A Hybrid Model to Explore How a Hippocampal CA1 Neuronal Network Is Affected by Faulty Molecules of an Intraneuronal CREB Signaling Network

A. Emadi[1], A. Abdi[1,2], M. Migliore[3] and C. Mazzara[3]

1. Department of Electrical and Computer Engineering, New Jersey Institute of Technology, Newark, NJ, USA
2. Department of Biological Sciences, New Jersey Institute of Technology, Newark, NJ, USA
3. Institute of Biophysics, National Research Council, Palermo, Italy
{ae378, ali.abdi}@njit.edu, michele.migliore@cnr.it, camille.mazzara@ibf.cnr.it

***Abstract*—Analysis of intraneuronal signaling networks using systems biology approaches provides insights on how various molecules within each neuron may affect the behavior of the neuron under normal and pathological conditions. However, memory formation, learning and cognition are sophisticated functions of the human brain that not only depend on intraneuronal molecules and systems, but also emerge from the collective behavior of neurons in complex neuronal networks and the interneuronal processes among them. Therefore, understanding psychiatric and mental disorders where learning, memory or cognition are impaired, requires a hybrid modeling approach where both intraneuronal and interneuronal processes are included. In this paper, a hybrid model is introduced where a hippocampal CA1 neuronal network is considered, together with an intraneuronal signaling network that regulates the CREB (cAMP Response Element-Binding) protein. CREB is a transcription factor that is highly involved in cognitive and executive function of the human brain. Upon using the hybrid intraneuronal/interneuronal model, together with fault diagnosis analysis, we determine how neuronal excitability in the context of a neuronal network is affected, when there is one faulty – mutated or dysfunctional – molecule, or two concurrently faulty molecules. The hybrid approach allows to classify molecules into different classes, depending on how much they affect a neuronal network, when they are faulty. This has important applications in target discovery, since analysis of the hybrid model reveals which molecules or pairs of molecules result in substantial deviation from the normal network behavior, when they are faulty. Such molecules may be considered as proper targets, to develop effective therapeutics.**

Keywords— molecular networks, neuronal networks, CREB, signaling molecules, spike time dependent plasticity

I. INTRODUCTION

Memory formation, storage and retrieval involve various processes which are active areas of research, even though a large body of knowledge is accumulated over the past decades. Understanding such processes is highly relevant to memory-related disorders, to develop effective and proper therapeutic solutions. Nowadays, there is an ongoing challenge to bridge the gap between neuronal level molecular findings at one end and memory formation and learning in neuronal networks at the other end. Signaling molecules, including receptors, neurotransmitters, etc., play important roles in regulating neuronal functions, and their dysfunction can contribute to pathological conditions such as depression, schizophrenia and many more psychiatric and cognitive disorders. Discovering highly important signaling molecules using a variety of systems biology methods to analyze intracellular signaling networks is an effective approach to identify proper therapeutic targets [1]. Combining such intraneuronal studies and findings with neuronal network modeling and analyses is the goal of this paper, and has the potential to open new doors to target discovery and development of proper therapeutics.

Previously, we introduced a hybrid model [2] that combined interneuronal parameters such as neuronal activity and synaptic weight or strength with signaling responses of an intraneuronal network in which five neurotransmitters regulated the transcription factor cAMP Response Element Binding protein (CREB), where cAMP stands for cyclic adenosine monophosphate. CREB is known to be highly involved in cognitive functions of the human brain, learning and memory processes. The hybrid model was analyzed using an experimentally-verified molecular fault diagnosis approach [3] that assessed the impact of faulty – mutated or dysfunctional – signaling molecules on long term potentiation (LTP), which is an interneuronal process [4]. LTP is essentially the synaptic strength increase over a period of time among neurons, typically induced by an activity in the brain that generates high frequency stimulations.

In this paper, we extend our prior study now to a network of 100 neurons [5], to characterize the effect of faulty signaling molecules on neuronal excitability, in the context of a neuronal network.

The rest of the paper is organized as follows. The hybrid interneuronal-intraneuronal model for a single neuron, that is, the basic LTP-signaling model, is reviewed in Section II. Section III has three subsections and discusses the architecture and synaptic plasticity equations of the considered hippocampal CA1 neuronal network, followed by the introduced network hybrid LTP-signaling model, to compute the effect of faulty

molecules on synaptic plasticity and action potential spike counts of the network. Some concluding remarks are given in Section IV.

