Effects of Glia on the Synchronized Bursting in Cultured Neuronal Networks

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The phenomenon of spontaneous firings induced by a low Mg$^{2+}$ condition in primary cortical neuronal cultures with different amounts of glial cells are used to study the effects of glia on the dynamics of the network. Single cell patch-clamp measurements have shown that the firing patterns during spontaneous firings are different for networks with and without glia. In general, the synchronization of networks with glia is better than that of those without glia. Furthermore, originally glia-free cultures can be made to produce better synchronization and recover the firing patterns similar to those from cultures with glia after glia have been added to the network. Our finding indicates that glia are interacting with neurons in the network to coordinate the firings. Furthermore, our experimental findings are consistent with a mean field model for synchronized bursting.

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I. INTRODUCTION

There are two main types of cells in our brain; namely neurons and glia. Traditionally, the computational aspect of the brain has only paid attention to the neurons. Glia are treated only as supportive, such as insulating an axon, maintaining ionic balance, and supporting neuro-transmission. Absence of glia will reduce the neuronal survival rate. Almost all the modelings of the cognitive functions of our brains do not include glia. However, it is known that several neural diseases are related to malfunctions of glia [1, 2]. For example, multiple sclerosis is due to the damage of oligodendrocytes in the brain [3] and glia are known to be a major factor for the cause of a certain type of epilepsy [4]. In past decades, effects of glia (especially astrocyte) on neuronal activities have already attracted a lot of attention and some significant roles of glia cell have been reported. It is now recognized that the presynaptic and postsynaptic neurons are wrapped by astrocytes, which not only affect the synapse maturation and maintenance but also signal trafficking [5]. Astrocytes can receive signals, mainly calcium, from the presynaptic neuron and respond by releasing

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gliotransmitters such as glutamate, ATP, D-serine, and GABA [6–11] through vesicular exocytosis or membrane ion channels and pumps [12]. These gliotransmitters can have very different effects. For example, molecule glutamate is a major extracellular messenger and will increases neuronal activities. D-serine is an agonist of the NMDA-type glutamate receptor, and it will increase the activation probability of a NMDA receptor and enhance the synaptic long-term potentiation [13]. Also, ATP will suppress synaptic transmissions and plays several functions in neural networks [14]. Furthermore, astrocytes can also communicate with each other by calcium waves [15]; this calcium wave can propagate through gap junctions and extracellular ATP signaling. Although the concept of a tripartite synapse has been proposed, the effect of glia on the activities of a neuronal network are still far from clear. From the viewpoint of dynamics, it is also of interest to investigate how non-excitable elements, such as glial cells, can play important roles in modulating the collective dynamics of a network consisting of excitable cells such as neurons.

In neural tissues, glia and neurons intermingle together to form a network. Most of the studies of the neuron-astrocyte interaction are based on observations of a single or a few cells in such tissues. Since the functions of the tissues can usually be obtained only from the collective dynamics of the networks, it is difficult to conclude on the role of glia played in functions of the tissue, and it is most interesting to understand how these local astrocytes networks affect the collective activities or functions of the networks. In this study, we examine the synchronized bursting (SB) in neuronal cultures with and without astrocytes. Synchronized bursting [16–18] is a cooperative network phenomenon in cultures which could be induced by a low Mg$^{2+}$ condition [19]. During SB, more or less the whole cultures could fire synchronously within a period of time. It is known that SB is mediated by the synaptic currents [20], which are controlled by network properties. Therefore, the phenomena of SB is a good candidate for exploring the effects of astrocytes on network dynamics. Up till now, the bursting of a neuron is usually considered to be a single cell phenomenon, as the neuron can be considered to be intrinsically endowed with specific ion channels [21]. A study on the astrocyte modulation in SB could help to clarify the role of glia in the burst generation. Furthermore, since synchronization of neuronal networks can be related to the induction of long-term plasticity (LTP) [22] (formation of long term memory), this study would provide vital information on the importance of glia related to long term memory.

