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Experimental validation of a synapse model by adding synaptic conductances to excitable endocrine cells in culture

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Abstract. The purpose of the present work is to study and investigate the physiological behaviour of artificial synapses in excitable non-neural cells. We have supplied a numerical synapse model to a frog melanotrope cell by using either the current-clamp technique or a home-made easy-to-use application to achieve dynamic-clamp recordings in real time conditions. From results obtained with the conventional biophysical AMPA/NMDA model of glutamatergic neurotransmission, we have introduced changes in order to adapt the model to the electrophysiological responses of the recorded cell. Further developments are in progress to extend such hybrid connections to multi-component neural networks.

1 Introduction

For pluricellular organisms to form and operate, cellular behaviour must be vastly more complex than what is seen at the single-cell level. Cells must not only regulate their own growth and activity, they also have to communicate and interact with their neighbours in order to ensure the correct coordination of the entire organism.

The synaptic transmission is the main mechanism by which neurons communicate. The efficacy of synaptic transmission varies both in time and from one synapse to another, and is indicated by changes in post synaptic responses. This phenomenon is commonly called synaptic plasticity and can be divided into two classes: short-term [1, 2] and long-term [3, 4] modulation. These two categories are relevant for learning and computation in neural networks [5, 6]. For instance, short term plasticity may play an important role in oscillatory [7, 8] and synchronized bursting properties [9]. Long term plasticity is considered to be the basic phenomenon underlying learning and memory [10, 11]. However, neurons do not represent the only excitable cells in organisms. In melanotrope cells from the frog hypophysis for instance, hormone release is coupled to spontaneous action potential firing. A variety of external nervous inputs, originating from the hypothalamus, are known to shape their firing pattern [12]. But, locally, these non neural cells do not form any chemical synapse to each other.

The purpose of the present study is to create an artificial recurrent glutamatergic synaptic contact in a single melanotrope cell in culture. Our approach consists in an electrophysiological recording of the electrical activity of the cell, a computer-performed simulation of the pre- and postsynaptic responses and a re-injection of the calculated currents into the cell. By using of this computer-

synthesized feedback loop, we intend to investigate the mechanisms regulations, and potentially the plasticity, of a pacemaker activity after the introduction of a single synapse in a living cell. We would like to know if melanotrope cells actually modify their electrical response as a function of their immediate or previous experience.

2 Material and methods

2.1 Simulations, data collection and software development

Simulations were performed using Matlab *(The Mathworks, Inc)* or Scilab* software. To be accurate, the differential equations system has been solved by the Runge Kutta method with a step fixed at 50μs corresponding to the sampling rate of the biological recordings. The acquisition setup is based on an Axopatch 200B patch-clamp amplifier interfaced to a 1,5 GHz computer via a Digidata 1322 analog-to-digital converter by an SCSI bus *(Axon instruments, Foster City, CA).* On-line data processing was performed using Axon Instruments software (pCLAMP 8). However, an easy to use application has been developed under Windows Xp, written in C++ language *(Visual studio 6.0, Microsoft, Corporation),* running in real time, with the same SCSI communication protocols, providing a functional switch to dynamic clamp mode.

2.2 Cell culture and electrophysiological recordings

Frog melanotrope cells (figure 1) were isolated and cultured as previously described [12]. Briefly, 8 neurointermediate lobes from frog (*Rana ridibounda*) pituitaries were dissected and enzymatically dissociated in a Leibovitz L-15 medium supplemented with 0,15 % collagenase for 20 min. The digested tissue was then mechanically disaggregated and dispersed cells were plated in the same culture medium supplemented with 10 % fetal calf serum at a density of 10 000 cells in plastic culture dishes. Electrophysiological recordings were conducted at 25-28°C using the standard patch-clamp technique in the whole-cell configuration (see figure 1) and the current-clamp mode. Signals were filtered at 5 kHz and digitized at a sampling rate of 20 kHz.

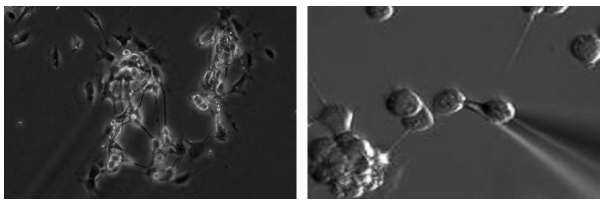

Fig. 1: Frog melanotrope cells in primary culture (Phase contrast, left). Patch pipette sealed to a melanotrope cell (Hoffman contrast, right).

