Prof. Włodzisław Duch
Department of Informatics,
Faculty of Physics, Astronomy and Informatics,
Nicolaus Copernicus University,
ul. Grudziądzka 5, 87-100 Toruń, Poland
Google: Wlodzislaw Duch

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Review of the PhD Thesis "Computational modeling of epileptic seizures in the brain", written by Mr. Damiano Gentiletti, under the supervision of dr hab. Piotr Suffczyński, Professor at the Institute of Experimental Physics, Warsaw University.

Physics has developed many branches useful to other scientific disciplines. Computational models in biophysics allow for investigation of complex systems, and help to understand pathological mechanisms responsible for many brain disorders. Such research is highly interdisciplinary, drawing on the biophysical models of neural functions, molecular physics, experimental data from neurophysiological experiments and psychiatric investigations, and medical research on therapeutic effects of drugs. In recent years computational neuroscience started to deliver detailed models that help to understand many brain functions and dysfunctions. Although such models cannot account for all biological details they can elucidate some mechanisms, reproduce experimental data, suggests new therapies and give insight into hypothetical scenarios that can be linked to real observations. Predictions of computational hypothesis may be validated using animal models of brain disorders, and human neuroimaging data or electrophysiological recordings. With the growing sophistication of software for neural modeling and the growing power of computers this field is certainly very important. Computational neuroscience is now at the foundations of many international, global projects, such as the Human Brain Project flagship of the European Union, Decade of the Brain and the BRAIN Initiative of the USA government, IEEE Brain, and other large-scale projects around the world.

The thesis of Mr. Gentilleti is devoted to the mechanism of epileptic seizure generation, investigated from computational perspective. This is one of the most important and promising subjects, because epilepsy affects about 80 million people worldwide. It is also found in most mammals, including dogs, cats and guinea pigs. This allows researchers to construct animal models of disease and investigate mechanisms of seizure generation in animal brains. Focal seizures are restricted to localized regions of the brain, while generalized seizures may spread over many brain areas. Not all kinds of epilepsy lead to seizures. Different types of epilepsy may result from abnormal brain activations in different regions and intensity, therefore over 15 different epilepsy syndromes have been identified. These syndromes do not cover more than half of all epilepsy cases. As one of the eminent American

neurologists once told me people want to have their names attached to syndromes, but clear-cut diagnosis distinguishing different epilepsy types is usually not possible. In most cases of epilepsy seizures may arise spontaneously, but in reflex epilepsy they may be triggered by flashing lights, noises, hyperventilation or stress. One should therefore not expect that a single model will be able to explain all variants of epilepsy.

Unfortunately this has not been mentioned in the introduction, focused on the general approach to epileptogenesis. Initially it was quite natural to assume, that the excitation/inhibition imbalance in neuronal networks is responsible for uncontrolled activity discharges. Strong excitation and low inhibition may result in the development of an epileptic discharge. Pyramidal neurons provide such stimulation and may be involved in reflex epilepsy. However, already in 1980 epileptic discharges in hippocampus were observed in vitro in the absence synaptic transmission. It was sufficient to add some more calcium into the intercellular space. Now several publications point out to different mechanisms that may initiate epileptic activity. The importance of electric fields and ionic flows have been proposed in 2014 paper by Mingming Zhang et al., who has written many paper on that subject in recent years. Very recently Pinotsis and Miller (Neuroimage, June 2022) showed how stable electric fields may transfer information between different brain areas, so this may be indeed an important phenomenon.

In section 1.3, discussing types of neural models, a "lumped neural models" name is introduced. Such name has been rarely, if ever, used in this field. Population models have not been mentioned. This is strange, because many interesting papers have been written on epilepsy using population models of the whole brain, for example Virtual Epileptic Patient (VEP) of Jirsa et al. (2017). However, such models are not designed to explain detailed mechanisms of seizures' generation, the main topic of Mr. Gentiletti thesis. Previous computational models used for this purpose assumed constant concentrations of ions in intra- and extra-cellular space. "Potassium accumulation hypothesis" has been formulated already half century ago. Experimental data showed correlation of changes in the extracellular K^+ concentration with the occurrence of epileptic seizures. The main goal of research under review was to understand the role of ion concentration dynamics in epileptic seizure generation, progression, and termination, using data from the *in vitro* experiments. This has been done using NMODL, code generation framework for the well-known NEURON neural simulator.

Epileptic discharges induced in the experimental preparation closely resemble the activity seen in human temporal lobe focal epilepsy. Chapter 2 describes experimental data collected in Milan by neurophysiologists working on the brain of the guinea pig. Proconvulsant drugs are used to induce seizures in entorhinal cortex region. This approach allows for measurement of extracellular potassium concentration and electric potentials in the perfused brain with largely preserved connectivity. Such *in toto* experiments provide results that are close to the *in vivo* situation and are used as the reference

animal model of human focal epilepsy. Intracranial recordings in humans show similar characteristics as those obtained from guinea pig brain.

