Spontaneous Oscillations of Dressed Neurons: A New Mechanism for Epilepsy?

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(Received 7 April 2003; published 24 December 2003)

Most modeling studies of neurons and neuronal networks are based on the assumption that the neurons are isolated from their normal environment. Based on recent experimental data we put forward a model for neurons that incorporates the influence of the surrounding glia (*dressed neurons*). We predict seizurelike spontaneous oscillations in the absence of stimuli for strong coupling between neurons and astrocytes. Consistent with our predictions, a signature of this enhanced crosstalk, over expression of glutamate receptors in astrocytes, has been observed specifically in epileptic tissue.

DOI: 10.1103/PhysRevLett.91.268101 PACS numbers: 87.19.La, 05.45.Xt, 87.19.Xx

Neurons in most parts of the brain are outnumbered by glial cells by approximately a factor of 10. A realistic picture of brain tissue is therefore a mass of glial cells with embedded neurons. The most numerous type and best studied glial cells are the star-shaped astrocytes. Contrary to the long-time belief that the information processing in the brain is exclusively a task of the neurons, research in the recent years [1–3] has demonstrated that astrocytes listen to the neuronal chatter, respond to it and talk back to the neurons, thus modulating their functions. Astrocytes, in contrast to most neuronal cells, do not fire action potentials due to the lack of sufficient numbers of Na^+ channels [4]. Astrocytes do not connect to other astrocytes or neurons via long processes. Until recently it was believed that the role of the glia for brain function is to provide structural and chemical support for the neurons. Such support functions include, for example, the uptake of potassium after a neuronal action potential. Although it has been known for a long-time that synaptic astrocytes respond with depolarization to neuronal action potentials, this was thought to be a response caused by the increased extracellular K^{2+} concentration. The discovery by Porter and McCarthy [1] that astrocytes respond to neuronal action potentials by binding glutamate to the metabotropic glutamate receptor has changed the current thinking about the role of astrocytes dramatically. It is now clear that astrocytes are active members of the neuro glial communication system and can modulate neuronal dynamics at the same synapse or synapses at some distance. As neurons fire, glutamate is released into the synaptic cleft, which is partially bound to the metabotrobic glutamate receptors (mGluR) of the synaptic astrocytes. Metabotropic glutamate receptors are a subtype of glutamate receptors which regulate a variety of intercellular signaling pathways. The group-I is a subtype of metabotropic glutamate receptors that is coupled to polyphosphoinoside hydrolysis. A particular kind of polyphosphoinoside, inositol 1,4,5-triphosphate (IP_3), acts as a second messenger and is a key player in the release of calcium from internal stores and regulates calcium signalling. Upon binding of glutamate to the astrocyte, IP_3 is released into the intracellular space. IP_3 in turn binds to the IP_3 receptor in the endoplasmic reticulum (ER) and Ca^{2+} is released from the ER into the cytosol. Such Ca^{2+} release can occur in forms of intracellular Ca^{2+} waves. The Ca^{2+} wave can propagate across the cell membrane, through extracellular space into adjacent astrocytes (for a recent review on Ca^{2+} signaling, see [5]). Astrocytes with elevated Ca^{2+} concentrations release glutamate into the extracellular space [2] that copropagates with the Ca^{2+} wave front. Extracellular glutamate modulates the neuronal synapse by generating additional inward currents [2]. This feedback could, of course, also be inhibitory if enhanced Ca^{2+} concentrations in neighboring astrocytes are activating inhibitory interneurons. Thus, there is a bidirectional coupling between neurons and astrocytes. In spite of this known symbiotic arrangement between neurons and astrocytes almost the entire literature on neuronal dynamics ignores the effect of the glia. In this Letter, we put forward a model for a single neuron in contact with astrocytes, i.e., the dressed neuron. The model we are proposing is a minimal model that describes the neuron with a single compartment and the astrocytes by a homogeneous reservoir. Gradients in membrane potentials and ionic concentrations are thus neglected.

