Connectivities and Synchronous Firing in Cortical Neuronal Networks

L. C. Jia,[1,4] M. Sano,[2] Pik-Yin Lai,[1,3,5] and C. K. Chan[1,*]

[1]*Institute of Physics, Academia Sinica, Nankang, Taipei, Taiwan 115, Republic of China*
[2]*Department of Physics, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[3]*Department of Physics and Center for Complex Systems, National Central University, Chungli, Taiwan 320, Republic of China*
[4]*Institute of Medical Image, Yuanpei University of Science & Technology, Hsinchu, Taiwan 300, Republic of China*
[5]*Brain Research Center, University Systems of Taiwan, Taiwan, Republic of China*

(Received 3 June 2003; published 16 August 2004)

Network connectivities ($\bar{k}$) of cortical neural cultures are studied by synchronized firing and determined from measured correlations between fluorescence intensities of firing neurons. The bursting frequency (f) during synchronized firing of the networks is found to be an increasing function of $\bar{k}$. With f taken to be proportional to $\bar{k}$, a simple random model with a $\bar{k}$ dependent connection probability $p(\bar{k})$ has been constructed to explain our experimental findings successfully.

DOI: 10.1103/PhysRevLett.93.088101 PACS numbers: 87.19.La, 05.45.Xt, 84.35.+i

An important characteristic of interconnected systems [1] is that there are emerging properties of the systems [2] which are not properties of the individual constituents. For example, it is known that neurons are connected in different manners in our brains[3] to provide different functions. In principle, it is possible to deduce or design the functions of a network if detailed interactions among the constituents are known[4]. However, when such information is not available, one has to rely on modeling based on emerging properties. A good example is the synchronized firing (SF) observed in cortical neural cultures [5,6]. The neurons in the cultures form synchronized clusters (SC) which fire synchronously with a bursting frequency f of the order of 0.1 Hz when the extracellular concentration of Mg^{2+} ($[Mg^{2+}]$) is lowered. Obviously, a thorough understanding of the underlying mechanism of SF will be valuable in the understanding of interactions among the neurons during growth [7]. Unfortunately, the basic mechanism for SF is not clear [8].

It is known that SF can be observed only when there are enough connections in the cultures[9]. Obviously, the structure of the cultures is important. Usually, the structure of a network is measured by the *characteristic node connectivity* $\bar{q}$ [1]; the average of q over all nodes in the network where q of a node is defined as the number of other nodes to which it is directly connected. Since connections between neurons are difficult to identify and characterized by their synaptic strength, the geometrically defined $\bar{q}$ cannot be measured for the SC. However, during SF, the correlated activities of the neurons allow the measurement of a *correlation connectivity* $\bar{k}$ for the SC where $\bar{k}$ is defined similarly to $\bar{q}$ but with connections among nodes being determined by their correlated activities not their physical contacts. Note that $\bar{k}$ is related to the effective connections of the network.

In this Letter, we present results on experimental studies of SF, which are designed to probe the effects of changes in $\bar{k}$ on SF by the fact that extracellular Mg^{2+} can block the N-methyl-D-aspartate (NMDA) channels of a neuron [3], leading to the reduction of effective connections in the network. We find that the bursting frequency f during SF is an increasing function of $\bar{k}$. With f taken to be proportional to $\bar{k}$, a simple stochastic model with a $\bar{k}$ dependent connection probability $p(\bar{k})$ has been constructed to explain our experimental findings.

Neuronal culture samples are prepared [10] from the cerebral cortex of embryonic days $17 \sim 18$ ($E17 \sim E18$ with E0 as the breeding day) embryos of Wistar rats similar to other works [5]. Observations of the culture are carried out in a fluorescence microscope while the cultures are kept in buffered salt solution (BSS) [5]. Spontaneous firing of the cultures is induced by reducing $[Mg^{2+}]$ in the BSS. Firings of the networks are monitored by the changes in intracellular Ca^{2+} concentration $[Ca^{2+}]$, which is indicated by the fluorescence intensity of the Ca^{2+} fluorescence probe (Oregon Green from Molecular Probe Inc.) and recorded by an intensified charge coupled device (CCD) video camera (Cohu Inc.) at 30 frames per second with a resolution of 400×400 pixels. The higher speed of a photodiode array [11] is not used here because the present spatial resolution of such devices is not high enough for the reconstruction of spatial networks needed in this work.