In this article, we report on the results of our experiments designed to study the effects of glia on the SB of a neuronal culture induced by a low Mg$^{2+}$ condition. The effects of glia on SB are studied in our experiments by controlling the number of glial cells in the system by using glia suppressed and enhanced (post-plating) cultures. Both methods of calcium imaging and electrophysiology are used. Our main finding is that SB is mostly absent in cultures without glia and it could be restored by the addition of glia; suggesting that the glia-neuron interaction can also provide a new mechanism for bursting. Finally, our experimental findings are consistent with a mean field bursting model that incorporates the effects of glial cells.
II. MATERIALS AND METHODS

Neuronal cultures are prepared from cerebral cortices of E17 Wistar rats embryos. The cortices are dissociated with trypsin/EDTA, and plated onto the poly-D-lysine coated glass coverslips at a density of 250–300 cells/mm$^2$. Cultures are maintained with DMEM containing 5% FBS, 5% HS, and 1% Penicillin-Streptomycin at 37 °C in a 5% CO$_2$ incubator. Cultures are changed to Neurobasal medium supplemented with 2% B27 and 0.5mM GlutaMax after 24 h. Three types of neuronal cultures are prepared in the present study: (1) Normal Glia Neuronal Cultures (NGNC, the neuron-glial mixed cultures) as described above. (2) Glia-Suppressed Neuronal Cultures (GSNC) as described above except that they are treated with cytosine arabinoside (Ara-C, 5 µM) after 48 h to suppress the proliferation of glial cells. After treating with Ara-C, one cover-slip is prepared for GSNC and three others plated with glia are cultured side by side in a culture dish. This method allows neurons to grow in glia-conditioned medium (GCM) without substantial contact with glia, and experiments can be performed in the absence of glia. (3) Glia-Postplated Neuronal Cultures (GPNC) are prepared and cultured in the same manner as GSNC, except that glia are then added into the GSNC within 7 DIV and cultured confluently to be studied.

The cultures are fixed with 4% Paraformaldehyde in phosphate-buffered saline (PBS) at 37 °C for 20 min. After the cells have been perforated with 0.2% Triton X-100 for 10 min, the cultures are blocked with 10% Goat Serum (GS) for 1 h at room temperature. Cultures are then stained with anti-MAP2 (1:500) and anti-GFAP (1:500) overnight at 4 °C. After washing with PBS, the cultures are incubated for 1 h at room temperature with fluorescence-conjugated secondary antibodies: Goat-anti-rabbit IgG FITC (1:200) for the GFAP and Goat-anti-mouse IgG Rhodamine TRITC (1:200) for the MAP2. The cultures are then imaged by using a Zeiss LSM 510 inverted confocal microscope.

Neuronal cultures are loaded with Ca$_{2+}$ indicator (Oregon Green-488 BAPTA 1-AM ester) and performed on an inverted microscope (Zeiss Axiovert 25) using 10X, 20X, or 40X objectives in room temperature. Images are collected at 30 frames per second by an ICCD video camera, which is composed of an image intensifier (Lambert II) and a CCD camera (Prosilica GE680). Matlab analysis software is used to detect the positions of neurons and graphic processing.

Electrophysiological measurements are performed with a Multiclamp 700B amplifier (Molecular Devices, Foster City, CA) and Digidata 1322A with pCLAMP 9 (Axon) software and analyzed off-line using Clampfit 9.2. Whole-cell patch clamp recordings are performed using 3-5 MΩ glass (Shutter Instruments, Novato, CA) pipettes with internal solution containing (in mM): 136.5 K-gluconate, 17.5 KCl, 9 NaCl, 10 HEPES, 0.2 EGTA, 4 Mg-ATP, and 0.3 Na-GTP (pH 7.3). All experiments are done at room temperature (22–24 °C). Cells are visualized with a 20X objective on an inverted microscope (Zeiss Axiovert 25). Voltage clamp measurements are performed at −70 mV. In the current clamp recording, the membrane potentials are continuously monitored and adjusted. For dual-patch recording, two neurons are patched within a typical distance of about 200 µm. All results are recorded with basal saline solution (BSS) containing (in mM): 130 NaCl, 5.4 KCl, 1.8 CaCl$_2$, 5.5 Glucose, 20 HEPES at a low (0 mM) and high (1.6 mM) Mg$^{2+}$ condition.
III. RESULTS

Figure 1 shows confocal images of immunostaining with glia (NGNC) and without glia (GSNC). It can be seen that the NGNC (Fig. 1A) has both neurons (red) and glia (green). However, for the GSNC, it can be seen from Figure 1B that nearly no glia are present. On the other hand, immunostaining verified the presence of glial cells for GPNC preparations, and the neuron morphologies are similar to that of the NGNC, as shown in Figure 1C. These pictures show that our protocols for the sample preparation produce desirable results. In the discussions below, cultures with a similar number of neurons with and without glia are used for the comparison in their spontaneous synchronized firing properties.