At the onset of seizures in such preparation strong firing of the local interneuron is recorded, with no excitatory activity in pyramidal cells. The main hypothesis investigated in this thesis is summarized in Fig. 2.5. While strong interneuron activity inhibits excitatory neurons, Na^+ channels in interneurons open, allowing sodium ions to enter intracellular space and depolarize it. This leads to the opening of K^+ channels, sharp increase of the extracellular potassium concentration with respect to the baseline level, and repolarization of the cell membrane. After a delay of a few seconds pyramidal cells, stimulated by high K^+ concentration (Fig. 2.7), start irregular spiking. This activity changes into fast spiking, inducing seizures, and slowly reduces, due to synchronization with other neurons, into rhythmic bursting activity.

Chapter 3 describes minimal biophysical model used to analyze dynamics of the seizure generation. It is composed of a single pyramidal neuron and an inhibitory interneuron, embedded in a common extracellular space. The pyramidal cell has 6 compartments: one representing the soma, three representing the apical dendrites, and two the basal dendrites. The interneuron is also made of 6 compartments: one for soma, three for principal dendrites, and two for the remaining dendrites. Why model cells in Fig. 3.2 show only one dendrite? This construction allows for control of the flow of ion concentrations, including K^+, Na^+, Ca^{2+} and Cl^- due to the diffusion and Na/K pump processes, KCC2 transporter (neuron-specific chloride-extruder membrane protein), and glial buffering of extracellular K^+ ions. All these aspects are discussed in details, justifying the choices of parameters and approximations used in simulations. For example, three rate equations are needed for potassium buffering of the glial potassium uptake system. Thus, despite involving only a pair of neurons this model has many parameters, many types of currents, ion concentrations, synaptic and non-synaptic coupling mechanisms. Adding an Appendix with all parameters and equations written in one place could help to understand all aspects of this model (as it has been done in the bioRxiv preprint of Mr Gentilleti et al.). Not all parameters are well explained at this stage. For example, description of the model neurons does not mention specific receptors, like $GABA_A$ that inhibits pyramidal cells. More detailed drawing of all processes considered in these simulations would be helpful.

Section 3.6 presents simulation results of the minimal model. It is hard to estimate whether all parameters of such model are realistic, or how difficult it is to generate results that are qualitatively similar to experimental data. Values of parameters are given in real units, but they have to be rescaled and it is not clear how model was matched with the data. For example, initial depolarizing current injected to the soma of the interneuron drives it to fire spikes at about 300 Hz rate. The initial current was 1.3 nA, decreasing to 0.5 nA at the end of the simulation (60 seconds). It has an influence on the spiking rate and this particular choice has not been justified. Experimental value is 400 Hz and is significantly decrease after about 20 seconds, – why not increase current to match it? In case of

extended model the current was 0.8 nA, decreasing to 0.02 nA – how were these values established? Last time I have used simulator GENESIS (similar to NEURON) real values had to be rescaled for the model, allowing for some flexibility.

Results of the minimal model simulations are summarized in Fig. 3.3 to 3.11. Extracellular potassium concentration changes are quite similar to experimental, with sharp rise of the firing patterns of both cells that resemble experimental measurements. There are no surprises here, various components of currents behave as expected. The model allows for analysis of the role of glial buffer in clearance of extracellular potassium (Fig. 3.8), and diffusion of potassium to the brain bath (Fig. 3.9), showing interesting behaviors that cannot be experimentally investigated. Fixing concentrations of ions, and leaving only synaptic interactions blocks activity of pyramidal cells completely, failing to reproduce the experimental seizure patterns.

Minimal model does not account for the bursting phase that appears in the late part of experimental seizures. Chapter 4 is devoted to the extended model. In hippocampal circuits interneurons are about 10 times less frequent than pyramidal cells. To avoid high complexity of the model 4 pyramidal cells are used, with the same construction as in the minimal model. There is no information on computational cost of such simulations, how much time adding 9 cells will approximately require? The interneuron structure has been simplified here. It is composed of a single soma compartment, as its dendrites did not contribute significantly to the simulation results. The model has now 5 cells, embedded in the bath representing remote brain areas. To achieve electroneutrality impermeant anions are introduced. The size of the common extracellular space, determined by the ratio of extracellular to the total volume (including intracellular space), was fixed at experimental value 0.131 (was the same value also used in the minimal model?). This model has more complicated structure, presented in Fig. 4.4. All pyramidal cells receive 5 Hz background inputs in form of a Poisson spike trains, and have inhibitory $GABA_A$ synapses connected to the interneuron, that receives excitatory feedback through four excitatory AMPA synapses. In addition, each pyramidal neuron sends spikes to excitatory AMPA synapses in the other three neurons, simulating mutual excitatory interactions. Many detailed assumptions had been made, related to: radial diffusion of ions between shells around each neuron, calcium pump and buffer, dynamics of cell volume changes, initial concentrations of ions, spatial parameters used in calculations of local field potentials (LFP) based on integration of transmembrane currents in all cells. Altogether, this is really complex model that includes many experimentally determined parameters.

