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Production and diffusion model of Nitric Oxide for bioinspired spiking neural networks.

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***Abstract*— Nitric Oxide (NO) is an intracellular messenger whose diffusive properties enable an unconventional type of communication between neurons in the central nervous system that bypasses their anatomical connectivity. In this work, we modeled NO production and diffusion from a single source and investigated the range of action of the NO signal within a bioinspired spiking neural network. We found that a single active source will produce only a local effect on the individual synapse. While if multiple closely-located sources are active at the same time, NO will act more like a volume transmitter and influence even inactive synapses within that area. We focused our attention on the cerebellum's input layer, where NO is produced by the granule cells. In the granular layer, NO acts as a retrograde second messenger able to enhance pre-synaptic currents in the mossy fiber – granule cell synapses, thus potentiating them with long-term effects (LTP).**

I. Introduction

More than 30 years ago, the scientific community began to be aware of the fundamental role that Nitric Oxide (NO) plays inside the human body. Although NO could have noxious effects, in extremely controlled doses it is not only beneficial, but even necessary. It has been identified as a signalling molecule in many physiological processes, spacing from muscular relaxation to immune response, from vascular dilatation to neurovascular coupling and coordination of long-term memory. The latter implies that NO is somehow involved in plasticity mechanisms at the level of the central nervous system (CNS). Since the discovery of NO acting as an intracellular messenger in the brain [1], there is growing evidence that NO is responsible for the coordination of synaptic activity, both excitatory and inhibitory [2].

Cellular types that can produce NO molecules have been found in the cerebral cortex [3], hippocampus [4] and in the cerebellum [1], [5]. As NO synthesized in response to an external stimulus diffuses freely across the cell membrane, spreading rapidly in the extracellular space, it is able to provide a type of neural communication that goes beyond the mere synaptic transmission [6]. In the past few years, an increasingly number of studies (reviewed in [2]) suggested that certain stimulation patterns of a closely-packed group of neurons, containing neuronal NO synthase (nNOS) enzyme, may generate a diffuse cloud of NO, thus acting as a volume transmitter, with a relatively large area of influence. On the other hand, isolated stimuli would just lead to a local effect of the NO signal, exerting classical communication through single anatomical synaptic connection [7].

The aim of this work was to replicate the unique properties of NO as a second diffusible messenger [8] and study the volume transmitter effect in a spiking neural network. Therefore, we developed a model able to simulate the production and diffusion of NO molecules and study the NO signal in a realistic case scenario by placing NO sources in a 3D spiking cerebellar model [9]. We focused on the granular layer of the cerebellum, where NO notably acts as a retrograde messenger, being produced in the Granule Cells (GrCs) and regulating the neurotransmitter release probability of the mossy fiber (mf) terminals [5].

In the following section we present in details the procedure we adopted to build the model. Firstly, the set of differential equations that simulate the NO synthesis are illustrated, followed by the diffusion equation and its computation. Afterwards, we described how we placed the NO sources following the geometry of the cerebellar model developed in [9]. In Sec. III, we present the results obtained for the production equations, by comparing the results with simulations performed in NEURON environment [10], followed by the results obtained from simulations of NO diffusion when single sources are stimulated at different frequencies. We also report the results of the NO sources placement within the cerebellar granular layer. In the last section (Sec. IV), we discuss the issues related to the implementation of this model and the implications in studying NO diffusive effects.

II. Materials And Methods

All the equations and simulations have been implemented using Python 3.6.

Production Equations

The dynamic of NO production depends on complex biochemical reaction cascade[1] [8]. We split the reaction cascade in two parts and represented them with two differential equations: we designed eq. (1) for describing the Ca^{2+}/calmodulin

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[1]A spike train stimulates NMDA receptors. Ca^{2+} enters in the intracellular space through the NMDA receptor, increasing the intracellular concentration of Ca^{2+}. Ca^{2+} react with calmodulin and the concentration of Calm2C (calmodulin/calcium bounded) increase. This induces a catalytic activation of nNOS enzyme that starts producing NO using oxygen and reducing NADPH to catalyse the conversion of arginine to citrulline

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binding, and eq. (2) to describe the activation of nNOS enzyme.

$$\frac{dCalm2C(t)}{dt} = -\frac{Calm2C(t)}{\tau_c} + [Ca^{2+}]\delta_{spike}. \quad (1)$$

Where τ_c is a time constant describing the decay of Calm2C concentration after a spike of Ca^{2+} has entered the cell, $[Ca^{2+}]\delta_{spike}$ in the equation. We chose $\tau_c = 150$ ms and $[Ca^{2+}] = 1$.