In order to detect differences in the spatial distribution of firing patterns during SB for these two types of cultures, calcium fluorescence images are recorded, as there will be large variations of intracellular $\text{Ca}^{2+}$ concentration in the cells during SB. A typical fluorescence image of a culture is shown in Figure 2A. During SB, the fluorescence intensity at different locations of the image will be changed more or less periodically. Figure 2B shows the time course of the SB by using recorded images similar to that shown in Figure 2A. The vertical axis of Figure 2B is the index of neurons taking part in the synchronized burst determined from Figure 2A. Note that the ordering of the indices is arbitrary and does not represent geometrical locations. The firing rate (fluorescence intensity) is indicated by the gray scale. It can be seen that neurons taking part in the SB are firing more or less simultaneously with a period of about 10 seconds and the firing burst lasts about 4 seconds. A remarkable feature of spontaneous firing in NGNC is that the cells in the field of view oscillate as one big cluster, and therefore one can see in Figure 2B that the amplitudes of oscillation are more or less constant from one oscillation to next. Details of these phenomena can be found.
FIG. 2: Measurements of the calcium concentration variations in cultured cortex neurons (10DIV). A, Fluorescence image of a NGNC culture loaded with calcium dye during SB. Size:4mm². B, Time dependent fluorescence intensity of different neurons for a NGNC sample under the low Mg²⁺ (0 mM) condition. C, Same measurements with a GSNC. An arrow is used to indicate the local-synchronized fluorescence activities in GSNC. Scale bar : 10 s.

in [17–19]. Figure 2C is a similar measurement as those shown in Figure 2B but with a GSNC sample. There are three major differences in GSNC networks. First, the oscillations are less periodic. Second, there are large variations in the amplitudes from one oscillation to another. Third, the activities of neurons are not fully synchronized. Both global (all neurons) synchronized firing and local synchronized (small population of neurons) firing can be observed. It seems that, different from that of NGNC, the cells in the field of view seem to form not only one single cluster. These data suggest that the firing patterns in the spatial and temporal domain in NGNC and GSNC are quite different. It indicates that the presence of glia provides some kind of organization of the network such that the synchronization seems to be better in NGNC.
Electrophysiological measurements have also been used to characterize the details of SB in the NGNC and GSNC samples. First of all, it is important to see if the neurons in these two different types of cultures possess similar electrophysiological properties. To investigate the characteristics of individual neurons in different cultures, we need to isolate the neurons from the network. Since SB is induced in a low Mg\(^{2+}\) condition, an increase of Mg\(^{2+}\) will effectively eliminate the communication of neurons from each other [17]. To test this idea, we have performed current injection experiments in both NGNC and GSNC at high Mg\(^{2+}\) (1.6 mM) conditions. Figure 3A shows the result of whole-cell current clamp at \(I=+50\) pA for a neuron in a NGNC. Figure 3B shows a similar measurement but for a GSNC. It can be seen that the response of the neurons in GSNC or NGNC are basically the same under the high Mg\(^{2+}\) condition. This last finding suggests that the neurons in GSNC and NGNC exhibit a similar function and will be capable of bursting if a large enough inward current is present.

After we have established that the neurons in both the NGNC and GSNC are similar, we proceed to perform SB experiments. Figure 4A is the measurement of the membrane
potential during SB in NGNC by whole-cell patching (current-clamp mode) with two neurons (typical separation within 200 µm) being patched at the same time. It can be seen that the firings of the cells during SB consist of a series of bursts with a period of about 4 second as the Ca\(^{2+}\) oscillation shown in Figure 2B. The characteristic of a burst is that there is a more or less constant depolarization (plateau, shown by arrow) together with action potentials. Note that during SB only the bursts are synchronized between different cells, but the spikes are not synchronized. Figure 4B shows the details of the firing during one burst together with the recording of the second cell being changed to voltage clamp mode (clamped at −70 mV). It can be seen that during the burst, there is a large slow inward current flowing into the cell. Since the dynamics of different cells are burst synchronized, we can safely assume that the firing pattern shown in the upper trace is induced by a similar current shown in the lower trace.