II. The Single Neuron Hybrid LTP-Signaling Model

In Figure 1, we present our previously introduced neuronal hybrid model [2], where a single neuron responds to an input stimulation and its output depends on the activity level of the transcription factor CREB, which is regulated by an intraneuronal signaling network of molecules. CREB is the output of the network, whereas the network inputs are these five important neurotransmitters: dopamine, serotonin, acetylcholine, adenosine and glutamate. The network itself consists of 51 intermediate molecules and 136 molecular interactions, all shown in [2], Figure 2. These 51 molecules are categorized into seven classes, according to the effect of each single faulty molecule on the CREB activity level [2], Figure S2A. Additionally, based on how much each pair of concurrently faulty molecules affect the CREB activity level, all the 1275 pairs of molecules are divided into twenty nine classes [2], Figure S2B. Looking at multiple concurrently faulty molecules is supported by the pharmaceutical industry findings that multi-target drugs, e.g., multi-kinase inhibitors, may serve as more effective treatments [1].

To form the hybrid model [2], the Bienenstock-Cooper-Munro (BCM) model of synaptic plasticity is used, by modulating the LTP threshold using various CREB activity levels. This allows to compute changes in an important interneuronal parameter such as the synaptic weight, for single and double faulty molecules [2], Figure 7. The results indicate that there are some single and pairs of molecules whose dysfunction can cause noticeable departure from the control or wild-type case.

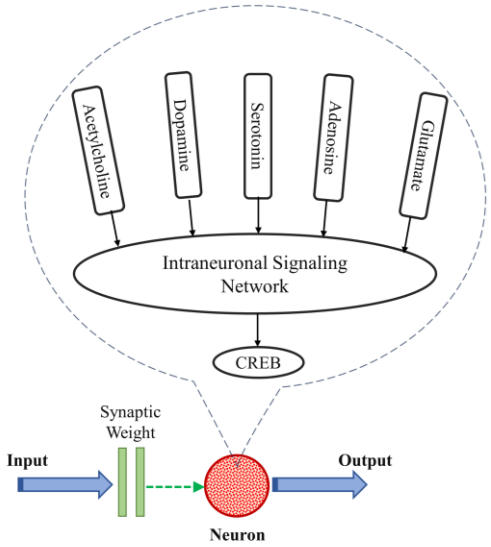


Figure 1. Schematic of the introduced hybrid interneuronal-intraneuronal model of a neuron.

While the above results are of interest, they are limited only to a single neuron, and it is unknown how to compute and study them for a network of neurons. It is the main goal of this paper to develop and analyze a network level hybrid LTP-signaling model, as detailed in the next section.

III. The Hippocampal CA1 Neuronal Network

A. Network Architecture

The considered network [5], Figure 1A consists of 100 CA1 neurons and four different types of inhibitory neurons where each one is responsible for a specific function in memory formation. There are three major inputs: 100 excitatory inputs of CA3 Schaffer collateral, 20 excitatory inputs from EC and 10 inhibitory inputs from septum [5], Figure 1A. According to [6] and [7], the excitatory inputs, CA3 and EC, are modeled as bursts of synaptic excitation in gamma rhythm with an average frequency of 40 Hz that rides on the top of 100 cycles of a 4 Hz theta rhythm, and the septal input is modeled as a burst of synaptic excitation with an average frequency of 50 Hz and a length of 1/3 of a theta cycle.

The CA1 neurons in the network of [5] follow the structure of [8], and each cell involves several compartments: axon, soma, two sections for basal dendrites, three sections for trunk, and three sections for distal dendrite for either of the two branches [5]. All the ionic currents are taken from [9]. To simulate the network, we use its NEURON implementation available at http://senselab.med.yale.edu/ModelDB/.

