Growth of cortical neuronal network *in vitro*: Modeling and analysis

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We present a detailed analysis and theoretical growth models to account for recent experimental data on the growth of cortical neuronal networks *in vitro* [Phys. Rev. Lett. **93**, 088101 (2004)]. The experimentally observed synchronized firing frequency of a well-connected neuronal network is shown to be proportional to the mean network connectivity. The growth of the network is consistent with the model of an early enhanced growth of connection, but followed by a retarded growth once the synchronized cluster is formed. Microscopic models with dominant excluded volume interactions are consistent with the observed exponential decay of the mean connection probability as a function of the mean network connectivity. The biological implications of the growth model are also discussed.

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I. INTRODUCTION

The fundamental units constituting our brain and nervous system are neuron cells. The basic physical properties of an individual neuron were well characterized more than half a century ago by the classic work of Hodgkin and Huxley [1]. However, the complex emerging properties [2] of highly connected networks [3] of neurons and the function of the brain are still not fully understood. As we know, most neuron cells do not divide and the number of living neurons in an organism will not increase, only the connectivity between the neurons changes in the course of the development. Thus the sophisticated behavior and functions in the brain are largely determined by the neuronal connections. In principle, it is possible to deduce or design the functions of a neuronal network if the neuronal connections are known in detail [4]. It is known that neurons are connected in different manners in the brain [5] to provide different functions. Such neuronal networks are often highly complex, for example, a single neuron is connected to about 10^4 neurons in the vertebrate cortex, and in the mammalian brain there are around 10^{11} interconnected neurons [5]. Detailed information such as the interactions among the constituents, connectivity of the network, the synaptic strengths, etc., are often not precisely known, and one has to infer this microscopic information from the macroscopic emerging properties. Presumably, a deeper understanding of the growth process of the connections of neuron networks *in vitro* might provide some information about the early developmental stage of the brain. Although there are significant differences between neuronal culture *in vitro* and brain developmental processes *in vivo*, there are some interesting features that are common in both cases. For example, some features such as the high degree of synchrony observed in a primitive form of network-driven activity in early development for immature neurons *in vivo* [6], are also observed in cultured neurons *in vitro*. As a first step to probe the growth of connections in such neuronal networks, we recently reported experimental observations [7] of the growth of cortical neuronal networks *in vitro* and the associated synchronous firing (SF) phenomenon when the network is sufficiently well connected. And it has been suggested that such synchronized activity plays an important role in the strategy followed by the developing brain in turning to an active state that possesses highly diversified electrical signals and selective synapses [6]. The basic idea in our experiments [7] was to use the SF frequency as a mean to probe the network connectivity at different growing stages. In this paper, we present a detailed analysis of the experimental data in [7] and theoretical growth models to account for the observations. It should be emphasized that in this work we focus on the growth model of the neuronal network, rather than on the mechanisms giving rise to the SF phenomenon. In Sec. II, the experimental observations reported in [7] are summarized with further analyses to establish the relation between the SF frequency and the mean network connectivity. Section III presents the model of an early stage accelerated growth followed by an exponentially retarded growth when SF is achieved. The values of the growth parameters of the model are also estimated. Section IV analyzes the system from the microscopic interaction viewpoint, presenting some possible mechanisms that could give rise to the growth model. Finally, some biological implications are discussed in the last section.

II. SUMMARY OF THE EXPERIMENTAL OBSERVATIONS

Cortical neuron cells are implanted and incubated in a Petri dish, forming a quasi-two-dimensional neuronal net-

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work. These cortical cultures are networks of growing neurons. Synchronized firing has been observed in these cortical neural cultures under suitable conditions [8,9]; typically for cell density of the order 10^4 cell/mm^2, it takes about 6 to 8 days for synchronous firing to be observed. In our recent work [7], we reported synchronized firing frequency data. The synchronous firing is in bursts. A large part of the neurons in the culture can be seen to form synchronized clusters (SCs) which fire synchronously with a bursting frequency f of the order of 0.1–1 Hz when the extracellular concentration of Mg^{2+} is lowered. The size of the SC is more or less fixed during many periods. Because of the variability of samples from different dissections, results reported were taken usually from samples of the same dissections. However, the synchronized firing phenomena can be observed in all the dissections, while the quantitative data were taken from the average of many samples. The major findings can be summarized as follows.

(i) SF occurs only when the incubation time [measured in days *in vitro* (DIV)] exceeds some critical value t_c.

