Effects of astrocytic mechanisms on neuronal hyperexcitability

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Abstract— **While originally astrocytes have been thought to only act as support to neurons, recent studies have implicated them in multiple active roles, including the ability to moderate or alter neuronal firing patterns and to possibly be involved in both the prevention and propagation of epileptic seizures. In this study we propose a new model to incorporate pyramidal cells and interneurons (a common neural circuit in CA3 hippocampal slices) as well as a model of astrocyte. As both potassium and calcium ions have been shown to potentially affect neuronal hyperexcitability, the astrocytic model has both mechanisms – the clearance of potassium through potassium channels (such as K_{IR}, K_{DR} and sodium-potassium pump), and the influence of astrocyte in the synapse (forming the tripartite synapse with calcium-glutamate interactions). Preliminary findings of the model results show that when potassium conductances in the astrocyte are decreased, it results in the accumulation of extracellular potassium, leading to both spontaneous discharges and depolarization block, while the alteration of normal calcium response in the astrocyte can lead to just hyperexcitable conditions without the depolarization block.**

I. Introduction

Despite the fact that astrocytes are by far the most prevalent type of cell in the brain, their function has been mysterious. Originally thought to only provide support to neurons, such as protection and nutrients, recent findings in the past two decades have shown that astrocytes have a greater role in neural circuits. Astrocytes have been implicated in synapse formation via multiple signalling synaptogenic molecules, synapse maturation and pruning [1]. They also help manage signal-to-noise ratio in neural networks through calcium-glutamate feedback mechanism [2]. Finally, astrocytes have been shown to be able to moderate or alter neural firing patterns in response to local environment conditions [3].

Dysfunction in various astrocytic processes has been shown to be correlated with many neurophysiological disorders including epilepsy [4]. Several experiments and studies have explored the notion that elevated extracellular potassium concentration can lead to epilepsy and generate seizure-like activity [5]. Under normal circumstances with low level of neural activity potassium that is released into the extracellular space during action potentials is reabsorbed via Na^+/K^+-ATPase pump [4]. However, during periods of higher neural activity, sodium-potassium pumps get overwhelmed and extracellular K^+ concentration is maintained through a) spatial diffusion of extracellular potassium to areas of lower concentration [6] and b) glial potassium moderation, using inwardly rectifying Kir4.1 channels [3] or $Na^+/K^+/Cl^-$ cotransporter NKCC1 [7].

It has been suggested that elevated $[K^+]_o$ can lead to a bi-stable system in the neural circuit with alternating states of slow spiking with large number of neurons firing (driven by steady depolarization) and fast bursting activity, both characteristic of seizure-like events [8]. Additionally, an increase in intracellular chloride due to elevated $[K^+]_o$ appears to extend seizure duration and severity [9].

Additional influence that astrocytes exert on neural networks is in the area of synaptic connections. Astrocytes, via their processes connect to both the presynaptic and postsynaptic terminals forming the so-called "tripartite" synapse. Astrocytic processes react to glutamate release from presynaptic terminal into the synaptic cleft and release Ca^{2+} from ER stores due to glutamate-initiated IP3 production. Increase in the concentration of intracellular Ca^{2+} in the astrocytes causes glutamate release which results in the increase of presynaptic Ca^{2+} concentrations [2] and further depolarization of postsynaptic neuronal membrane [10]. On the other hand, several studies have shown that astrocytes in hyperexcitable networks respond with massive calcium release [11]. Moreover, they have been implicated in generating seizure-like events through excessive glutamate release, and antiepileptic drugs were found to suppress glial calcium signalling [6].