Simulation results of the extended model are presented in section 4.10. Simulation times go on to 180 seconds, about 3 times longer than in the minimal model. Injected currents are weaker and although potassium concentration rapidly grows seizure-like activity is delayed on about 10 seconds. Is there relation between network size, current strenght and increased timing? Burst activity appears and slows down with decreasing inhibitory current, finally leading to a 30-second period of very low amplitude

fluctuations, until background activity returns. Comparison with experimental results (Fig. 4.7) is quite convincing, matching LFP signal characteristics, pyramidal cells firing patterns and changes in concentration of extracellular potassium.

Stimulation of pyramidal cell did not result in LFP increase, in contrast with stimulation of interneuron and increase of K^+ concentration. Influence of chloride dynamics proved to be very important. Fixing the level of chloride concentration results in inhibition of the pyramidal cells by the interneuron discharge, so there is no excitatory activity. Bursting behavior appears when high K^+ concentration reduces currents responsible for membrane repolarization, and enhanced excitability leads to the generation of bursts, sustained by both transient and persistent sodium currents; longer depolarization activates the slow noninactivating muscarinic K^+ current I_{KM} in the soma, which increases during a single burst and eventually repolarizes the membrane, terminating the discharge. High concentration of K^+ ions initiates the next burst and the cycle repeats. Termination of this phase depends on the actvity of the Na/K pump (as summarized in Fig. 4.19). Neuronal excitability and high K^+ concentration is decreased due to the pump hyperpolarizing current in the bursting phase, followed by the intracellular sodium concentration build-up.

This complex behavior is observed when all factors are included in the model. Influence of individual components of the model was assessed by excluding them from simulations. Reduced models show that radial diffusion of K^+ ions is essential for generation of seizures, while diffusion of Na^+ ions between shells has small influence on simulation results. Leaving only synaptic interactions and strong inhibition by the interneuron blocks strong excitations and the development of seizures. Analysis of the interburst interval near the end of ictal period showed the exponential decay of the bursting frequency, quite similar to human temporal lobe epilepsy recordings, and guinea pig brain recordings.

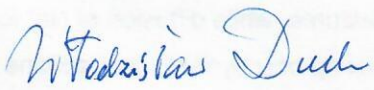
Simulations presented in this work lead to conclusions that control of the potassium ion concentration may be the key to control epileptic attacks. Nanoparticles that absorb such ions are one possibility. It is also worthwhile to analyze the influence of a diet. Several papers from the Gary Yellen laboratory at Harvard point to such possibility (for example, Ma, Berg, & Yellen, (2007). Ketogenic Diet Metabolites Reduce Firing in Central Neurons by Opening KATP Channels. J. of Neuroscience, 27, 3618).

Results of the research described in this thesis have been presented as posters at the BMC Neuroscience in 2015 and 2017, a longer book chapter (Suffczynski, Gentiletti, Gnatkovsky, de Curtis. In: Computational Neurology and Psychiatry, vol. 6 of Springer Series in Bio-/Neuroinformatics), and in the the International Journal of Neural Systems paper (2017), where Mr Gentiletti is the first author. The latest preprint article in bioRxiv is probably submitted as a journal paper somewhere, but there is no information about it.

This thesis is not an easy reading. It lacks a list of acronyms and abbreviations, such as various types of currents. Not all choices of model constructions and parameter values are justified. Not all values of parameters are provided. Instead, reference to the preprint in BioArxiv is given, making reading of this thesis harder than it could have been. This preprint presents all information in a more orderly manner. Based on the information provided it may not be possible to replicate simulations described here. It would help to summarize information about relative importance of different mechanisms included in simulations scattered in different sections.

Despite these shortcomings I do appreciate the amount of work that has been done to create such complex models, implement them, perform simulations and analyze them in details. This thesis is concerned with very important topic, and offers insight into mechanisms that may have an impact on our understanding of generation of seizures, and even point out potential therapies. It is a good example of interdisciplinary approach that requires integration of knowledge from biophysics, mathematical modeling, computational neuroscience, neurology and brain research. Therefore, I have no doubts that this thesis fulfills requirements for a doctorate dissertation, and Mr Gentilleti deserves to proceed with further steps to award him the PhD degree.

Sincerely yours,



Head of the Neuro-informatics and Artificial Intelligence group, University Center of Excellence "Dynamics, Mathematical Analysis and Artificial Intelligence.
Dept of Informatics, Faculty of Physics, Astronomy and Informatics,
Nicolaus Copernicus University, Toruń, Poland
Fellow, International Neural Network Society
Past President, European Neural Network Society