(ii) The data on the synchronized firing frequency f as a function of time can be well fitted by the empirical relation

$$f = f_c + f_0 \ln\left(\frac{t}{t_c}\right), \quad t > t_c. \tag{1}$$

For $t_c \lesssim t$, the above gives a linear increase of f with t, $f \simeq f_c + f_0(t/t_c - 1)$.

(iii) f_c is roughly independent of the mean cell density of the culture, ρ.

(iv) The critical age t_c scales with ρ as

$$t_c \sim \rho^{-\beta}, \quad \beta \approx 0.44 \pm 0.08. \tag{2}$$

(v) The SF frequency f is roughly proportional to the mean effective connectivity k.

First of all, we shall examine (v) more closely. There is a strong correlation between the synchronous firing f and the mean network connectivity k, which is defined as the average number of physical connections of a neuron to the others in the network. Notice that k as defined here is not exactly the same as the correlation connectivity in [7]. The correlation of the network depends on both k and the synaptic strengths of the connections. From empirical observations, both f and k increase with time and hence f also increases with k. More intuitively, one can rationalize from the fact that as the mean connectivity of the network increases, the average communication time between the neurons decreases, which would set the collective time scale of the system to be faster. More quantitative dependence of f on k can be obtained from the data of killing neurons with a UV laser. Figure 1 shows the variation of the SF frequency as a function of the number of neurons being killed (n_{kill}) in the SF cluster. Suppose the original SF cluster contains N neurons; then the mean connectivity of the network varies with n_{kill} as $k \simeq (1/N)(N - n_{kill})(N - n_{kill} - 1)P_0$, where P_0 is the mean probability that any two neurons are connected before any neuron is killed. Notice that the killing process increases n_{kill}, but P_0 would be unaffected. The data in Fig. 1 would be well fitted by assuming f to vary linearly with k, $f = a + bk$ for some constants a and b. Then a fitting of the data in Fig. 1 with a quadratic dependence in n_{kill} gives $a \sim 0.1$Hz, which is of the order of the error bars in f [7]. Hence, to a first approximation, one can take $f \propto k$ and the mean connectivity k also has a logarithmic time dependence, $k = k_c + k_0 \ln(t/t_c)$ for $k \geqslant k_c$, similar to Eq. (1), with k_c being the connectivity at t_c, which is a constant independent of ρ.

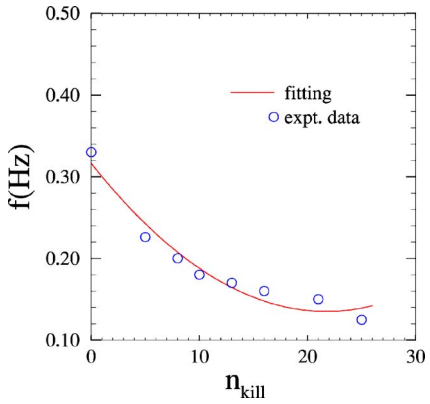

FIG. 1. (Color online) Synchronized firing frequency as a function of number of neurons being killed by a UV laser. The data are taken from the inset of Fig. 2 in Ref. [7]. The curve is a fitting of the experimental data to a quadratic function.

III. THE GROWTH MODEL

Here we propose a theoretical model for the growth of a quasi-two-dimensional neuronal network *in vitro*. This model is aimed at providing a quantitative description of the growth behavior in the neuronal culture. The model provides a phenomenological description while the microscopic interaction mechanisms that leads to the observed growth behavior cannot be inferred from this model. Some thoughts on the nature of these microscopic interactions are presented in Sec. IV.

Let N_d be the number of cells in a region, if the probability that any two cells are connected is P, then the mean coordination number (microscopic connections) of a cell is given by $k \approx PN_d(N_d - 1)/N_d \approx PN_d$. In general, P depends on k. Suppose the cells are deposited on a two-dimensional plane with a uniform cell density ρ; consider a domain of radius d with mean connectivity k. As the neurons reach out for their connections in the next time step, the domain radius increases by δd; then the increase in the mean connectivity, δk, is given by

$$\frac{\delta k}{\delta d} \simeq 2\pi P(k)\rho d, \tag{3}$$

where $P(k)$ is the probability of two neurons being connected. Equation (3) implicitly assumes that the growth of connection is irreversible since the increase δk depends on the probability of connection of the previous step. Although the physical connections of the neurites are irreversible, the synaptic strength of a neural connection can be enhanced or weakened by neuronal firing activities. The synaptic strengths averaged over all the connections are slowly varying on the time scale of the SF frequency measurement. Fur-