B. Synaptic Plasticity in the Network

Synaptic strength or synaptic weight modeling in this paper follows a spike time dependent plasticity (STDP) rule where LTP depends on the timing of pre and post synaptic activities. As an asymmetrical temporal type of Hebbian rule, STDP can be triggered by the correlation of the pre and post synaptic activations, and specifies the peak synaptic conductance changes [4]. The variable peak conductance $g_{peak}(t)$, a measure of synaptic weight changes, is updated as follows:

$$g_{peak}(t) = w(t) + g_{peak}(0), \qquad (1)$$

where $w(t)$ represents the degree of potentiation such that:

$$w(t) = w(t-1)\left(1-(2\pi)^{-1/2}(d/\tau_{-})e^{-\frac{(\Delta t-\tau_M)^2}{2\tau_{-}^2}}\right), \Delta t < 0, \quad (2)$$

$$w(t) = w(t-1) + p\left(g_{\text{peak}}^{\max} - g_{\text{peak}}(0) - w(t-1)\right)e^{-\frac{\Delta t}{\tau_{+}}}, \Delta t > 0. \quad (3)$$

Note that $\Delta t = t_{\text{post}} - t_{\text{pre}}$, $\tau_{-} = 5$ msec, $\tau_M = -22$ msec, $\tau_{+} = 10$ msec, and p and d are the potentiation and depression constant parameters over a 16 sec time window, respectively, all coming from the experimental findings of [10].

C. *The Introduced Network Hybrid LTP-Signaling Model to Compute the Effect of Faulty Molecules on Synaptic Plasticity Parameter and Action Potential Spike Counts of the Network*

To incorporate the effect of various faulty – mutated or dysfunctional – signaling molecules on the considered neuronal network, we modulate the important parameter of maximum peak conductance [5] – a measure of synaptic weight – using a parameter β , as follows:

$$g_{\text{peak}}^{\max} = 1.64\,\beta\, g_{\text{peak}}(0) \ , \quad (4)$$

where for the initial value of the peak conductance we have $g_{\text{peak}}(0) = 0.45$ nS [5]. When there is no faulty molecule, we have $\beta = 1$ and Equation (4) reduces to the wild-type case in [5]. The parameter β is the normalized synaptic weight introduced and computed in [2], Figure 8A, for the seven different classes of single faulty molecules mentioned in the first paragraph of Section II. All the β values are listed in Table I, along with the associated $g_{\text{peak}}^{\max}$ values. We note that except for class 2 of single faulty molecules, all other classes of single faults introduce some changes in β , compared to the wild-type case.

Table 1. Seven Classes of Single Faulty Molecules, the Associated Synaptic Plasticity Parameters and Spike Counts

Class of single faulty molecules	β	$g_{\text{peak}}^{\max}$	**Spike count (Number of action potentials)**
wild-type	1	0.738	107
1	1.115	0.823	116
2	1	0.738	107
3	1.034	0.763	111
4	0.705	0.520	94
5	0.795	0.587	97
6	0.68	0.502	92
7	0.707	0.522	94

To study how a faulty molecule may affect the considered neuronal network behavior, we look at the membrane voltages. Figure 2 demonstrates the last 20 cycles of the entire 25 sec simulation for the somatic membrane voltage recorded from one of the CA1 pyramidal cells of the network, in response to 20 EC inputs and 100 CA3 inputs, obtained using the network simulator of [5]. We notice less spikes for the faulty case, compared to the fault-free (control or wild-type) case. In fact, the total number of spikes for the control case and over the entire 25 sec simulation is 107, whereas it is 92 when a molecule of class 6 is faulty in the intraneuronal signaling networks within the neurons. These and other spike counts are listed in Table I as well. We observe different changes in the spike counts, depending on which molecule is faulty. More specifically and compared to wild-type, classes 1 and 3 exhibit more spikes, classes 4-7 result in less spikes, and class 2 generates the same number of spikes. They correspond to $\beta > 1$, $\beta < 1$ and $\beta = 1$, respectively. Changes in the number of action potential spikes caused by CREB changes are studied and considered in papers such as [11], [12], [5], without looking into the roles of intraneuronal signaling molecules.

Using Table 1, the number of spikes, also known as the number of action potentials (APs), is graphed in Figure 3A for different classes of signaling molecules. As explained in the previous paragraphs, depending on which signaling molecule is faulty, the number of APs can change differently. To relate these AP changes to the