Despite these findings and general interest in the deeper insight into the biophysiological mechanisms behind epileptic seizures, computational modelling efforts have been focused more on complex neural networks without the significant inclusion of astrocytes. Nevertheless in the past decade new models with neuron-glial interactions have emerged. Kager and colleagues presented a model where the glial tissue enfolds the extracellular space and the neuron [12], and another model represented glial potassium buffering simply as a phenomenological effect [13]. Additionally, Nadkarni, Jung, and Levine presented a mathematical model for synaptic interactions between presynaptic and postsynaptic terminals and astrocytes [2], which was then followed by a more general model of tripartite synapse [10]. Several other variations of models exist each focusing on various aspect of neuron-glial processes. However, no current model fully accounts for potassium-calcium interactions in hyperexcitable conditions, especially considering the population of calcium-dependent K^+ channels in astrocytes [14] and complex interplay of stochastic effects in both potassium clearance and calcium-

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glutamate feedback loop in the tripartite synapse. By combining a computational representation of an astrocyte with various potassium clearance pathways and detailed processes involved in glutamate driven calcium dynamics with a prevalent minimal neural network, we aim to produce a biologically detailed model that would allow us to quantitatively assess both the degree of influence each factor has on neuronal hyperexcitability as well as their interactions.

II. Experimental Procedure

In order to evaluate our hypotheses we developed a neural circuit based on a previously described model by Stanley et al [15]. The circuit is a minimal neuronal network comprised of two neurons – specifically a pyramidal cell (PY) and an interneuron (IN). Both neurons consist of a soma and unbranched apical and basal dendrites, compartmentalized as per Traub's specifications [16,17]. In addition to the two neurons, an astrocyte is also added. The astrocyte is connected to both neurons as well as act as a sink for extracellular potassium concentration. All of the modelling was done in GENESIS 2.3 environment with standard simulation parameters.

A. Neuron Models

To describe the dynamics of neurons we used two modified compartmentalized models – for a hippocampal pyramidal cell (PY) and an interneuron (IN) – first described by Traub [16, 17 respectively]. Several key differences exist between Traub's models and what is used in this study. Dendrites are compartmentalized but are not branched for simplicity; they contain Ca^{2+} channels, as well as several different potassium channels – several K channels – delayed rectifier potassium channel (K_{DR}), afterhyperpolarization channel (K_{AHP}), A-type transient potassium channel (K_A), and calcium-dependent potassium channel (K_{Ca}). Altogether, each neuron contains thirteen compartments – 7 apical dendrites, 3 basal dendrites, 2 axon segments and a soma (see Fig. 1A).

To better represent the effect of sodium on the dynamics in the neural network, the generic sodium channel is replaced by the duo of transient (Na_T) and persistent (Na_P) sodium channels. Additionally, AMPA, GABA and NMDA receptors are added to both somatic and terminal dendritic compartments. This allows for proper glutamate signaling between the neurons. As reported by Stanley et al [15], NMDA receptors are also needed to represent low-frequency subthreshold noise in interneurons, while AMPA receptors allow for higher frequency neuronal activity.

In order to have stochastic subthreshold noise-like activity in the network (an important precursor to neural calcium dynamics), the majority of channels in the interneuron are designed to have Markovian kinetics. The details are covered fully in the modeling paper [15]. In this paper it will suffice to say that Markovian kinetics are represented by a Markov system with up to six states (Fig. 2) with precise number of states depending on the type of channel. As seen in (1), with the Markov system the ionic current is determined either by single channel conductance

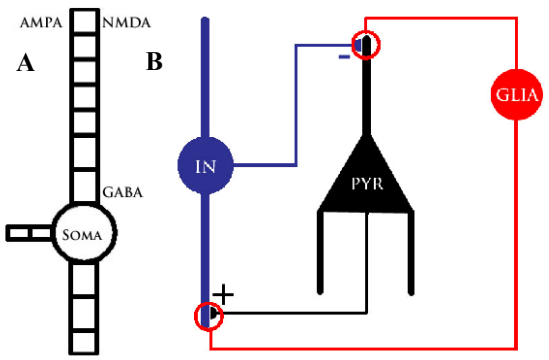

Figure 1: **A**) Schematic of a single neuron model; **B**) schematic of the whole neural network with interneuton (IN), pyramidal cell (PYR) and astrocyte (GLIA)