To model the neuron, we use the ionic-conductance based Hodgkin-Huxley [6] model. This model is not particular to cortical neurons, yet we use it, as it is a standard, well known, model that exhibits the main features such as the generation of action potentials and refractoriness. The transmembrane potential v, normalized to zero in the resting state, is described by the following set of equations

$$C_m \frac{dv}{dt} = -g_K n^4 (v - v_K) - g_{\mathrm{Na}} m^3 h (v - v_{\mathrm{Na}}) - g_l (v - v_l) + I_{\mathrm{ext}} + I_{\mathrm{astro}} \quad (1)$$

$$\frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m; \qquad \frac{dn}{dt} = \alpha_n (1 - n) - \beta_n n,$$

$$\frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h, \quad (2)$$

where $m^3 h$ denotes the fraction of open Na^+ channels, n^4 the fraction of open potassium channels, and

$v_K = -12.0$ mV, $v_{Na} = 115.0$ mV, $v_L = 10.6$ mV the reversal potentials for the potassium, sodium, and leakage system, respectively. The maximal conductance of the sodium, potassium, and leakage channels is given by $g_{\mathrm{Na}} = 120\ \mathrm{mS/cm^2}$, $g_K = 36.0\ \mathrm{mS/cm^2}$, and $g_L = 0.3\ \mathrm{mS/cm^2}$, respectively, and the membrane capacitance by $C_m = 1\ \mu\mathrm{F/cm^2}$. The opening and closing rates of the respective gates are given by

$$\begin{aligned} \alpha_m &= 0.1\frac{25-v}{\exp[\frac{25-v}{10}]-1}; \qquad \beta_m = 4\exp\left[\frac{-v}{18}\right], \\ \alpha_h &= 0.07\exp\left[\frac{-v}{20}\right]; \qquad \beta_h = \frac{1}{\exp[\frac{(30-v)}{10}]+1}, \\ \alpha_n &= 0.01\frac{10-v}{\exp[\frac{10-v}{10}]+1}; \qquad \beta_n = 0.125\exp\left[\frac{-v}{80}\right], \end{aligned} \tag{3}$$

where the transmembrane potential v is given in units of mV and time t in units of msec. The current I_{ext} denotes injected or synaptic current inputs. The current I_{astro} introduces feedback from the astrocytes to the neuron and will be discussed below.

When a neuron fires, it releases quantal amounts of neurotransmitters into the synaptic cleft [7]. As neurotransmitters bind to the mGluRs on the astrocytes, triggering the release of IP3 intracellularly, we assume that this amount is also quantized. The production of intracellular IP_3 in the astrocyte is modeled by

$$\frac{d[Ip_3]}{dt} = \frac{1}{\tau_{IP_3}}([Ip_3]^* - [Ip_3]) + r_{IP_3}\Theta(v - 50\,\mathrm{mV}), \tag{4}$$

where $[Ip_3]^*$ is the equilibrium concentration of IP_3. We use here the recently determined values [8] of $\tau_{IP_3} = 0.000\,140$ (1/msec) and $[\mathrm{IP_3}]^* = 160.0$ nM, respectively. The parameter $r_{\mathrm{IP_3}}$ determines the production of IP_3 in response to a neuronal action potential. The production term is activated when the membrane potential of the neuron is larger than $+50$ mV via the step function $\Theta(x)$. Since the production of intracellular IP_3 is proportional to the activated mGluRs, the parameter $r_{\mathrm{IP_3}}$ is proportional to the density of mGluRs in the membrane of the synaptic astrocytes.

Production of IP_3 in the intracellular space of astrocytes triggers the release of Ca^{2+} from internal stores, most notably the endoplasmic reticulum (ER). This process has been modeled intensively over the last ten years or so. The cytosolic Ca^{2+} concentration can change due to Ca^{2+} flux from the ER through the IP_3 release channels, leakage flux from the ER into the cytosol, and pump-flux from the cytosol into the ER. Transport of Ca^{2+} through the cell membrane is not taken into account. We choose the Li-Rinzel model [9]. The Ca^{2+} concentration in the intracellular space, $[Ca^{2+}]$, is described by the set of two equations

$$\frac{d[\mathrm{Ca^{2+}}]}{dt} = -J_{\mathrm{channel}} - J_{\mathrm{pump}} - J_{\mathrm{leak}}, \tag{5}$$

$$\frac{dq}{dt} = \alpha_q(1-q) - \beta_q q, \tag{6}$$

where q is the fraction of activated IP_3Rs, J_{channel} denotes the calcium flux from the ER to the intracellular space through the IP_3R channel, J_{Pump} the calcium flux pumped from the intracellular space into the ER, and J_{Leak} the leakage flux from the ER to the intracellular space. The expressions for the fluxes are given by