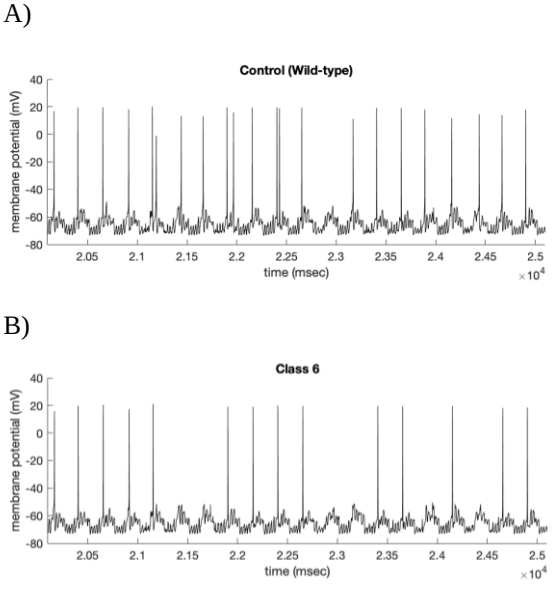


Figure 2. Somatic membrane voltage for one of the CA1 neurons showing the last 20 cycles of a 25 msec time course - cycles 80 to 100: **A)** Control or wild-type or fault-free case. **B)** The case where a molecule of class 6 is faulty in the intraneuronal signaling networks.

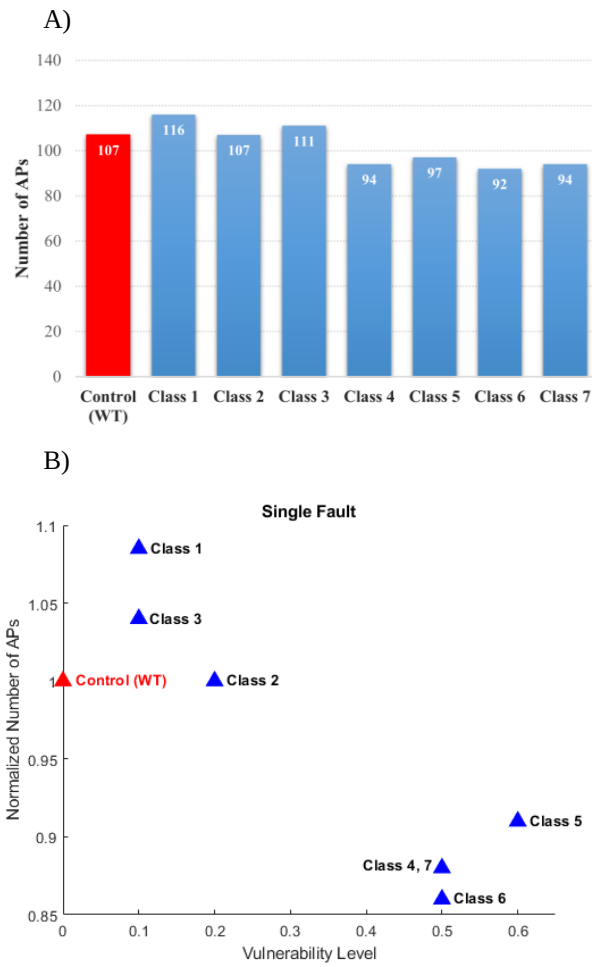


Figure 3. Neuronal excitability results of a hippocampal neuronal network [5], when there is one faulty molecule in the intraneuronal CREB signaling network. A) Number of action potentials (APs) for each class of single faulty molecules, as well as the control (wild-type) case. B) Normalized number of APs versus the vulnerability level for each single-fault class and the control case (where all the molecules are normal and fault free).

various roles that molecules play in the CREB intraneuronal signaling network, they are graphed in Figure 3B, in terms of the vulnerability levels of single-fault classes of molecules. The hybrid model analysis presented in Figure 3B is a combination of intraneuronal signaling network analysis and the interneuronal APs of a neuronal network.

The vulnerability levels of single faulty molecules of the CREB signaling network are computed and presented in [2], Figure S3A, and reproduced on the horizontal axis of Figure 3B here. They are computed using an experimentally-verified molecular fault diagnosis approach [3] [13]. A molecule is faulty (mutated or dysfunctional), when it fails to respond to the input signals properly, so that its output activity turns out to be incorrect. A high vulnerability level for a molecule means that the signaling network response is incorrect with high probability, when that specific molecule is faulty.

Inspection of Figure 3B reveals that higher faulty molecular vulnerabilities typically correspond to more changes in the number of APs. For instance, class 1 of faulty molecules with 0.1 vulnerability results in about 8% change in the number of APs, whereas class 6 with 0.5 vulnerability produces about 14% change.