thermore, in our experiments, the culture is relatively quiet until it is induced to fire at a certain time. Therefore, at the mean field level, the overall change in the correlations of the network is dominated by the change in the physical neurite connections, and so the growth of the "correlation connectivity" can also be taken to be irreversible in a first approximation for simplicity [7]. The dependence of $P(k)$ is expected to be in general a decreasing function of k for large k since more connections would reduce the probability of a new connection simply due to the finite metabolic rate of a neuron. We shall show below that in order to account for the experimental observations, the search range of the axons is modeled to behave as

$$d = ut, \quad t < t_c,$$

$$d^2 = Dt, \quad t \geqslant t_c, \tag{4}$$

where u and D are the active search speed and diffusive search coefficient, respectively. Furthermore, in order to account for the experimentally observed growth law given by Eq. (1), $P(k)$ is modeled to be

$$P(k) = \begin{cases} P_<(k), & k < k_c, \\ P_c \exp\left(-\dfrac{k-k_c}{k_0}\right) & k \geqslant k_c, \end{cases} \tag{5}$$

for some function $P_<(k)$, where $P_c \equiv P(k_c)$ and k_0 are constants. This form of $P(k)$ implies that if there is a large number of connections present, the probability of new connections forming is very small. This kind of feedback mechanism between the firing activity and effective connections has also been reported in the homeostatic plasticity in some developing neural systems [10]. Although the explicit form of $P_<(k)$ is not known, we shall make the reasonable assumption that $P(k)$ is continuous at $k=k_c$, i.e., $P_<(k_c)=P_c$. Since there are no direct experimental data for $t<t_c$, we shall take the simplest case of $P_<(k)=P_c=$const whenever an explicit form of $P_<(k)$ is needed for quantitative calculations.

A. Enhanced growth model for connectivity toward the synchronized cluster: $t<t_c$

In this regime, there is no direct experimental data on the connectivity as a function of t, but we can still attempt to construct a theoretical model that is consistent with what the experimental data would suggest. The neurons tend to grow their neurites very fast before they achieve a SC; the search range of the neurites is proposed to be given by the active growth manner: $d(t) \simeq ut$ for some characteristic search speed u. Without invoking any explicit form of $P_<(k)$, we shall derive a relation between $k_c \equiv k(t=t_c)$ and t_c. The only assumption is that the only scale characterizing $P_<(k)$ is k_c, i.e., $P(k)=\tilde{P}(k/k_c)$ for some function $\tilde{P}$. Using Eq. (3) and the active growth law, one gets

$$\delta k = 2\pi\rho u^2 t\,\delta t P_<(k), \tag{6}$$

and upon integrating one gets[1]

$$k(t) = k_c \Phi^{-1}\left(\Phi(1)\frac{t^2}{t_c^2}\right) \tag{7}$$

where

$$\Phi(x) \equiv \int_0^x \frac{dx}{\tilde{P}(x)} \tag{8}$$

and k_c is given by

$$k_c = \pi\rho u^2 t_c^2/\Phi(1). \tag{9}$$

One expects u to be insensitive to ρ since in the early stage of the search, k is small and a neuron does not know how many neurons are out there. SF occurs when $k \simeq k_c$ for some fixed value of k_c which is independent of ρ [from experimental observation (iii)]. Hence one has $t_c \propto 1/\sqrt{\rho}$ consistent with the experimental result.

In this picture, where the neurons tend to grow their neurites very fast before they achieve a SC, we can examine the model from the viewpoint of the connectivity growth rate $\mathcal{R} \equiv dk/dt$. From Eqs. (6) and (1), one easily obtains

$$\mathcal{R} \simeq 2u\sqrt{\pi\rho k_c \Phi(k/k_c)}\,\tilde{P}(k/k_c). \tag{10}$$

There are no direct experimental data for $t<t_c$ and little empirical information about the form of $P_<(k)$ for $k<k_c$, but one can still make some further reasonable assumptions and proceed. Assuming $P_<(k)=P_c=$const for $k<k_c$, then $\Phi(x)=x/P_c$ and k_c is given by

$$k_c = \pi\rho u^2 P_c t_c^2 \quad \text{or} \quad u = \frac{1}{t_c}\sqrt{\frac{k_c}{\pi\rho P_c}}, \tag{11}$$

and $\mathcal{R}$ becomes $\mathcal{R}=2u\sqrt{\pi\rho P_c k}$ for $P_<(k)=P_c$. Furthermore, it is easy to show that [for $P_<(k)=P_c$]

$$\frac{d^2k}{dt^2} \equiv \frac{d\mathcal{R}}{dt} = 2\pi\rho u^2 P_c > 0, \quad t < t_c, \tag{12}$$

suggesting an accelerated growth of the neurites toward a synchronized cluster.