$$\begin{aligned} J_{\mathrm{channel}} &= c_1 v_1 m_\infty^3 n_\infty^3 q^3([\mathrm{Ca^{2+}}] - [\mathrm{Ca^{2+}}]_{ER}), \\ J_{\mathrm{pump}} &= \frac{v_3[\mathrm{Ca^{2+}}]^2}{k_3^2 + [\mathrm{Ca^{2+}}]^2}, \\ J_{\mathrm{Leak}} &= c_1 v_2([\mathrm{Ca^{2+}}] - [\mathrm{Ca^{2+}}]_{ER}), \end{aligned} \tag{7}$$

with

$$\begin{aligned} m_\infty &= \frac{[\mathrm{IP_3}]}{[\mathrm{IP_3}] + d_1}; \qquad n_\infty = \frac{[\mathrm{Ca^{2+}}]}{[\mathrm{Ca^{2+}}] + d_5}, \\ \alpha_q &= a_2 d_2 \frac{[\mathrm{IP_3}] + d_1}{[\mathrm{IP_3}] + d_3}; \qquad \beta_q = a_2[\mathrm{Ca^{2+}}]. \end{aligned} \tag{8}$$

The parameters of the model are $c_1 = 0.185$, $v_1 = 6\,\mathrm{sec^{-1}}$, $v_2 = 0.11\mathrm{sec^{-1}}$, $v_3 = 0.9\ \mu\mathrm{Msec^{-1}}$, $k_3 = 0.1\ \mu\mathrm{M}$, $d_1 = 0.13\ \mu\mathrm{M}$, $d_2 = 1.049\ \mu\mathrm{M}$, $d_3 = 0.9434\ \mu\mathrm{M}$, $d_5 = 0.08234\ \mu\mathrm{M}$, and $a_2 = 0.2\ \mu\mathrm{M^{-1}\ sec^{-1}}$. Conservation of Ca^{2+} within the cell implies the constraint $[\mathrm{Ca^{2+}}]_{ER} = (c_0 - [\mathrm{Ca^{2+}}])/c_1$ with $c_0 = 2.0\ \mu\mathrm{M}$.

This simplified model resembles the Hodgkin-Huxley model for electrically excitable membranes if the concentration $[Ca^{2+}]$ is replaced by the transmembrane potential. The driving force for Ca^{2+} fluxes is the concentration gradient $([\mathrm{Ca^{2+}}] - [\mathrm{Ca^{2+}}]_{ER})$ while the driving force for the ionic currents in the Hodgkin-Huxley equation is the voltage gradient. Equations (5)–(9) predict that stimulation of astrocytes with glutamate released into the synaptic cleft triggers intracellular astrocytic Ca^{2+} signals. This prediction is well described in the literature, *in vitro* [10] and *in vivo* [11].

In the absence of neuronal stimulus, i.e., $[\mathrm{IP_3}] = [\mathrm{IP_3}]^*$, Eqs. (5)–(8) predicts Ca^{2+} oscillations for $0.345\ \mu\mathrm{M} < [\mathrm{IP_3}] < 0.644\ \mu\mathrm{M}$ and steady states otherwise. The transitions from steady-state to oscillations and from oscillations to steady-state are due to supercritical Hopf-bifurcations. Ca^{2+} oscillations are frequently observed in astrocytes, hepatocytes, pancreatic island cells, and various types of epithelia. In our model, the concentration of IP_3 in the astrocytes is controlled by neuronal firing and thus becomes another dynamic variable.

To complete the model, we use experimental data [2] that relate the Ca^{2+} concentration in the astrocytic environment to additional inward currents in neurons (see Fig. 1). The recordings were made from a single neuron grown on microislands of astrocytes and can be fitted by the function of inward current versus astrocytic Ca^{2+} concentration

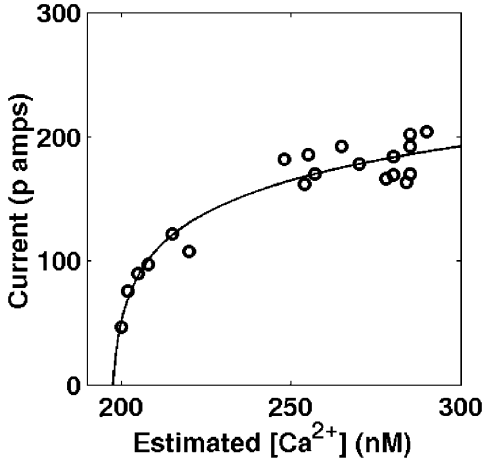

FIG. 1. Simultaneus recordings (data from Fig. 5B of [2]) of astrocytic calcium and total inward current in the neuron are shown by open circles while the fit by Eq. (9) is shown as a solid curve.

$$I_{\text{astro}} = 2.11\Theta(\ln y)\ln y, \qquad y = [\text{Ca}^{2+}]/\text{nM} - 196.69 \quad (9)$$

with the Heaviside function $\Theta(x)$. The recorded total current (measured in pA) was converted to a current density measured in $\mu\text{A/cm}^2$ by assuming a spherical neuron with 50 μm diameter.