One important application of the hybrid analysis presented in Figure 3B is that it allows us to distinguish among the molecules that exhibit the same vulnerability. This has important implications for therapeutic target discovery. For example, classes 4, 6, and 7 have the same vulnerability level of 0.5. From the intraneuronal vulnerability analysis perspective, these classes and their molecules are equivalent. However, using the hybrid analysis and as shown in Figure 3B, class 6 induces more changes in the number of APs, when a molecule from this class is faulty. Therefore, class 6 may suggest a more relevant set of molecules to consider for targeting, to develop therapeutics, since it seems to contribute more to the observed abnormal neuronal network behavior.

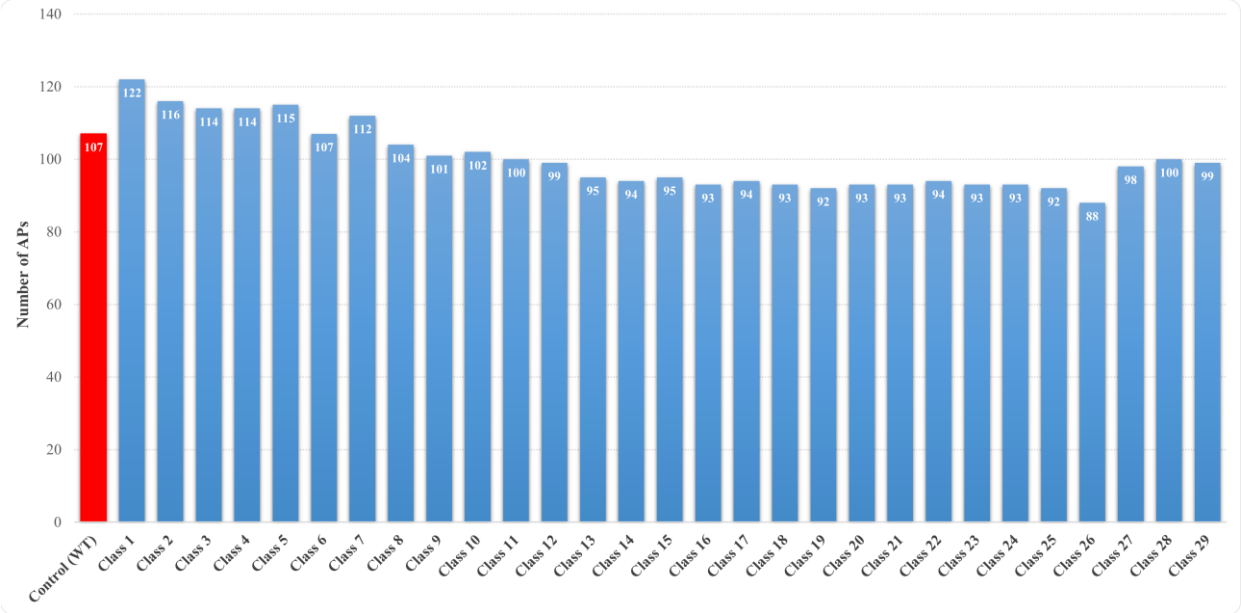
This capability of the proposed neuronal network hybrid analysis becomes more profound, when looking at two concurrently faulty molecules. Following the same method explained at the beginning of Subsection III.C to simulate the neuronal network of [5] for single faults, we perform simulations to study the effects of double faults. The parameter β in Equation (4) is taken from [2], Figure 8B, for the twenty nine different classes of double faulty molecules mentioned in the first paragraph of Section II. The number of APs is graphed in Figure 4A for these twenty nine classes of double faults. Compared to the single fault results in Figure 3A, for the double faults we observe larger variations. In fact, the number of APs for double faults in Figure 4A changes from 88 to 122, while for single faults in Figure 3A, it varies from 92 to 116.

To understand how these AP changes are associated with various double faults in the CREB intraneuronal signaling network, they are graphed in Figure 4B, in terms of the vulnerability levels of double-fault classes of molecules. The vulnerability levels of two concurrently faulty molecules of the CREB signaling network are computed and presented in [2], Figure S3B, and reproduced on the horizontal axis of Figure 4B here. They are computed using an experimentally-verified molecular fault diagnosis approach [3] [13]. We observe that higher double faulty molecular vulnerabilities

usually correspond to more changes in the number of APs. For example, while the class with 0.05 vulnerability in Figure 4B results in about 6% change in the number of APs with respect to the control case, class 26 with 0.8 vulnerability generates about 18% change.