B. Retarded growth model for the connectivity after SF: $t>t_c$

At $t \approx t_c$ ($k \approx k_c$), the neurons have made enough connections among themselves and cooperativity begins. Presumably a neuron gets enough signals from other neurons such that it surmounts some threshold signaling that there are enough connections for cooperativity and there is no need for further increase of connection. Thus for $t_c \lesssim t$, motivated by the experimental observation of early linear dependence of f (and hence k) on t, and from Eq. (3), we model the search range of the neurites to form connections by a diffusive be-

[1] $d\Phi^{-1}(y)/dy = \tilde{P}[\Phi^{-1}(y)]$.

havior: $d^2 \simeq Dt$ (D is a kind of diffusion coefficient).[2] Invoking Eqs. (1) and (3) together with the diffusive behavior for the search range, d, one finally has

$$P(k) = P_c \exp\left(-\frac{k-k_c}{k_0}\right) \tag{13}$$

and $P_c \equiv P(k_c)$ has to satisfy

$$k_0 = \pi\rho D P_c t_c. \tag{14}$$

Using Eq. (9), one gets

$$k_c/k_0 = u^2 t_c/[DP_c\Phi(1)]. \tag{15}$$

One expects D to be a decreasing function of ρ as the density effect will be important as the neurites will encounter many obstacles as they grow in this regime. If one assumes $P_<(k) = P_c =$ for $k < k_c$, then one has $k_c/k_0 = u^2 t_c/D$. After the SC has formed, $P(k)$ decays rapidly for a sufficiently large cluster. From the viewpoint of the rate of growth, $\mathcal{R} \equiv dk/dt$ is governed by

$$\mathcal{R} \equiv dk/dt = \frac{k_0}{t_c}\exp\left(-\frac{k-k_c}{k_0}\right) \quad \text{for } k \geqslant k_c. \tag{16}$$

Equation (16) together with the initial condition of $k(t=t_c) = k_c$ will give the observed empirical relation in Eq. (1). It is easy to show that $d^2k/dt^2 = -k_0/t^2 < 0$ and the growth is retarded once the SC has formed.

As suggested by the model (and the data), the decrease in $\mathcal{R}$ is rather strong, namely, exponential in k, and a neuron with connectivity much greater than a characteristic k_0 has a negligible $\mathcal{R}$. We shall briefly examine the implication of an exponential decay of $\mathcal{R}$ in this regime. First, we assume that the neurons are passive in response to the increasing connections, i.e., the neuron will not do anything extra in response to an increase in k. Then $\mathcal{R}$ will be roughly a constant (set by the nominal metabolic rate of the neuron) and the mean connectivity will increase linearly with t. Thus, it is quite possible that the neurons (and also some active components in the medium such as the glia cells) play an active and effective role in decreasing the connection growth rate via some sort of feedback mechanism, which is related to the plasticity of developing neural systems [10,11]. It is plausible that the system tries to homogenize the connection number for each neuron, and it does so in an effective way: $\mathcal{R} \sim \exp[-(k-k_c)/k_0]$ so that neurons with $k \gg k_0$ basically stop the growth of new connections. If one assumes that, to lowest-order approximation, the decrease in $\mathcal{R}$ is proportional to both $\mathcal{R}$ and the increase in k, i.e., $d\mathcal{R} \propto -\mathcal{R}\, dk$, then one obtains the consistent result of (16).

1. More results from the model

From Eqs. (9) and (14), one gets

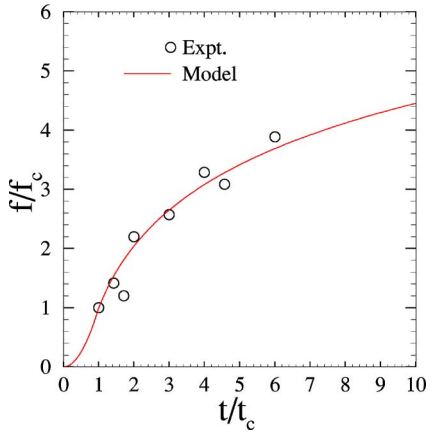

FIG. 2. (Color online) The reduced bursting frequency of SF f/f_c vs the reduced DIV t/t_c. The experimental data are fitted with Eq. (18) with $f_0/f_c \simeq 1.5$.