Figure 4C to 4E shows a similar measurement as that of Figure 4A but for a GSNC sample. It can be seen that the forms of the firing patterns are quite different between NGNC and GSNC. Three types of firing patterns can be observed in the GSNC samples. The first type is burst-only (Fig. 4C) and their dynamics are similar to that of NGNC. The second type is a mixture of burst and spike (Fig. 4D). The last one is spike-only (Fig. 4E). However, only a small population of GSNC samples is burst-only but, the duration and period of the bursts vary. In most of the cases, the plateau for the NGNC mentioned above is missing. Spikes without plateau can be observed in the culture. Details of the current measurement similar to those of Figure 4B have also been performed for GSNC, and the result is shown in Figure 4F. It can be seen that the firing pattern consists of randomly distributed spikes. There are isolated small and fast currents for each spike. The statistics for the occurrence of these three different cases of firing patterns for both NGNC and GSNC are shown in Figure 5. It can be seen that almost all of the cultures of NGNC show bursting except for one data set (Fig. 5A). Comparing to the NGNC culture, less than half of the GSNC cultures are bursting (Fig. 5B). From the double patch recording, all of the NGNC cultures are synchronized, but less than half of the GSNC culture are synchronized (Fig. 5C). This ratio is similar to the ratio of GSNC with burst.

As mentioned above, we have also created a glia post-plated culture (GPNC) from GSNC by post-plating of glia at various DIV into the GSNC. Before the addition of glia, the GSNC will not burst under a low Mg\(^{2+}\) condition. However, after glia is being added, the GPNC shows similar behavior to NGNC under a low Mg\(^{2+}\) condition. Therefore, it seems that the different forms of firings of the neurons under low Mg\(^{2+}\) conditions are strongly related to the presence of glia in the culture and the effects can be restored by the addition of glia.

IV. DISCUSSIONS

In an attempt to understand theoretically the role of glial cells on the firing dynamics of the cultured neuronal network, we have constructed a model [23] to investigate the properties of SB under the influence of the glia and somatic channels by considering the
FIG. 4: Differences in spontaneous electrical activities between NGNC and GSNC under the low Mg$^{2+}$ (0 mM) condition (10 DIV). A, Paired current-clamp recordings between two cells from the NGNC. B, Similar experiment as in A (NGNC) but with one of the cell being voltage-clamped at −70 mV to provide current recordings (bottom trace). Note that there is a large slow inward current during SB. C∼E, Three different types of activities were recorded between two cells from the GSNC, burst-only C), burst and spike D), and spike-only E). F, Recordings similar to those shown in B but with spike only GSNC sample, and also the two traces are not recorded at the same time. Note that the absence of the large slow inward current as seen in F.
FIG. 5: Summary of firing patterns in spontaneous electrical activities between NGNC and GSNC under the low Mg$^{2+}$ (0 mM) condition. A, Result of NGNC samples. Only one sample did not show burst. B, Result of GSNC samples. Less than half of the samples show burst. C, Probability of synchronized burst recorded by paired current-clamp. Less than half of the samples are synchronized. The probability of being synchronized is similar to the probability of burst in A and B.

interactions of glial release with the extra-synaptic receptors on the nearby neurons. In this mean-field model, the dynamics of the synchronized network can be effectively represented by a two-compartment neuron, consisting of distinct somatic and dendritic parts, with feedback to both dendrite and soma in the presence of glia. The governing equations for
this model are given by:

\[ C_m \frac{dV_S}{dt} = -I_{K-DR} - I_{Na} - I_{K(Ca),S} - I_{Ca,S} - I_{L,S} - \frac{g_c}{p}(V_S - V_D) + I_{\text{noise}}, \quad (1) \]

\[ C_m \frac{dV_D}{dt} = -I_{\text{NMDA}} - I_{Ca,D} - I_{K(Ca),D} - I_{KA} - I_{L,D} - I_{\text{AMPA}} - \frac{g_c}{1-p}(V_D - V_S), \quad (2) \]

\[ \frac{dC_{a,S}}{dt} = f_S(-\beta_S I_{Ca,S} - k_{Ca,S} C_{a,S}), \quad (3) \]

\[ \frac{dC_{a,D}}{dt} = f_D(-\beta_D I_{Ca,D} - k_{Ca,D} C_{a,D} - I_{Ca,NMDA}), \quad (4) \]

where \( V_S \) and \( V_D \) are the membrane potentials for the soma and dendrite parts, respectively. \( C_{a,S} \) and \( C_{a,D} \) are the dimensionless intracellular free calcium concentration in the soma and dendrite, respectively, and \( k_{Ca,S} \) and \( k_{Ca,D} \) are their corresponding decay rates. The \( I \)'s are various ionic currents, and \( g \)'s are the various channel conductances. \( p \) and \( 1-p \) are the ratios of the total neuron area occupied by the soma and dendrite. The other parameters have their usual meaning and their values can be found in [23]. For neuronal transmission, the glutamate-gated currents on the dendrite can be written as:

\[ I_{\text{NMDA}} = \frac{g_{\text{NMDA}}}{(1 + [Mg]_K^{Mg} \exp(-V_D Q))} (V_D - V_{\text{NMDA}}), \quad (5) \]

\[ I_{\text{Ca,NMDA}} = \frac{g_{\text{Ca,NMDA}}}{(1 + [Mg]_K^{Mg} \exp(-V_D Q))} (V_D - V_{\text{Ca}}), \quad (6) \]

\[ I_{\text{AMPA}} = g_{\text{AMPA}} (t - t_e) \exp(-(t - t_e)/0.5)(V_D - V_{\text{AMPA}}). \quad (7) \]