The utility of the proposed hybrid model and approach becomes more visible, when it comes to distinguishing among pairs of molecules that have the same vulnerability level. For example, there exist 34 pairs of molecules in classes 27, 24, and 26, all with the same highest vulnerability of 0.8 in Figure 4B. However, by

A)



B)

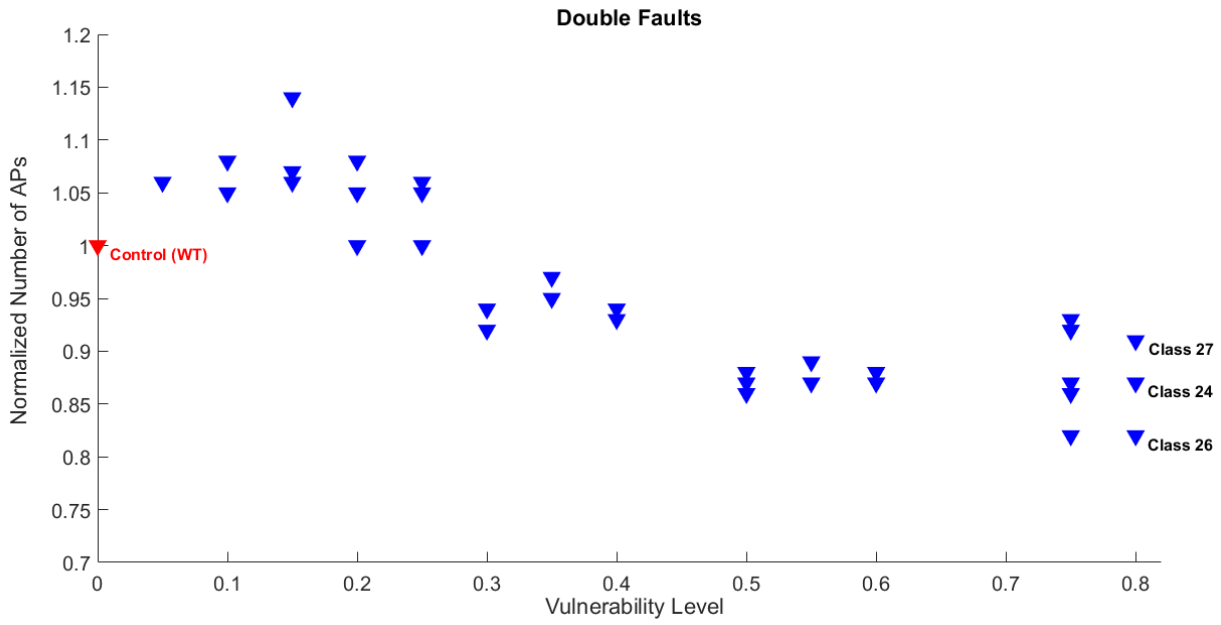


Figure 4. Neuronal excitability results of a hippocampal neuronal network [5], when there are two concurrently faulty molecules in the intraneuronal CREB signaling network. A) Number of action potentials (APs) for each class of double faulty molecules, as well as the control (wild-type) case. B) Normalized number of APs versus the vulnerability level for each double-fault class and the control case (where all the molecules are normal and fault free).

using the normalized number APs as a parameter to quantify the roles of various molecular pairs, we note that the pairs in class 26 render more changes in the number of APs, when they are faulty. Therefore, they may serve as better candidates for therapeutic targeting. This is of particular importance in target discovery, given the usefulness of multi-target drugs such as multi-kinase inhibitors [1].

Finally, in this paper we have focused on single and pairs of concurrently faulty molecules in a hybrid neuronal network model, to understand how they affect the network behavior. Using the approach of [14], one can extend this study to three or more concurrently faulty molecules.

IV. Limitations and Future Work

This study offers a comprehensive computational model to understand how disruptions in CREB signaling can impact the hippocampal CA1 neuronal network. However, the absence of direct experimental validation is a limitation. Collaborating with experimental labs in the future will be essential to confirm our findings and strengthen the model's reliability. Additionally, while we chose the BCM model and STDP to simulate synaptic plasticity, future studies could explore other plasticity models and test our approach on larger, more complex networks. Addressing these areas will help us expand our model's impact and biological relevance.

V. Conclusion

This paper introduces a hybrid model which is developed to explore the effect of various molecules of a CREB intracellular signaling network, in the context of a hippocampal CA1 neuronal network. The transcription factor CREB plays an essential role in cognitive and executive functions of the human brain. Analysis of this hybrid model allows to classify the intraneuronal molecules into different classes, on the extent to which their faulty behaviors affect certain aspects of the neuronal network, for example, the action potential spike counts.