The activation of nNOS enzyme is modelled with the following equation.

$$\frac{dnNOS(t)}{dt} = -\frac{nNOS(t)}{\tau_{n1}} + \frac{1}{\tau_{n2}}\left(\frac{Calm2C(t)}{Calm2C(t)+1}\right). \quad (2)$$

Where: $\tau_{n1} = 25$ ms and $\tau_{n2} = 200$ ms. Here we assumed that the amount of NO produced by a source is proportional to the amount of activated nNOS, given by eq. (2).

$$\frac{dNO(t)}{dt} = A\frac{dnNOS(t)}{dt}. \quad (3)$$

Where $A = 1.35\ 10^{-9}$. We chose parameter values that better replicate the simulations in NEURON (see Sec. III).

Diffusion Equation

To model NO diffusion, we used the heat diffusion equation, as in [11], [12], [13], where the solution is the NO concentration $C_{no}(\mathbf{x},t)$, in $\mathbf{x} = (x,y,z)$ at time t.

$$\frac{\partial C_{no}(\mathbf{x},t)}{\partial t} = D\nabla^2 C_{no}(\mathbf{x},t) - \lambda C_{no}(\mathbf{x},t) + S(\mathbf{x},t) \quad (4)$$

Where:

D is the diffusion coefficient. Thanks to low molecular weight and non-polarity, the NO can be considered to diffuse isotropically through the tissue, meaning that the diffusion coefficient is constant scalar. As reported in [12], we used a diffusion coefficient of $8.48 \times 10^{-10}\ m/s^2$.

$\lambda C_{no}(\mathbf{x},t)$ is the inactivation term. It represents a first-order reaction that governs the NO consumption in the brain tissue [7], and it is defined by a rate constant of $\lambda = 150\ s^{-1}$, equivalent to an half-life for the NO molecule of 4.6 ms (values taken from [12]). This inactivation term can be seen as a simple global loss function that allow to consider all background reactions involving the NO, i.e., with oxygen species and metals as well as with the *Heam* group of target sGC proteins.

$S(\mathbf{x},t)$ is a function describing the dynamics and location of NO sources (see Sec. II).

In order to solve eq. (4), we had to adopt some simplification on the geometry of the problem. First of all, we modelled each individual source of NO as a point source, from which the NO diffuses uniformly in all directions. Thus, we can safely assume radial symmetry to compute the diffusion profile in space. We are going to compute C_{no} with respect to the distance r from the source, not to $\mathbf{x}$ (3D Cartesian coordinates). This means that the source will have a fixed location in $r = 0$ and it will be described by just its evolution in time, hence $S(t)$. To represent the action of multiple sources we will simply sum each of their contribution, with respect to a given point of observation. Moreover, due to the rapid decay in the NO concentration (high inactivation rate), we can assume a finite domain with boundaries condition being $C_{no}(r,t) \approx 0$.

We computed the solution of eq. (4) by using the Green's function [14]. In our case given:

$$\frac{\partial C_{no}(r,t)}{\partial t} - D\nabla^2 C_{no}(r,t) + \lambda C_{no}(r,t) = S(t) \quad (5)$$

The corresponding Green's function is:

$$G(r,t) = H(t)\left(\frac{1}{4\pi Dt}\right)^{\frac{3}{2}} \exp\left[\frac{r^2}{4Dt} - \lambda t\right] \quad (6)$$

We have numerically integrated eq. (5) over time and obtained the spatiotemporal profile of NO signal from a single source. Thanks to the linearity of the problem, we can sum the contributions of each NO source, and evaluate the effect of the overall NO concentration in a specific point of the network.

Placing NO sources in a realistic cerebellar model

The production and diffusion model compute the NO signal for single sources. In order to replicate the volume transmitter properties of NO signal, as suggested in many researches [7], [2], [6], we started by placing the NO sources inside a network inspired by a realistic anatomy. Therefore, we adopted a recently developed cerebellar model [9], and extract information about the geometry and connectivity of the granular layer, where nNOS has been abundantly found [12]. We focused on the mossy fiber - granule cell synapse (mf-GrC) since it has been found to be implied in a long-term plasticity mechanism (LTP) that depends on NO concentration [5].

We know that nNOS is physically tethered to NMDA receptors through the PSD5 protein, 18 nm inside the cell membrane [7]. Considering the granular layer, nNOS is located in the postsynaptic terminals of GrCs that form synaptic connection with specialized structures called glomeruli. In [9], each glomerulus represents a mossy fiber terminal, and it is modelled as a neuron with a soma radius of 1.5 μm. The very last geometrical information we need to correctly locate the NO source is the one related to the synaptic cleft: about 20 nm [12].

Therefore, from the model topology we extracted the 3D coordinates of the center of each GrC and glomerulus soma, computed the directrix connecting the two centers, and place the NO source on this directrix, $1.5\ \mu m + 20\ nm + 18\ nm$ away from the glomerulus center, as shown in Fig. 1.