To start an experiment, cultures are first loaded with Oregon Green for 30 min at 37° C. The cultures are then washed by a Mg^{2+} free medium [BSS (−)] several times and then placed in a perfusion chamber on the microscope which is temperature controlled at 37° C. Fluorescence images of the culture are then recorded while $[Mg^{2+}]$ of the medium is controlled by the perfusion system. The origin of the age (t) or growth time of the cultures is set at the time of plating and measured in units of DIV (days in vitro). Data reported below are the results of more than 20 dissections. Every dissection on average gives 15 samples. Because of the variability of samples from different dissections, results reported are taken usually from samples

of the same dissections. However, phenomena reported below can be observed in all the dissections. By comparing the SF frequencies from samples with and without previous exposure to the fluorescence imaging, the toxicity of fluorescence imaging is found to be not affecting the frequency of SF of the cultures in any significant way.

The lower inset of Fig. 1 shows a typical measured time dependence of the fractional changes ($\Delta F/F$) of total fluorescence intensity (F) during SF. It indicates that intracellular $[Ca^{2+}]$ of the whole network is changing with a period of about 4 sec. The increase in $[Ca^{2+}]$ has been found to be associated with the firings of neurons [5]. Typical durations of the bursts (t_B) and the silences in between (t_S) are 4 sec and 6 sec, respectively, at small DIV. However, both t_B and t_S decrease with DIV. Note that t_S becomes basically zero after 5 DIV and then t_B is the same as the period of SF. Although the periodic behavior shown in the inset is obtained from the total fluorescence intensity over many neurons visible in the video picture, measurement of individual neurons in the picture also reveals similar behavior. This last finding shows that the size of the SC is more or less fixed during many periods. For SF, it is known that f and the size of SC increases with time [9]. Presumably, the connections in the network also increases with time. Figure 1 shows a typical time dependence of f for such samples. It can be seen that f increases with t linearly at early time and its time dependence can be fitted to a logarithmic form: $f = f_c + f_0 \log(t/t_c)$; with a minimum f (f_c) and a threshold of growing time (t_c) or the onset time for SF. Note that f_c (~ 0.1 Hz) seems to be independent of the plating density but t_c is found to be a function of ρ as shown in the upper inset of Fig. 1. The ρ dependence of t_c can be fitted to the form: $t_c \sim \rho^{-\beta}$ as shown in the inset with $\beta = 0.44$. The χ^2 of the fits in Fig. 1 and its upper inset are 0.095 and 0.67, respectively.

After the culture medium of the sample has been changed to BSS(−) to induce SF, it is found that the bursting rate f will decrease exponentially with a time constant of about 15 h as shown in Fig. 2. This exponential decay can be observed in other cultures of different plating densities at different DIV with more or less the same decay constant. If f is related to the connections in the network, this finding suggests that the total number of connections in the network is a decreasing function of time. This decrease in the number of connections might be due to the death/nonfunctioning of a neuron and/or a connection because the physiological conditions of the culture are not being maintained, different from other experiments [12]. Different functional forms have also been used to fit the data in Fig. 1 and 2 but they do not give better fits.

In the experiments, the $[Mg^{2+}]$ in BSS can be changed in steps by the perfusion system. Figure 3 shows the changes of f when $[Mg^{2+}]$ in the BSS of a culture is changed every 15 min. The time of the experiment is limited to within 3 h to avoid the decay seen in Fig. 2.