The hybrid intraneuronal/interneuronal model is analyzed when there is only one faulty – dysfunctional or mutated – molecule, or when there are two concurrently faulty molecules. The results complement and expand the knowledge and information that can be obtained via analyzing only the intraneuronal signaling network. Looking at the roles of molecules in a two-dimensional intraneuronal/interneuronal feature space can provide a better understating of complex psychiatric and mental disorders such as depression, schizophrenia, bipolar disorder, etc. It can also assist with identifying potentially better therapeutic targets.

Acknowledgments

MM acknowledges a contribution from the Italian National Recovery and Resilience Plan (NRRP), M4C2 and funded by the European Union - NextGenerationEU (Project IR0000011, CUP B51E22000150006, EBRAINS-Italy.

References

[1] M. Ozen, E. S. Emamian and A. Abdi, "From data to knowledge: A mini-review on molecular network modeling and analysis for therapeutic target discovery," *Arch. Pharmacol. Ther.*, vol. 5, pp. 36-43, 2023.

[2] A. Emadi, M. Ozen and A. Abdi, "A hybrid model to study how late long term potentiation is affected by faulty molecules in an intraneuronal signaling network regulating transcription factor CREB," *Integrative Biology*, vol. 14, pp. 111-125, 2022.

[3] A. Abdi, M. B. Tahoori and E. S. Emamian, "Fault diagnosis engineering of digital circuits can identify vulnerable molecules in complex cellular pathways," *Science Signaling*, vol. 1, ra10, 2008.

[4] L. Benuskova and N. Kasabov, "Modeling L-LTP based on changes in concentration of pCREB transcription factor," *Neurocomputing*, vol. 70, pp. 2035-2040, 2007.

[5] D. Bianchi, P. D. Michele, C. Marchetti, B. Tirozzi, S. Cuomo, H. Marie and M. Migliore, "Effects of increasing CREB ‐dependent transcription on the storage and recall processes in a hippocampal CA1 microcircuit," *Hippocampus*, vol. 24, pp. 165-177, 2014.

[6] L. Fuentemilla, W. D. Penny, N. Cashdollar, N. Bunzeck and E. Düzel, "Theta-coupled periodic replay in working memory," *Current Biology*, vol. 20, pp. 606-612, 2010.

[7] J. Lisman, "Working memory: The importance of theta and gamma oscillations," *Current Biology*, vol. 20, R490-R492, 2010.

[8] V. Cutsuridis, S. Cobb and B. P. Graham, "Encoding and retrieval in a model of the hippocampal CA1 microcircuit," *Hippocampus*, vol. 20, pp. 423-446, 2010.

[9] D. Bianchi, A, Marasco, A. Limongiello, C. Marchetti, H. Marie, B. Tirozzi, et. al., "On the mechanisms underlying the depolarization block in the spiking dynamics of CA1 pyramidal neurons," *Journal of Computational Neuroscience*, vol. 33, pp. 207-225, 2012.

[10] M. Nishiyama, K. Hong, K. Mikoshiba, M. M. Poo and K. Kato, "Calcium stores regulate the polarity and input specificity of synaptic modification," *Nature*, vol. 408, pp. 584-588, 2000.

[11] M. Lopez de Armentia, D. Jancic, R. Olivares, J. M. Alarcon, E. R. Kandel and A. Barco, "cAMP response element-binding protein-mediated gene expression increases the intrinsic excitability of CA1 pyramidal neurons," *Journal of Neuroscience*, vol. 27, pp. 13909-13918, 2007.

[12] A. Emadi and A. Abdi, "A study of how abnormalities of the CREB protein affect a neuronal system and its signals: Modeling and analysis using experimental data," in *Proc. IEEE Signal Processing in Medicine and Biology Symp.*, Philadelphia, PA, 2022, pp. 1-6.

[13] I. Habibi, E. S. Emamian and A. Abdi, "Advanced fault diagnosis methods in molecular networks," *PLoS One*, vol. 9, p. e108830, 2014.

[14] M. Ozen, E. S. Emamian, and A. Abdi, "Exploring extreme signaling failures in intracellular molecular networks," *Computers in Biology and Medicine*, vol. 148, 105692, 2022.