We characterize the prediction of our dressed neuron model by the time course of the relevant observables, the neuronal membrane potential v, the calcium concentration in the astrocytic environment $[\text{Ca}^{2+}]$ and the IP_3 concentration $[\text{IP}_3]$. We stimulate the neuron by a dc current, $I_{\text{ext}} = 10\ \mu\text{A/cm}^2$. In response to this current the neuron exhibits a periodic spike train. The concentration of IP_3 is initially at its experimentally determined resting value of 0.16 μM. In Fig. 2, top panel, we show the time course of the variables with an IP_3 production rate of $r_{\text{IP}_3} = 0.2\ \mu\text{M/s}$). While the neuron is stimulated (40 s), the concentration of IP_3 builds but does not become large enough to induce Ca^{2+} oscillations. After 40 s, the dc stimulation of the neuron is turned off. As a consequence the concentration of IP_3 and Ca^{2+} decreases and the neuron stops firing. At larger values of the IP_3 production rate, Ca^{2+} oscillations can be induced and there is a delay between the end of the dc stimulus and the termination of neuronal firing since the neuron stops firing only when the concentration of Ca^{2+} falls below a threshold. In Fig. 3, top panel, we show the bifurcation diagram for $r_{\text{IP}_3} = 0.2\ \mu\text{M/s}$ which resembles the bifurcation diagram of the isolated HH-neuron. Periodically repetitive action potentials coexist with a steady-state membrane potential for $\equiv 6.2\ \mu\text{A/cm}^2 < I_{\text{ext}} < 9.66.2\ \mu\text{A/cm}^2$. For $I_{\text{ext}} > 9.66.2\ \mu\text{A/cm}^2$ the steady state becomes unstable. What is interesting is that the coexistence regime of the steady state and the limit cycle extends to smaller values of the injected current. This means that the dressed neuron is more likely to spontaneously oscillate with smaller stimuli. If the generation

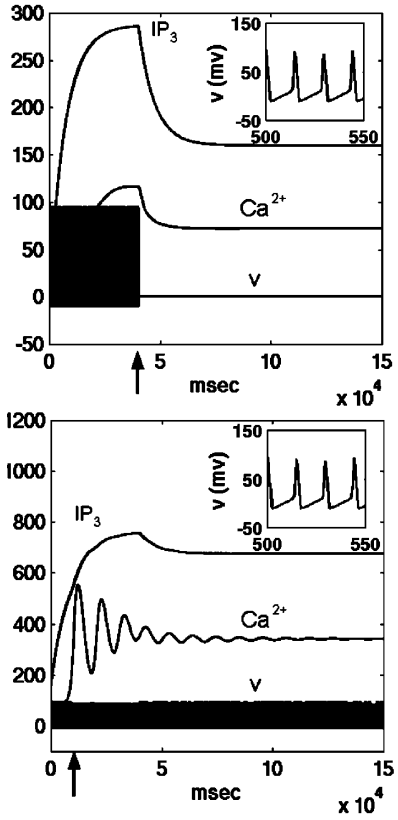

FIG. 2. The time course of the IP_3 and Ca^{2+} concentrations (in nM) in the astrocyte are compared with the neuronal membrane potential v (in mV). Top panel: $r_{\text{IP}_3} = 0.2\ \mu\text{M/s}$, the neuron is stimulated with a dc current, $I_{\text{ext}} = 10\ \mu\text{A/cm}^2$ for 40 s. Bottom panel: $r_{\text{IP}_3} = 0.8\ \mu\text{M/s}$. The neuron is stimulated with a dc current, $I_{\text{ext}} = 10\ \mu\text{A/cm}^2$ for 10 s. The arrow indicates the end of the stimulation period. The inset describes a small time segment of the neuronal firing.