Calcium dynamics is essential here, since the burst will be terminated by the elevation of \([\text{Ca}^{2+}]_s\). \text{Ca}^{2+}\text{-exchange between the soma and dendrite through diffusion is assumed to be negligible. Effects of Mg}^{2+} \text{ are described through its concentration ([Mg]) and the constant } K_{Mg}. q \text{ is a fixed voltage scale on the dendritic compartment. Stimulation on the dendrite is assumed to affect mainly on the NMDA channels, whose conductance } g_{\text{NMDA}} \text{ is governed by:}

\[ \frac{dg_{\text{NMDA}}}{dt} = \begin{cases} -\lambda g_{\text{NMDA}} + |g_a|, & t = t_e, t_a \\ -\lambda g_{\text{NMDA}}, & \text{otherwise} \end{cases} \quad (8) \]

with \( g_{\text{NMDA}} \geq g_0 \), where \( g_0 \) is the conductance in the absence of stimulation. \( t_e \) and \( t_a \) correspond to evoked and asynchronous release times, respectively, and \( \lambda \) is the rate of
NMDA receptors responding to the neurotransmitters. Since glutamate binding on NDMA receptors is stochastic, \(| g_a |\), can be modeled by the following Gaussian process:

\[
<g_a(t)> = 0 \quad \text{and} \quad <g_a(t)g_a(t')> = 2 \cdot (0.15 \cdot G_N) \delta(t - t'),
\]

where the noise amplitude \(G_N\) represents the total fluctuations due to the availability of recovered neurotransmitters vesicles and that of the network connectivity.

The key idea of the above model is that stimulation from the self-synapse not only affects the dendrite, but also triggers the somatic glutamate channels through the diffusive chemicals in the extra-synaptic spaces which were released from the glia. The regulation of the collective dynamics in a network consisting of excitable cells by other non-excitable elements through the diffusion of signaling molecules is also quite common in biological systems, such as in the phenomenon of quorum sensing [24]. The stochastic binding of the released chemicals on the receptors is assumed to be a random process, and together with other environmental noises on the cell body, thus the total environment influence on the soma can be modeled by an Ornstein-Uhlenbeck process which is represented by \(I_{\text{noise}}\) in Eq. (1). A detailed description of the model and the values of parameters can be found in [23] and the appendix therein. Due to the coupling between the neuron and glia, our model can also be used to simulate the situation in which there is no or little glia in the network. Figure 6 displays some typical firing patterns computed using the model for both the NGNC and GSNC condition, which can be compared with the experimental results in Figure 4, indicating that the essential features of the firing patterns can be captured, at least qualitatively. Our model can reproduce most of the reported experimental observations of SB for both the NGNC and GSNC networks. The importance of the triggered glial release and the somatic glutamate channels are tied together in our mean field two-compartment model to account for the change of firing patterns in neuronal networks. The glial release in this model provides an environmental signal in response to the synaptic activities of the neuron, and the information in extra-synaptic space can thus be sensed by the somatic glutamate channels.

From the above results, it is clear that both the NGNC and GSNC are capable of producing spontaneous firing activities under the low \(\text{Mg}^{2+}\) condition. However, the probability of emergence of bursting is decreased without the help of glia. In fact, the originally more or less randomly distributed spontaneous activities in GSNC are synchronized into bursting when glia are added to form a GPNC. After glia is added to GSNC, a new time scale, namely the bursting period, is created. In other words, glia seems to be playing a role of a coordinating agent which couples the activities of different neurons to produce a new collective firing pattern. This finding is consistent with the fact that glia can synchronize nearby neurons [25].