* Free software for simulations developed by (*INRIA,* France) see www.scilab.org

3 Model

3.1 Presynaptic compartment.

Herein, it has been assumed that each spike evokes a change in the conductance of the postsynaptic membrane. The model used is based on the concept of a limited pool of synaptic resources available for transmission noted (R) [13]. The synaptic resources available for transmission evolve according the following differential equation (eq.1).

$$\frac{dR}{dt} = \frac{(1-R)}{\tau_{rec}} - U_{SE} * R * \delta(t - t_{sp}) \tag{1}$$

$$\begin{cases} \dfrac{dU_{SE}}{dt} = (U_1 + seuil) * (A_{norm} - U_{SE}) / \tau_{facile\,1} & \text{(if spike)} \\ \dfrac{dU_{SE}}{dt} = \dfrac{-U_{SE}}{\tau_{facil\,2}} & \text{(otherwise)} \end{cases} \tag{2}$$

The recovery time constant, τ_{rec} determines the rate of return of resources to the available pool. Note that the constant time of releasing the neurotransmitter and the returning to available mode are not the same. Changes have thus been introduced (*i.e.* a 2nd time constant) in order to be more rigorous with the dynamics of the cells.

The second differential equation describes the facilitation dynamic of the synapse, where U_{SE} becomes a dynamic variable which increases at each presynaptic spike and returns to the initial state in absence of spike. τ_{facil} and U_1 are the constants of this differential equation which determine the dynamic characteristic of it.

3.2 Postsynaptic compartment

The post synaptic compartment is modeled like an electrical circuit (figure 2), the equation 3 describes the synaptic current flowing through AMPA-receptor ionophores [14].

$$\begin{aligned} I_{AMPA} &= g_{syn}(t) * (V_m - E_{synn}) \\ \frac{dg_{syn}}{dt} &= -\frac{gsyn}{\tau_{syn}} + G\max_{syn} * \delta(t - t_{sp}) \end{aligned} \tag{3}$$

Where τ_{rec} is decay constant time applied in both AMPA/NMDA receptors with different values see table1. The synaptic NMDA current (eq.4) is described by product of $g_{\max}(t)$ and voltage-and magnesium–dependant Boltzmann term [15].

$$I_{NMDA} = \frac{1}{1 + \eta * [Mg^{2+}] * e^{-\gamma * V}} g_{syn}(t) * (V_m - E_{synn}) \tag{4}$$

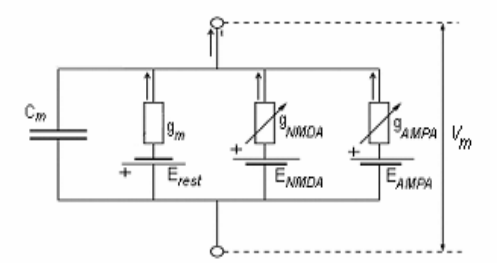

Fig. 2: the electrical circuit corresponding to synaptic conductances.

4 Experimental Results

As shown in the representative trace of the figure 3-B, melanotrope cells recorded in the whole-cell current-clamp mode of the patch clamp technique exhibited spontaneous action potentials. Similar recordings were used to adapt constant membrane parameters of our model. In particular, the range for τ_{rec}, initially described from depressing synapses in pyramidal cells of rat somatosensory cortex, was inadapted. In the same manner, For facilitating synapses, the experimental ranges of U_1 , τ_{rec} and τ_{facil} are 0.012-0.086, 104-694 ms and 550-3044 ms, respectively [16]. In melanotrope cells, we have used other values, obtained by fitting the model responses to recordings. In addition, we have introduced a second time constant for, τ_{rec2} and τ_{facil2} as presented in Table 1.

Presynaptic compartment					
τ_{rec1}(ms)	2000	τ_{facil1}(ms)	1800	U_1+Seuil	150
τ_{rec2}(ms)	500	τ_{facil2}(ms)	200	A_{norm}	100
Postsynaptic compartment					
	Membrane			**AMPA**	**NMDA**
C_m(μF/cm^2)	1	g_{max}(nS)		6	23
G_m(mS/cm^2)	0.1	τ_{syn} (mS)		2	100
E_{rest}(mV)	-62	E_{syn}(mV)		-30	-30
	-	η (mM)		-	0.33
	-	[Mg] (mM)		-	2
	-	Γ (mM)		-	0.11

Table 1: Constant parameters of model.