$$\frac{f_0}{f_c} = \frac{k_0}{k_c} = \frac{D\Phi(1)P_c}{u^2 t_c}. \tag{17}$$

The time dependence of k or f can be put into the form

$$\frac{f}{f_c} = \frac{k}{k_c} = \begin{cases} \Phi^{-1}\left[\Phi(1)\left(\dfrac{t}{t_c}\right)^2\right], & t < t_c, \\ 1 + \dfrac{D\Phi(1)P_c}{u^2 t_c}\ln\left(\dfrac{t}{t_c}\right), & t \geqslant t_c. \end{cases} \tag{18}$$

The relation between the microscopic growth parameters can be obtained from a fitting of the experimental data. Figure 2 shows the fitting of Eq. (18) with the data of $\rho = 10^4/\text{mm}^2$ in [7] [taking $P_<(k) = P_c$], one gets $f_0/f_c = D/(u^2 t_c) \simeq 1.5$. The mean connectivity k is continuous at $t = t_c$, but $\mathcal{R}$ can in general be discontinuous at t_c with

$$\mathcal{R}(t_c^-) = 2u\sqrt{\pi\rho k_c \Phi(1)}P_c = 2\pi\rho u^2 t_c, \tag{19}$$

$$\mathcal{R}(t_c^+) = \frac{k_0}{t_c} = \pi\rho D. \tag{20}$$

However, due to the collective nature of SF, fast variations in the growth rates are expected to be smoothed out; hence we expect the discontinuity in $\mathcal{R}$ at t_c will not be large. This can be checked in our model by computing $\mathcal{R}(t_c^-)/\mathcal{R}(t_c^+) = 2u^2 t_c/D = 2f_c/f_0$. From the fitting of experimental data $f_0/f_c \simeq 1.5$, one gets $\mathcal{R}(t_c^-)/\mathcal{R}(t_c^+) \simeq 1.35$. The rate of growth is switched to a lower value once the synchronized cluster is formed.

2. Estimates of u and D

Here we attempt to obtain an order of magnitude estimate for the microscopic growth parameters u and D of the model using information from further experiments. Recall that u is the mean active search speed of a neuron before the synchronized cluster is formed and D is the mean diffusion constant of the diffusive search of a neuron after the network is synchronized. Using an UV laser, we also performed experiments of isolating a circular interior domain from the rest of

[2]One can include the effect of excluded volume interaction by writing $d^2 \simeq \tilde{D}t^{2\nu}$ with self-avoiding walk exponent ν [=3/4 in two dimensions (2D)], then all calculations will also follow with $D \to \tilde{D}$ and $k_0 \to k_0/(2\nu)$.

the network when the culture has grown to the SF stage [12]. We then determined the minimal radius r_c of the isolated domain such that SF still occurred in it. From the experimental data of cultures of $\rho=10^4/\mathrm{mm}^2$, we found r_c is of the order 0.1–0.2 mm. For a rough order of magnitude estimate, we shall assume $r_c\sim 0.15$ mm. Denoting $N_c=\pi\rho r_c^2$ to be the number of cells in the the threshold domain, N_c can be regarded as the minimal number of neurons in a connected cluster for collective behavior to emerge and this is achieved by the early stage of growth in a period of t_c. Invoking the active search growth law, then one has $(ut_c)^2\rho\sim N_c\sim r_c^2\rho$. Thus one gets $u\sim r_c/t_c$. Using $t_c\sim 6$ days for $\rho\sim 10^4/\mathrm{mm}^2$ and $r_c\sim 0.15$ mm, one has $u\sim 25$ μm/day. Also, using Eq. (17) [with $P_<(k)=P_c$] and $D\sim u^2t_cf_0/f_c$ and from fitting of the experiment, $f_0\sim 1.5f_c$, one gets $D\sim 0.0056$ mm^2/day. It is also worth noting that $N_c\sim 300$.