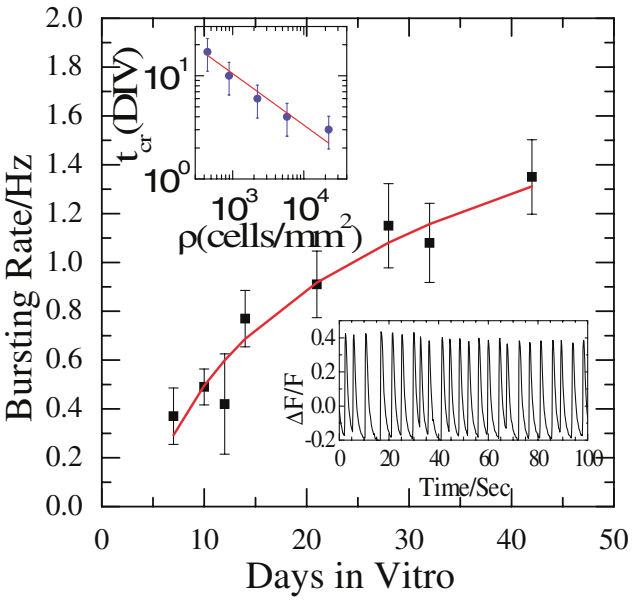

FIG. 1 (color online). Time dependence of bursting rate for a culture with a plating density of $\rho = 10^4$ cells/ mm^2 in BSS(−). Lower inset shows the time dependence of fluorescence intensity on 4 DIV and the upper inset shows the plating density dependence of the onset time of synchronous firings. Solid lines in the figure and the upper inset are fits of the data to forms described in the text. Error is estimated from two sets of data obtained from two different dissections.

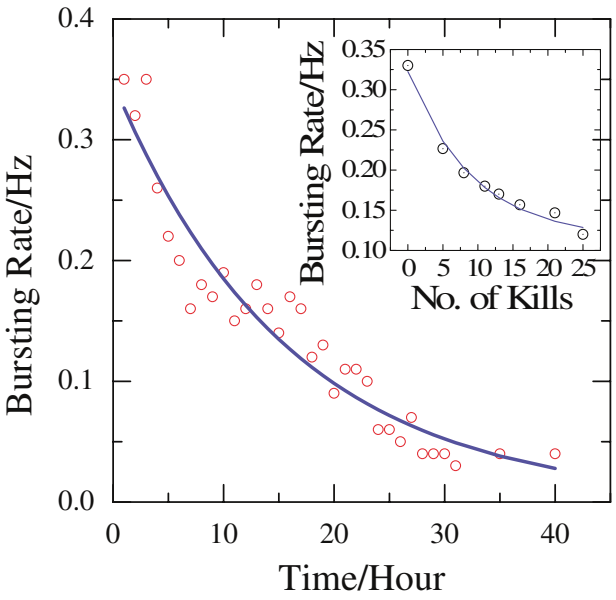

FIG. 2 (color online). Time dependence of the bursting rate of SF of a dying culture with a plating density of $\rho = 10^4$ cells/ mm^2 on 6 DIV in BSS(−). More than ten samples have been observed to give similar results but the bursting rates of different samples can be different by as much as 30%. Inset shows the effects on the bursting rate as the neurons in the SF cluster are being killed in a similar sample.