The effects of the glia on the dynamics of the network can also be seen in the inward currents recorded in voltage clamp experiments when spontaneous activities are induced under the low \(\text{Mg}^{2+}\) condition. For the isolated spikes of GSNC, it can be seen that the inward currents consist of isolated fast pulses Figure 4F, indicating that the currents are coming from the individual firing of neurons in the network. However, in the case of NGNC (Fig. 4B) or GPNC, it can be seen that the inward current which caused the bursting
FIG. 6: Typical membrane potential $V_S$ computed using the model [23] described in the text for cases of A) NGNC (compared with Fig. 4A), and B) GSNC (compared with upper panel of Fig. 4F), inset shows the magnified scale. C) Current computed under voltage-clamp condition for the case of GSNC (compared with lower panel of Fig. 4F), inset shows the magnified scale.

consists of two parts: i) fast isolated pulses similar to those in GSNC (Fig. 4F) and ii) a large slow varying part which has a maximum value of about 400 pA with a duration of the order of one second. Since the dynamics of neurons are intrinsically fast, this large slow inward current (SIC) presumably comes from the results of glio-transmission triggered by the increase of synaptic activities in the network [26].

Since it is known that the slow inward currents (SIC) are mediated by NMDA receptors [26] on the neurons in NGNC triggered by glia-mediated transmission, a possible explanation for the lack of the SIC in GSNC is that not enough NMDA receptors in the GSNC are activated. To test this idea, we have also performed experiments by applying enough NMDA in the GSNC under the low Mg$^{2+}$ condition to activate most of the NMDA receptors in GSNC. The result shows that bursting can be induced in GSNC by the application of NMDA. Also, there seems to be synchronization between the bursts similar to those from NGNC, even though these bursts are not periodic as in the case of NGNC. The implication of these results is that the NMDA receptors are present in the neurons of GSNC, and the lack of glia reduces the activation of the NMDA receptors under low Mg$^{2+}$ conditions.

It is interesting to note that the bursting induced by NMDA in GSNC is quite similar to that induced by the low Mg$^{2+}$ condition in NGNC except that there is no periodicity in the bursts. This last finding suggests that glia do not only activate the NMDA receptor of the neurons in NGNC to produce bursting but also modulate them into a periodic manner.
If we compare currents from these two types of bursting, it can be seen that there is also a slower component of inward current in GSNC. However, this slow component in GSNC has a time scale of the order of 100 msec, which is much faster than the SIC in NGNC. Most likely, the bursting of GSNC in NMDA is caused by the successions of this slower component. This last finding suggests that the presence of glia in NGNC prolongs the activation of the NMDA receptors. All these findings indicate that glia are playing an active role in the activation of the NMDA receptors in the neurons. The synchronization of GSNC induced by NMDA is not surprising because the NMDA is uniformly distributed in the culture and all the neurons in the culture will have the same environment. However, for the case of NGNC, the presence of glia seems to provide this uniform extra-synaptic environment as SB can also be observed in NGNC.

The picture emerging from the above discussion is that the neurons in GSNC and NGNC are basically the same, and glia are responsible for SB and the large SIC observed in NGNC. Since the neuronal activities of the whole network are synchronized during SB, one would therefore expect to have a larger SIC in our experiment as compared to studies reported earlier [6] when the cells are only locally stimulated. If this view is correct, the bursting mechanism induced by glia is very different from other bursting models. For example, there is no need of special channels on the neurons and thus any neuron is capable of bursting. With this picture, the firing of a neuron can be changed from isolated spikes into bursting if nearby glia which wrap its synapses are triggered to release chemicals to modulate the firings of neurons. If we compare the firing rates of neurons in GSNC and NGNC, it can be seen that the firing rates of NGNC are much faster than that of GSNC. Therefore, the glia do not only act as a signal amplifier to increase the overall firing rate of the neuron but also change the form of the firing from isolated spikes to bursting. Presumably, if bursting is a more robust means of communication, glia seem to be playing an active signal detector role by monitoring and modulating the amount of synaptic activities at the same time.

Neuronal bursting is an important phenomena in our brain. Electroencephalography (EEG) is a common method to study neuronal assemble activities in cognitive processes. Brain waves, which come from a population bursts, can be divided into several bands as alpha, beta, theta, delta, etc. These waves are strongly related to the state of our brain. For example, delta waves (< 4 Hz) are associated with deep sleep. Several studies suggested that the hippocampus requires delta waves to induce synaptic modifications in hippocampal circuitries that participate in memory consolidations. Bursting is not only related to cognition and memory but also to brain disorders such as epilepsy, sleep disorders, and chronic pains [4, 27]. Our study shows that the glia can improve neuronal communications and modulate bursting frequencies, suggesting that the glia should be considered as an important factor of brain functions.
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References