupling between molecular activity and local electrical excitability.

In the latest 3.2.0 release, we added an early support for large scale parallel simulation of stochastic molecular-electrophysiological events in full cell morphologies, thanks to the new developments of the TetOpSplit parallel reaction-diffusion solver and the PETSc EField solver. In the following 3.3 release, both the stochastic molecular solution (Operator Splitting) and the separate electrophysiological solution (Finite Volume) of a simulation are distributed across all computing cores. However, the two solutions exhibit different performance and scalability. Generally, the voltage solution EField (EF) is faster but scales poorly after ~100 cores, whereas the Reaction-Diffusion (RD) solution requires more computation but scales well to 1000s cores. The upcoming 3.4 release provides a splitting scheme where computing cores can be split into Reaction-Diffusion cores and EField cores for better loading balance. Early results indicate that the core splitting approach can further speedup our full Purkinje dendrite simulation on 2000 cores from more than a day to less than two hours by a 1900/100 (RD/EF) splitting scheme.

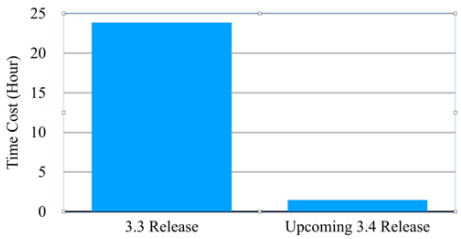

Figure 5. Time cost for a 60ms-long Purkinje cell dendrite simulation.

4) Stochastic simulation of the full cerebellar Purkinje cell: We simulated a Purkinje cell dendritic model (Anwar et al., J Neurosci, 2013) with synaptic (climbing fiber) activation in parallel STEPS. Whereas the 2013 simulations were run in serial STEPS on a scale of ~15% of the dendrite and in well-mixed compartments, with parallel STEPS we were able to simulate the entire dendritic tree to sub-micron resolution on a mesh of ~1M tetrahedrons. Even with the larger spatial scale and greater resolution, runtime was reduced by many orders of magnitude compared to serial STEPS.

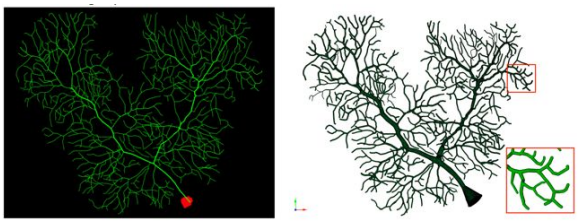

Figure 6. Left: Purkinje cell morphology (from neuromorpho.org, ID: NMO_35058). **Right:** Tetrahedral mesh reconstruction using the BBP mesh generation tool.

Similar to the smaller spatial scale of 2013, in the full dendrite we observed significant spatiotemporal variability in the voltage signal across trials (different random number seeds)

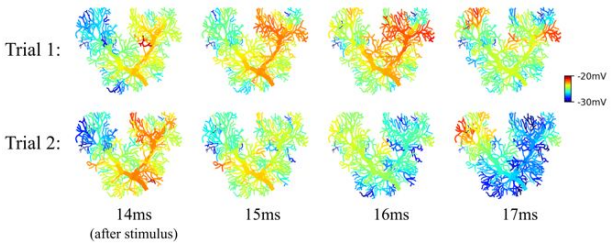

Figure 7. Trial-to-trial spatiotemporal variability in stochastic PC simulations. Two trials with an identical initial condition are shown.

STEPS is able to simulate full neuronal morphology with high geometrical accuracy and simulate chemical interactions, diffusion and voltage to sub-micron resolution. If appropriate HPC hardware is available, such neuronal models of high molecular detail can now be simulated in ~hours. Our early results indicate that this is a worthy avenue of research due to the high variability arising from stochastic processes in and around important signaling regions such as thin dendritic branches.

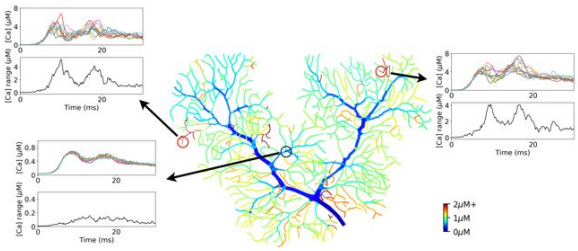

Figure 8. Strong trial-to-trial variability in the calcium signals in thin branches. Example calcium profile across the full dendrite for one trial at 17ms after stimulus, with insets showing the calcium profiles over 10 different trials (different colors) for the branches indicated, as well as the maximum range across those trials. The thinnest branches (red circles) show variability on the order of μM, although variability is lower for thicker branches (black circle).

Future improvements to our work will include utilizing a more detailed model and geometry, including spines and the somatic compartment. From a performance perspective, we will also pursue distributed meshing to reduce the memory footprint.

5．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計 4 件）

1. W. Chen and E. De Schutter (2017) Parallel STEPS: Large Scale Stochastic Spatial Reaction-Diffusion Simulation with High Performance Computers. *Frontiers in Neuroinformatics* 11: 13.
2. W. Chen and E. De Schutter (2017) Time to bring single neuron modeling into 3D. *Neuroinformatics* 15: 1-3.
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4. J. Myung, C. Schmal, S. Hong, Y. Tsukizawa, P. Rose, Y. Zhang, M. Holtzman, E. De Schutter, H. Herzel, G. Bordyugov and T. Takumi (2018) The Choroid Plexus is an Important Circadian Clock Component. *Nature Communications* 9: 1062.

〔学会発表〕（計 5 件）

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into functional subunits, Computational Neuroscience Meeting 2016 (Jeju, South Korea).
2. S. Hong, A. Takashima, E. De Schutter (2016) Impact of dendritic morphology on functional subunits in dendrites, Neuroscience 2016 (San Diego, CA, USA).
3. D. Han, S. Hong (2017) Heterogeneous layers stabilize propagation of a multiplexed spike signal in a feedforward network. Computational Neuroscience Meeting 2017 (Antwerp, Belgium).
4. S. Hong, D. Han, E. De Schutter (2017) Heterogeneous layers stabilize propagation of a spike signal in a feedforward network. Neuroscience 2017 (Washington DC, USA).
5. W. Chen, I. Hepburn, F. Casalegno, A. Devresse, A. Ovcharenko, F. Pereira, F. Delalondre, E. De Schutter (2017) STEPS 3: integrating stochastic molecular and electrophysiological neuron models in parallel simulation. Computational Neuroscience Meeting 2017 (Antwerp, Belgium).

〔図書〕（計　0　件）

〔産業財産権〕

○出願状況（計　0　件）

○取得状況（計　0　件）

〔その他〕
ホームページ等
1. STEPS project homepage: http://steps.sourceforge.net/STEPS/default.php
2. ChannelTune project homepage: https://github.com/CNS-OIST/channeltune

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