(γ) and number of open channels (N_O), or total ionic conductance and a percent of channels open.

$$I_{ion} = \gamma_{ion} * N_O * (V_m - E_{ion}) = g_{ion} * \frac{N_O}{N_T} * (V_m - E_{ion}) \quad (1)$$

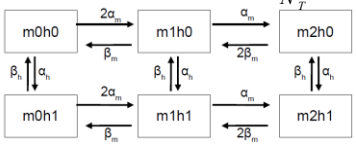

Figure 2: Representation of Markov system with six states, one of which (m2h1) is open [15]

C. Astrocyte Model

The astrocyte is represented by a single compartment soma with compartment size similar to that on a neuronal soma. The model includes only potassium channels with few notable exceptions – Na^+/K^+-ATPase pump which is modeled as a simple pump obeying Michaelis-Menten kinetics, and sodium-potassium-chloride co-transporter (NKCC1) which depends only ionic concentrations, as seen in (2) [7]. Other channels that are included in the model are - delayed rectifier potassium channel (K_{DR}), inwardly rectifying potassium channel (K_{IR}), A-type transient potassium channel (K_A), and calcium-dependent potassium channel (K_{Ca}). K_{IR} kinetics were described by Somjen [3] and are presented in (3) with minor modifications, while the rest of the channels have the same behaviour as their neuronal counterparts.

$$I_{NKCC\_1} = f\left(\left[K^+\right]_o\right) g_{NKCC\_1} \frac{RT}{F} \ln\left(\frac{\left[Na^+\right]_o \left[K^+\right]_o \left[Cl^-\right]_o^2}{\left[Na^+\right]_g \left[K^+\right]_g \left[Cl^-\right]_g^2}\right) \quad (2)$$

$$g_{Kir} = \frac{g_{\max}}{\sqrt{\left[K^+\right]_o \left(1 + \exp\left(\frac{V_m - V_h - E_K}{V_s}\right)\right)}} \quad (3)$$

Additionally, in order to properly model tripartite synapse, the astrocyte model contains synaptic calcium dynamics. Calcium dynamics are represented in three stages [10]. First of all, an ODE is used to model the production of secondary messenger (IP3) due to synaptic activity. Intracellular calcium is released in the astrocyte following the increase in the IP3. Finally, glutamate is released due to intracellular

calcium, and its effects both on the synapse (through elevation of pre-synaptic calcium) and the post-synaptic terminal through regular glutamate effects. In the latter case glial glutamate release effects are represented by an additional current to the postsynaptic neuron.

D. Minimal Neural Network

The minimal neural network is represented by a typical configuration found in CA3 region in hippocampus – a pyramidal cell connected to an interneuron with inhibitory feedback mechanisms. The astrocyte is connected to both synapses (PY to IN and IN to PY) as seen in Fig. 1B. The rest of the neural network system is represented by the stochastic Poisson process interacting with the pyramidal cell with a rate of 10 Hz. Several assumptions are made to simplify the network model. First of all, spatial diffusion is minimized (while still maintaining compartmentalized neuronal models) and only temporal changes are tracked. Additionally, in neurons it is assumed that the overall cation current is zero, meaning that Na^+ and K^+ currents are equal in magnitude. A third assumption is that there's a charge neutrality of extracellular space, which leads to the following representation of extracellular potassium concentration [7] in the form of (4), where $I_{K,g}$ is a sum of all glial potassium currents, including K_{IR} and sodium-potassium pump.

$$\frac{d[K^+]_o}{dt} = -\frac{d[Na^+]_o}{dt} - \frac{d[K^+]_g}{dt} = -I_{Na,T} - I_{Na,P} - I_{Na,leak} - I_{K,g} \quad (4)$$

Finally, unlike other models, equilibrium potentials for potassium channels dynamically calculated due to changes in extracellular potassium concentrations.