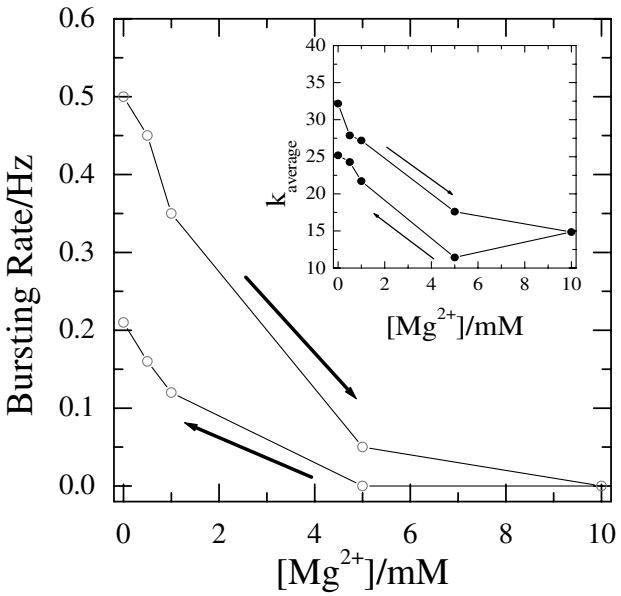

FIG. 3. Effect of $[Mg^{2+}]$ on the bursting rate of SF for a culture with a plating density of $\rho = 10^4$ cells/ mm^2 on 10 DIV. The upper branch are the data obtained during the decrease of $[Mg^{2+}]$, while the lower branch is obtained during the increase of $[Mg^{2+}]$. More than five different dissections have been observed to give similar results. The inset displays the variation of the number of links of the neural network as $[Mg^{2+}]$ is changed.

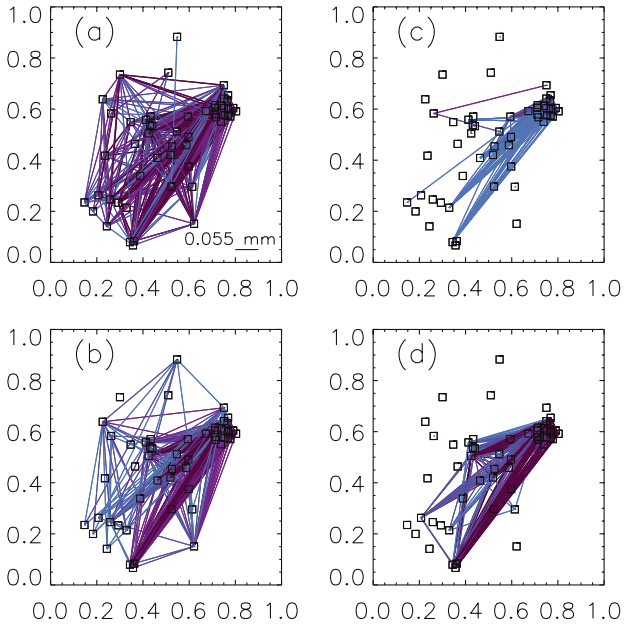

FIG. 4 (color online). Connectivity structures constructed from correlation matrix for the experiment in Fig. 3 when $[Mg^{2+}]$ equals (a) 0.0 mM, (b) 1.0 mM, (c) 5.0 mM, and (d) back to 0.0 mM again. Location of the neurons are denoted by squares and the coordinates of the axis are distance in real space. Note that there is hysteresis in the structure.

Also, the changes in $[Mg^{2+}]$ are designed to be in a cycle so that the $[Mg^{2+}]$ is zero again at the end of the experiment. It can be seen from Fig. 3 that f decreases with the increase in $[Mg^{2+}]$ and SF can be totally suppressed by 10 mM of Mg^{2+}. However, effects of Mg^{2+} are not completely reversible. From Fig. 3, it can be seen that SF cannot be restarted until $[Mg^{2+}]$ is further lowered to about 5 mM.

It is known that the effective connections in the culture are being changed by $[Mg^{2+}]$[6]. From Fig. 3, it is clear that the effects of the changes of $[Mg^{2+}]$ on f are similar to the changes in DIV. The advantage of using Mg^{2+} is that a single sample can be used while different samples must be used if the effects of DIV are studied [9]. If there are N firing cells in the video picture and $I_i(t)$ is the time dependence of the fluorescence intensity of the ith firing cells, a $N \times N$ correlation matrix [9] can be formed with the matrix element c_{ij} given by: $c_{ij} = \frac{1}{N_{ij}} \int \Delta I_i(t) \Delta I_j(t) dt$, $1 \le i, j \le N$, where $\Delta I_i(t) \equiv I_i(t) - \langle I_i(t) \rangle$ and $N_{ij} \equiv \sqrt{\int \Delta I_i^2(t) dt \int \Delta I_j^2(t) dt}$ is a normalization factor. Note that $-1 \le c_{ij} \le 1$ and a large value of $|c_{ij}|$ indicates that there are strong correlations (the weight of connection) between the ith and the jth neurons. Figure 4 is the visualized network structures for the experiment shown in Fig. 3. In Fig. 4, positions of the neurons are marked by squares and weights ($|c_{ij}|$) between two neurons are represented by the thickness of the line connected between them. Figure 4 agrees with our assumption that connections will be suppressed by the increase of $[Mg^{2+}]$. Note that when the $[Mg^{2+}]$ is reduced back to zero [Fig. 4(d)], the network structure does not recover to its original form [Fig. 4(a)].