rate of of IP_3, r_{IP_3}, is larger than 0.8 μM/s, the neuron can oscillate spontaneously without external stimulus. In Fig. 2, bottom panel, we show the time course of the concentrations of IP_3 and Ca^{2+} for $r_{\text{IP}_3} = 0.8\ \mu\text{M/s}$ and the neuronal membrane potential. We stimulate the neuron for 10 s starting at rest. As the concentration of IP_3 builds up, the Ca^{2+} concentration in the astrocytes starts to oscillate. The concentration of IP_3, however, increases beyond the second Hopf bifurcation of the Ca^{2+} dynamics described by the Li-Rinzel model and the oscillations cease. When the dc stimulation of the neuron is terminated, the feedback from the astrocyte to the neuron is strong enough to maintain the neuronal oscillations indefinitely. In the bifurcation diagram (see Fig. 3, bottom panel), the coexistence of the limit cycle (spiking neuron) extends now to vanishing external current and beyond. The dressed neuron can spike spontaneously turned on by perturbations or even noise.

It has been reported [12–14] that mGluRs are overexpressed in astrocytes from epileptic foci of humans suffering from temporal lobe epilepsy. Overexpressed mGluRs are associated with more glutamate binding

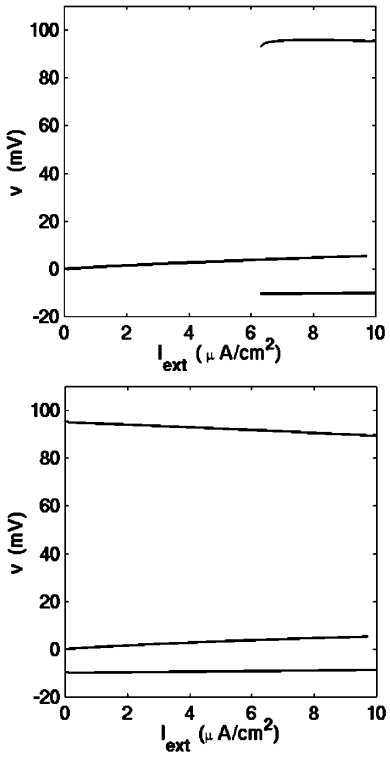

FIG. 3. The bifurcation diagram of the dressed neuron for $r_{IP_3} = 0.2\ \mu$M/s (top panel) and for $r_{IP_3} = 0.8\ \mu$M/s (bottom panel).

and thus enhanced production of IP_3, leading to an enhanced astrocytic Ca^{2+} response. This has been indeed observed in epileptic tissue [15]. In our model such a situation is described by a larger IP_3 production rate r_{IP_3}. In agreement with the observations, our model predicts an enhanced astrocytic Ca^{2+} response as r_{IP_3} is increased (compare Figs. 2 top and bottom panels). Our model further predicts that if the production rate of IP_3 is large enough, (i.e., the expression of mGluRs is large enough) the neuron in communication with the astrocytes can oscillate spontaneously in the absence of stimulation. Our hypothesis is therefore that enhanced expression of mGluRs can cause epilepsy. Although there is no direct evidence, there is circumstantial evidence that supports our hypothesis. First, epileptic astrocytes do exhibit more spontaneous intracellular Ca^{2+} oscillations at higher frequencies than normal astrocytes, implying that the Ca^{2+} response to glutamate is enhanced in epileptic astrocytes[15]. Second, slice studies have revealed that in pyramidal cells from hyperexcitable region with more Ca^{2+} active astrocytes, there is high level of spontaneous excitatory activity, [15] which, in a few cases become epileptic bursts. Third, high levels of astrocytic glutamate release trigger trains of action potentials in nearby neurons (in culture) [16].

Inhibition plays an important role for the dynamics of cortical neurons. In the absence of inhibition, pyramidal cells have a low threshold leading to spontaneous firing. Pyramidal cells activate interneurons that in turn inhibit the pyramidal cells, slowing down their spontaneous firing rate. It is important to note that such inhibition takes place on the time scale of ms while the astroytcic effects, described here, take place on the time scale of s to min. Thus, under the assumption that upregulated mGluRs in astrocytes do not modify the inhibitory mechanism, the astrocytic Ca^{2+} dynamics modifies the firing of an already inhibited cortical neuron. Inhibition will therefore shift the critical value of r_{IP_3} necessary to generate spontaneous oscillations.

The results we obtained here are not particular to the Hodgkin-Huxley model for the neuron. We have substituted the Hodkin-Huxley model by the Pinsky-Rinzel model [17]—which is a model that describes realistically CA3 pyramidal neurons and have found qualitatively similar results. This material is based upon work supported by the National Science Foundation under Grant No. IBN-0078055.

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