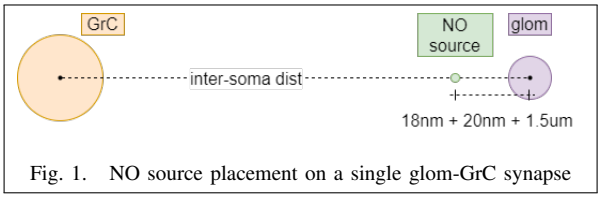

Fig. 1. NO source placement on a single glom-GrC synapse

Knowing the connectivity matrix between gloms and GrCs, we were able to place one source for each synapse:

since one glom is connected to a maximum of 28 different GrCs, each glom is surrounded by maximum 28 NO sources.

III. Results

Synthesis model

To validate the system of differential equations describing the synthesis process we compared the results with the ones obtained in a different simulation environment, NEURON [10]. This simulator allows to build detailed individual neuron properties, using compartmental models. In particular, we exploited its ability in simulating, with high level of realism, the biochemical reactions taking place in the intracellular space.

The biochemical cascade resulting in the NO synthesis has been triggered in both models by two 40 Hz burst stimuli 100 ms long, with a inter burst interval of 300 ms.

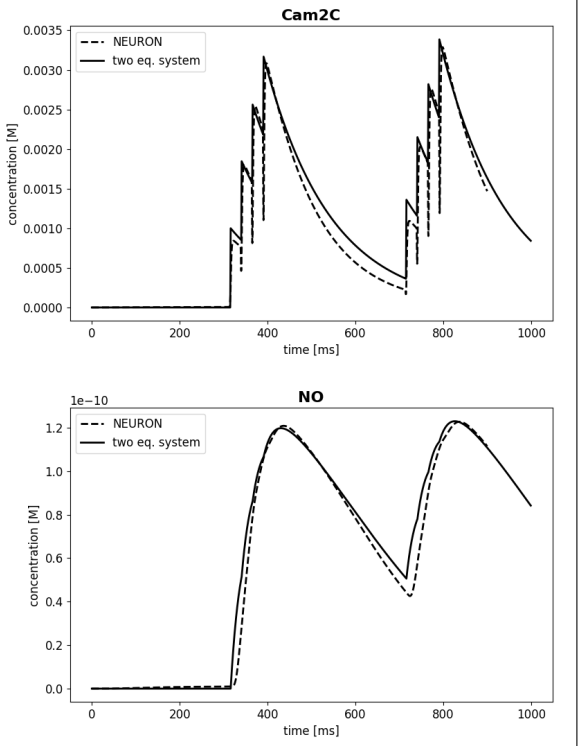

Fig. 2. Dashed lines refer to results obtained using NEURON simulation environment, while solid lines to our two-equations system. In the upper panel Ca2+/calmodulin concentrations are compared, while in the bottom panel the resulting NO concentrations produced by a single source.

Unlike the complex reaction cascade simulated in NEURON, our production function is able to replicate the synthesis process in two steps only, that depend on the timing of spike events, i.e., a binary variable. This implementation is compatible with information encoded in a spiking neural network: binary time series. Moreover, we are keeping the computational cost much lower with respect to the highly detailed simulation done in NEURON. Dealing with a large neuronal networks, the computational cost is required to be as low as possible.

Diffusion from a single source

Once we have obtained a reliable description of the production function, we simulated the diffusion from a single source. Fig. 3 shows the time and space profile of the NO signal, produced by single source stimulated at with different stimulation patterns: (1) single spike ($t_{spike} = 0$ ms); (2) 10 Hz burst 200 ms long; (3) 100 Hz burst 200 ms long; (4) 200 Hz burst 200 ms long. All stimuli have been delivered at $t_i = 0$ ms.