With c_{ij}, one can measure a correlation defined k and $\bar{k}$ for the cultures if one takes the value of $|c_{ij}| > 0.5$ as a threshold to indicate that the ith and jth neurons are *connected*. The inset of Fig. 3 shows the $[Mg^{2+}]$ dependence of the mean node connectivity (average over all sites) of the experiment shown in Fig. 3. It is similar to the bursting rate in our experiment. This last finding hints that f might be proportional to $\bar{k}$. Although the results of the threshold value of 0.5 is reported here, we find that the choice of the threshold in the range between 0.3 and 0.7 will produce similar results. It is important to note that $\bar{k}$ is not the same as physical connections among the neurons but they are related. To demonstrate this relationship, a pulsed UV laser (wavelength = 355 nm and pulse width = 50 ns) is used to kill the firing neurons in the SC during SF. The inset of Fig. 2 is the measurement of f as a function of the number of neurons being killed. Since the removal of nodes in a network will reduce the total number of connections, this result shows that the decrease of f and $\bar{k}$ are directly related to the decrease of physical connections.

One can consider the cultures as N random nodes (neurons) uniformly distributed in a space of size $L \times L$. A network can be created when nodes are connected if the distance between them is smaller than a critical length d. The dying process of Fig. 2 can then be simulated by

removing the nodes randomly from such a network. Simulations of such an operation with a constant removal rate of nodes for a network generated by $N = 200$, $L = 100$, and $d = 10$ show that the time dependence of the computed $\bar{k}$ is very similar to f of the experiment. This last result indicates that f might be proportional to $\bar{k}$ as suggested in Fig. 3. A growing network will be formed when d increases with t. Since the number of neurons within a region with radius d is proportional to d^2, we have $\bar{k} \propto d^2$. The linear increase of f at early time in Fig. 1 can be reproduced if $d \sim t^{1/2}$ and one assumes that $f \propto \bar{k}$. Since $d(t)$ is the search range of one neuron for another, the form $d(t) \sim t^{1/2}$ suggests that the growth of this search range is diffusive.

In this direct connection model, $\frac{\partial \bar{k}}{\partial d} \propto d$. If the probability $[p(\bar{k})]$ of connection between two neurons is $\bar{k}$ dependent, we will have $\frac{\partial \bar{k}}{\partial d} \propto p(\bar{k})d$. The logarithmic dependence of f on t can be modeled if we use $t \sim d^2$ as suggested above and choose $p(\bar{k}) \sim e^{-\bar{k}/k_o}$ with some characteristic k_o. In this model $d(t)$ is the length of a physical line originating from one site searching for another. However, in the neuronal cultures, it is not clear if d is really the length of a direct physical link between neurons and d could be just an effective distance separating two neurons, which can be considered as directly linked. Furthermore, if SF is determined by $\bar{k}$ in the cultures, SF will occur at $t = t_c$ when $\bar{k}$ has grown to some critical value $\bar{k}_c$. Since distances scale as $\rho^{-1/2}$, one will get $t_c \sim \rho^{-1/2}$ for a linear growth of d and $t_c \sim \rho^{-1}$ for a diffusive growth. From the upper inset of Fig. 1, it seems that our data favor the linear growth before SF takes place. The value of $\beta = 0.44$ is close to the prediction of 0.5 from the model. Since $d^2 \sim t$ is found after SF has taken place, it suggests that there might be two regimes for the growth of the cultures. At first, the growth is linear and then slows down to a diffusive one when there are enough connections in the network.