E. Model Validation

This network model is validated through analyzing its outputs compared both phenomenologically and statistically to the experimentally obtained recordings. After the model is calibrated using available physiological data, it is used to simulate hyperexcitable environment under various and varying conditions. Different conditions include disabling AHP potassium channel to both validate and investigate the role of that channel in blocking continuous depolarization; disabling active transport in the astrocyte to simulate compromised glial potassium uptake; high initial extracellular potassium and calcium concentrations; and varying rates of response of the astrocyte to calcium and glutamate release.

III. RESULTS

The results of the model for the first model validation stage can be seen in Fig. 3 and Fig. 4. During the normal functioning of the astrocyte, the pyramidal cell exhibits steady stochastic fluctuations of the somatic voltage with an occasional action potential in response to larger network (Fig. 3A). Since the interneuron in this minimal neural network is fast acting, it shows relatively steady and frequent bursting (Fig. 3B). One can also observe that there seems to be some sort of a cycle present in both pyramidal cell and the interneuron with around one second period – it is possible

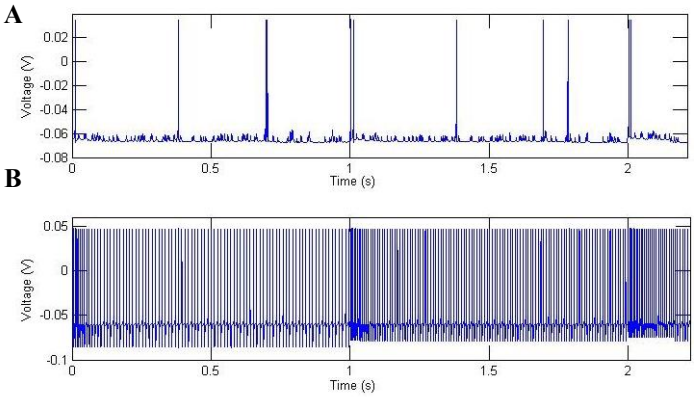

Figure 3: Normal recordings of somatic voltage in **A)** pyramidal cell and **B)** interneuron when connected in a minimal network with astrocytic effects

that this is a reflection of the influence of the astrocyte on the synapse and the signal (spike) transmission.

A. Potassium Clearance

After recording the physiological data, potassium clearance mechanisms in the astrocyte were compromised to represent the hypercitability of neural networks in epileptic models. While the $[K^+]_o$ started increasing almost immediately during hyperexcitable conditions (starting at 3 mM as per [7]), abnormal cell behavior can only be observed past the 1 second mark. We observed $[K^+]_o$ rising steadily, with spontaneous discharges beginning at around 10 mM; the concentration reached a plateau at 15.3 mM. As can be seen in Fig. 4A, the buildup of extracellular potassium is slow, as only after one second mark do frequent spike trains start appearing. Spontaneous discharges occur between around 1.1 and 3.2 seconds, at which point the depolarization block stops seizure-like activity.

Fig. 4B, 4C, and 4D show conductance for various channels. A-type potassium channel seems to have fluctuations in conductance prior to the onset of seizure-like activity (SLA) with a max of about 1 nS; during the SLA the general trend is transition from high individual conductance spikes (2 nS) to smaller trains of spikes. This is in contrast to Ca-dependent channel, which transitions from 10 nS small fluctuations to up to 100 nS during the later stage of spontaneous bursting. While the difference in conductance changes in AHP channel compared to other potassium channels is notable, it is important to highlight that both the onset and the cessation of SLA occurred when AHP conductance reached a plateau.