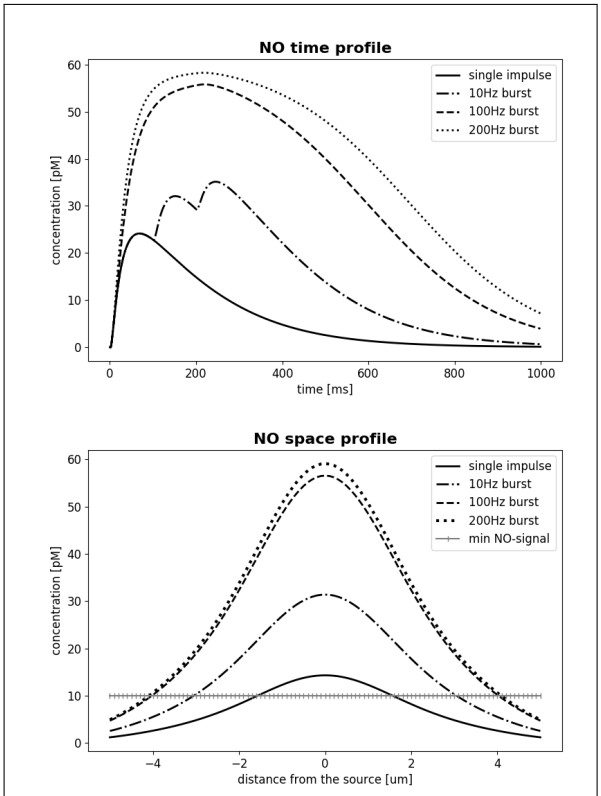

Fig. 3. Upper panel: Time profile of NO signal generated by a single source stimulated at different frequencies. The burst length is fixed at 200 ms. The observational point is located at 200 nm at the source. Bottom panel: Space profile of NO signal generated by a single source stimulated at different frequencies. All curves are referred to t = 210 ms. We reported the minimum concentration at 10 pM that is able to trigger NO dependent effects [7]

It is interesting to see how different stimulation frequencies result in a different distance covered by the NO signal (bottom panel in Fig. 3). In particular the higher the frequency, the more NO is produced, and the larger area is covered by the NO signal. However we reach a physiological saturation point in the amount of NO that can be produced and consequently diffused, between 100 Hz and 200 Hz.

This result is in accordance with the one obtained by [7], where a different method to compute the NO diffusion has been used.

Sources placement

Considering that average relative distance between the nearest mf-GrCs synapses is 11 μm [9], we investigated the area of influence of a single active source and compared

it with the area of influence of multiple active sources. A single active source, located closely to the GrC's dendritic membrane, will affect only the nearest mf terminal, even when stimulated at high frequencies. In Fig. 3 we can see that even when a GrC responds to a 200 Hz stimulus, lasting 200 ms, the amount of NO produced does not diffuse far enough to reach other synapses. However, if more than one GrC receives stimuli that are close in time the area of influence of the NO signal will expanse as a consequence of the sum of the amount of NO produced by neighbouring active sources depending both on the frequency of these stimuli and on the number of GrCs being simultaneously stimulated. This creates NO clouds affecting specific portion of the network, thus enabling the volume transmitter effect.

In Fig. 4 we report the resulting source placement for a 140 μm^3 granular layer cube.

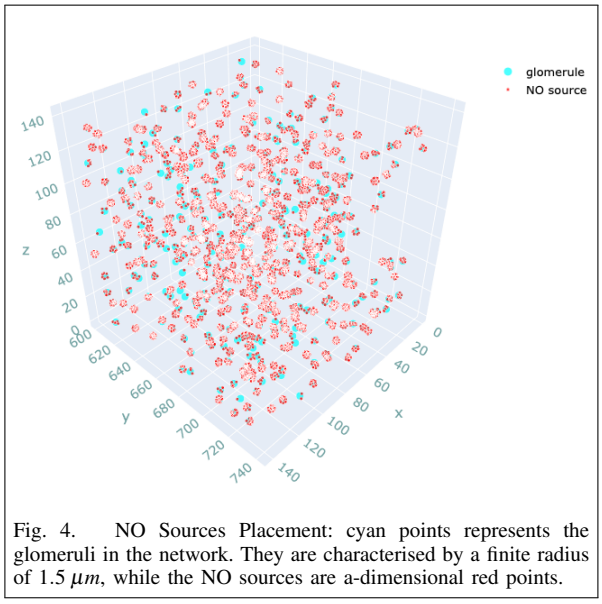

Fig. 4. NO Sources Placement: cyan points represents the glomeruli in the network. They are characterised by a finite radius of 1.5 μm, while the NO sources are a-dimensional red points.

IV. Conclusions

Given the unique type of intracellular communication exerted by NO molecules [15], and its implications in many physiological process, including synaptic plasticity [5], [2], mental health [6], development [16] and neurovascular coupling [17], many researchers have proposed different models for NO diffusion and effects within neural networks [11], [13]. However, these models suffer from a high computational cost in computing the diffusion equation, thus allowing to study the NO effect only on small non-biological inspired neural network. With this work we proposed a preliminary stage model able to compute the NO concentration in points of interest, resulting from the spiking activity of a realistic bioinspired neural network.

One critical issue we are still facing in validating such models is the poor data availability concerning the real NO concentration [12] that can be measured in the brain. There are many experimental studies, reviewed in [2], reporting picoMolar range, other reporting low nanoMolar range of NO being produced in the brain, but there are still conflicting results. However, with such models, we could have predictions of the NO concentration based on theoretical consideration (biochemical reaction cascade) at an individual synapse.

We are working toward the goal of creating a tool able to integrate plasticity and neurovascular coupling mechanisms with spiking neural network models and anatomical information, in order to simulate the network evolution due to NO dependent mechanisms.

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