It is clear that our model captures many features of our observations but the mechanism of SF remains unknown. Presumably, this mechanism triggers the periodic induction of SF. Pacemakers could have been responsible for SF but intracellular recording experiments suggested the contrary [5]. Also, it is known that properly coupled excitable systems [13] can be driven to synchronized states by the noise in the system. A likely source of such noises could be the tonically active NMDA channels in the neurons [5]. In principle, one will be able to distinguish these two possible mechanisms experimentally if firing activities from all the neurons during SF can be recorded [11]. If noise is the driving mechanism of SF, the firing rate of neurons during SF will probably be related to the connectivity of the system. Unfortunately, our present recording method cannot provide such detailed data.

This work has been supported by the NSC of Republic of China under the Grant No. NSC 90-2112-M001-027 and Grant No. NSC 92-2112-M001-52. P. Y. L. thanks the Brain Research Center of the University Systems of Taiwan for support. C. K. C. would like to thank Yoshida and Kikuchi for help during the early stage of this work.

*To whom all correspondence should be addressed. Electronic address: phckchan@ccvax.sinica.edu.tw

[1] For a review see, S. H. Strogatz, Nature (London) **410**, 268 (2001), and references therein.

[2] See for examples, M. Cross and Hohenburg, Rev. Mod. Phys. **65**, 851 (1993); H. Haken, *Principles of Brain Functioning: A Synergetic Approach to Brain Activity, Behavior and Cognition* (Springer-Verlag, Berlin, 1996); H. Kitano, Science **295**, 1662 (2002).

[3] E. Kandel, J. Schwartz, and T. Jessell, in *Principles of Neural Science*, edited by Eric R. Kandel *et al.* (Elsevier , New York, 1997).

[4] L. J. Landau and J. G. Taylor, *Concepts for Neural Networks: A Survey* (Springer-Verlag, Berlin, 1999).

[5] K. Muramoto, K. Kobayashi, and S. Nakanishi, Proceedings of the Japan Academy, Series B (Physical and Biological Sciences) **64**, 319 (1988); Hugh P. C. Robinson *et al.*,J. Neurophysiol. **70**, 1606 (1993).

[6] M. Canepari, M. Bove, E. Maeda, M. Cappello, and A. Kawana, Biol. Cybern. **77**, 153 (1997).

[7] R. Segev, M. Benveniste, Y. Shapira, and E. Ben-Jacob, Phys. Rev. Lett. **90**, 168101 (2003).

[8] J. Kowalski, G. Albert, B. Rhoades, and G. Gross, Neural Networks **5**, 805 (1992); Yoshinori Hayakawa and Yasuji Sawada, Phys. Rev. E **61**, 5091 (2000); R. Segev, Y. Shapira, M. Benveniste, and E. Ben-Jacob, Phys. Rev. E **64**, 011920 (2001); T. Tateno, A. Kawana, and Y. Jimbo, Phys. Rev. E **65**, 051924 (2002).

[9] Yoshida, Master thesis, Research Institute of Electrical Communication, Tohoku University, 2001.

[10] Briefly, isolated neurons from the cortex of embryos are prepared first in the form of a cell suspension in buffer solutions [5] with a concentration from 10^2 to 10^5cells/mm^2. Cultures are then prepared by plating a volume of 300 μl of the cell suspension onto the bottom of a Petri dish which has been precoated with polyetheylenimine. After the cells are allowed to adhere to the bottom of the Petri dish, samples are subsequently filled with 2 ml of culture medium [5], and maintained in a 37 °C incubator with 5% CO_2. Half of the medium is renewed twice a week. Samples can be typically maintained for up to a month.

[11] Z. Peterlin, J. Kozloski, B. Mao, A. Tsiola, and R. Yuste,Natl. Acad. Sci. lett. **97**, 3619 (2000).

[12] G. Turrigiano, K. Leslie, N. Desai, L. Rutherford, and S. Nelson, Nature (London) **391**, 892 (1998).

[13] Arkady S. Pikovsky and Jurgen Kurths, Phys. Rev. Lett. **78**, 775 (1997); O. V. Sosnovtseva, A. I. Fomin, D. E. Postnov, and V. S. Anishchenko, Phys. Rev. E **64**, 026204 (2001).