B. Tripartite Synapse

The response of the model to the altering of the effects of astrocytes and calcium-glutamate complex in the tripartite synapse is shown in the inset of Fig. 4. To achieve that, the magnitude of astrocyte response to synaptic activity

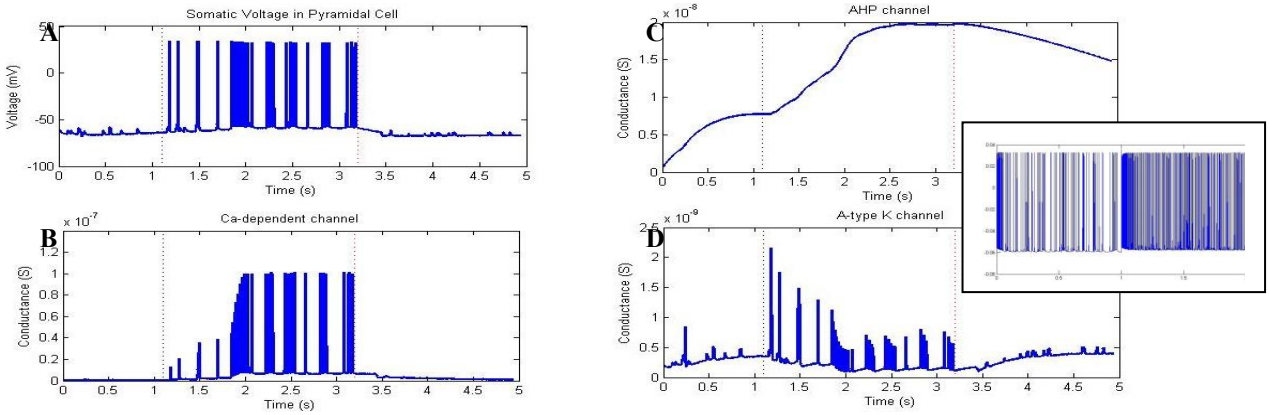

Figure 4: **A)** Somatic voltage from PYR, demonstrating spontaneous discharge region and the eventual cessation of seizure-like activity. **B-D)** Conductance recordings for calcium-dependent potassium channel, afterhyperpolarization (AHP) potassium channel, and A-type potassium channel respectively. In all graphs dotted vertical lines delineate seizure onset and offset. **Inset:** somatic voltage in PYR with altered calcium signaling in the astrocyte

and second messenger production are increased to simulate higher than normal calcium response. From the results, it appears that the somatic membrane voltage in the pyramidal cell behaves similarly to when it enters the spontaneous bursting phase due to the accumulation of extracellular potassium (frequent and relatively periodic spiking) with several key differences.

First of all, the change appears to occur at a much faster rate than during the potassium clearance failure, likely due to the fact that there's no ion accumulation, but the effects are directly on the spiking rate and sensitivity. Second, without the increased extracellular potassium (i.e. with normal clearance via astrocyte potassium channels), there appears to be no sign of depolarization block. This could help to explain the reasons behind the involvement of calcium in seizure prolongation and spread. Finally, it appears that there's a cycle in the somatic activity of the pyramidal cell with the period of about one second. This is similar to the normal activity; however each period appears to have a burst of activity (see around 0 – 0.05 seconds and 1 – 1.05 seconds). More insight might appear once the nature of the one-second cycle is studied to determine whether it is due to the astrocyte effects, positive feedback between the neurons or limitations of the model.

IV. Discussion

These results demonstrate that ionic homeostasis in the extracellular space of the neural networks is maintained, in part, by astrocytes – when astrocytic mechanisms are altered to invoke accumulation of ions (above 3-5 mM of K^+), epileptogenic conditions arise with rising channel conductances. This shines the light on the more impactful role that astrocytes could play in both generating hyperexcitability and affecting synaptic transmission. Our findings are further experimentally supported by recent research which shows that under epileptic conditions glial gap junctions are upregulated [18]. For further validation, model rhythms can be compared to epileptiform-related rhythms, looking for such parameters as reduced complexity, and gamma/high-frequency oscillations), all of which have been implicated in hyperexcitability.

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