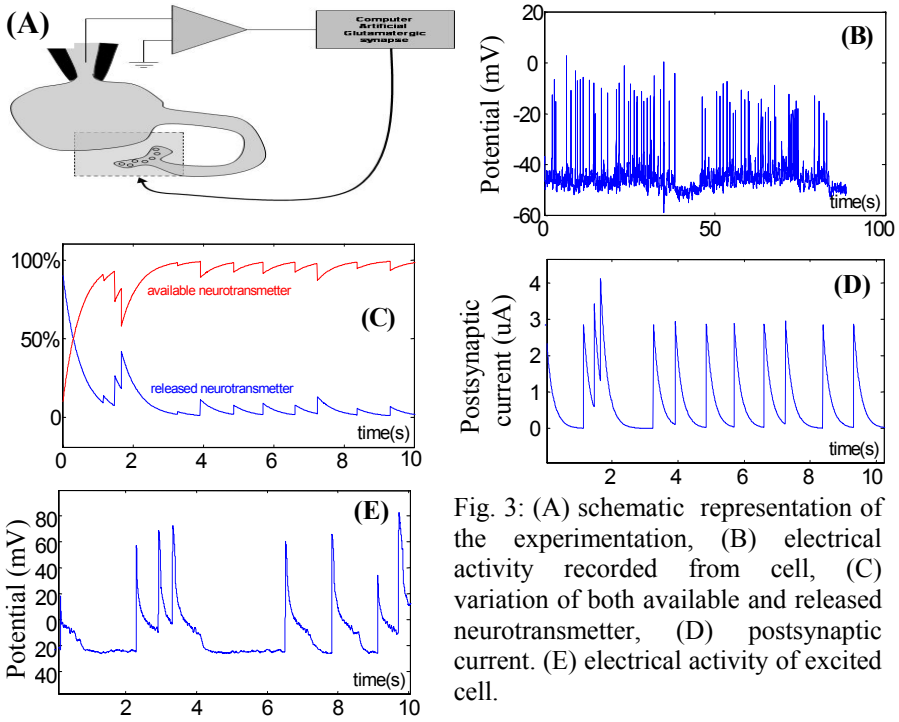

Fig. 3: (A) schematic representation of the experimentation, (B) electrical activity recorded from cell, (C) variation of both available and released neurotransmetter, (D) postsynaptic current. (E) electrical activity of excited cell.

The figures 3-B,C & D show the results of our application. The first 10-sec of the electrical activity have been used to compute (eq.1 & 2) the presynaptic glutamate available pool and the glutamate release (1-R) in the synaptic cleft. The corresponding time courses of R and 1-R are shown in figure 3-C. Notice that the calculated release of glutamate is correlated to the instantaneous spike frequency.

Finally, the corresponding AMPA and NMDA postsynaptic currents were calculated using (eq.3 & 4) and the sum (figure 3-D) was re-injected in the cell off-line as a stimulus file. The resulting electrical activity of the cell is presented (figure 3-E). The shape of electrical activity of the cell shown in figure 3-E reveals the existance of calcic currents.

5 Discussion

In the present sudy, we have synthesized a glutamatergic artificial recurrent synapse in a single melanotroph by using the model described in the figure 2. Such synapses are also called autapses . Herein, we demonstrate that it is possible both to simulate glutamate release and to add glutamatergic synaptic conductances in a single non-glutamatergic cell which lacks glutamate receptors. In addition, one of the main results of our study is that non-neural spiking cells change their own activity in response to artificial depolarizing inputs. Self-evoked glutamatergic currents are able to generate action potentials. Moreover, the analysis of the shape and time-course of the spikes strongly suggests the presence of calcium currents (figure 3-E).

The present approach is now used in our laboratory to develop real-time applications usable to create hybrid connections between neurons cultured on Multi Electrode Array and neural networks. In addition, the dynamic-clamp technique will used to modify in real time the repertoire of activated ionic channels in the recorded cell.

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