3. Coupling length between neurons

Intuitively, the probability of connection between two neurons should be some implicit function of their separation. This is because two neurons that are far apart will be difficult to connect because by the time there is a chance they can be connected, the connectivity of the two neurons has already grown to be too large, which decreases the probability of new connections. Here we attempt to deduce the mean probability $\mathcal{P}(\Delta)$ that two neurons initially separated by a distance Δ will be connected. The knowledge of $\mathcal{P}(\Delta)$ will be important in determining whether there is a finite coupling length between two neurons in a culture. Furthermore, it might provide information about the minimal size of a functioning synchronized neuron cluster. Adopting the model from the previous section, we first consider the small-Δ case, i.e., $\Delta<ut_c$. In this case, the two neurons are in active search and the connection will be achieved in a time $\sim\Delta/u<t_c$. From Eq. (7), the corresponding connectivity is $k=k_c\Phi^{-1}[(\Delta/ut_c)^2]$, and the connecting probability is given by $P_<(k)=\tilde{P}(k/k_c)$. Thus one obtains

$$\mathcal{P}(\Delta)=\tilde{P}\left\{\Phi^{-1}\left[\Phi(1)\left(\frac{\Delta}{ut_c}\right)^2\right]\right\}=\Phi^{-1\prime}\left(\frac{\pi\rho\Delta^2}{k_c}\right)\quad\text{for }\Delta<ut_c. \tag{21}$$

For $P_<(k)=P_c$, one has a constant $\mathcal{P}(\Delta)=P_c$. For $\Delta>ut_c$, diffusive search takes place after the active search; thus two neurons will meet at a time $\sim t_c+(\Delta-ut_c)^2/D$. At this time the connectivity is given by Eq. (18) with a probability given by Eq. (5). One finally gets

$$\mathcal{P}(\Delta)=\frac{P_c}{1+\dfrac{(\Delta-ut_c)^2}{Dt_c}}\quad\text{for }\Delta>ut_c. \tag{22}$$

Figure 3 shows the distributions of $\mathcal{P}(\Delta)$, illustrating that the coupling range increases with larger values of $D/(u^2t_c)$ for $\Delta>ut_c$. The "coupling length" Δ_c between two neurons can be characterized by the half-width of $\mathcal{P}(\Delta)$, i.e., $\mathcal{P}(\Delta)/P_c=\frac{1}{2}$, and one gets

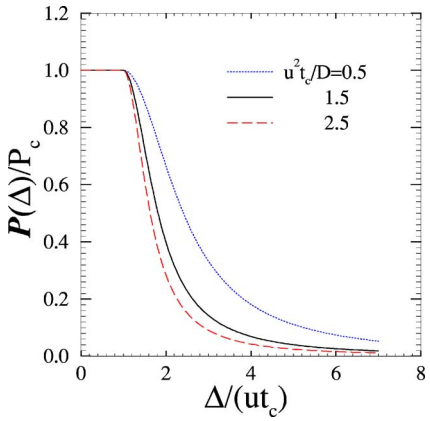

FIG. 3. (Color online) Reduced coupling length distribution $\mathcal{P}(\Delta)/P_c$ as given by Eq. (22) for various values of u^2t_c/D.

$$\Delta_c=ut_c+\sqrt{Dt_c}. \tag{23}$$

To get an estimate of the value of Δ_c, taking the estimated values of $u\sim 25$ μm/day, $t_c\simeq 6$ days, and $f_0/f_c=(u^2t_c)/D\simeq 1.5$, one gets $\Delta_c\sim 0.33$ mm. This is very close to twice the experimental estimated critical radius $r_c\sim 0.15$ mm, as one might have expected.

IV. SOME MICROSCOPIC MODELS FOR NETWORK GROWTH

Here we attempt to model the microscopic interactions between the neurons during the network growing process. These models merely provide a plausible coarse model to describe the qualitative behavior of the observed growth of neuronal connections in a quasi-two-dimensional network *in vitro*. Further direct detailed experiments on smaller length scales should be conducted to provide more quantitative information on the nature of these microscopic interactions.

A. A Phenomenological model

As a first step, we construct a phenomenological microscopic model consisting of local connection rules that mimics the exponential behavior of $P(k)$. Consider a system of N neurons; the ith neuron will have a probability of accepting a connection given by

$$p_i=\begin{cases}P_c, & k<k_c,\\ P_c\exp\left(-\dfrac{k_i-k_c}{2k_0}\right), & k\geqslant k_c,\end{cases} \tag{24}$$

where k_i is the degree of connection of the ith neuron. k_0 and k_c are parameters with similar meanings, but not exactly the same quantities as in previous sections, since p_i is a local probability whereas $P(k)$ in the previous sections is a mean field probability for the whole system. A connection will be formed between neurons i and j with probability p_ip_j. This model can be easily simulated and the time dependence of the average connectivity can be monitored and compared with experimental data. Figure 4 shows the simulation results of the reduced mean connectivity k/k_c as a function of the

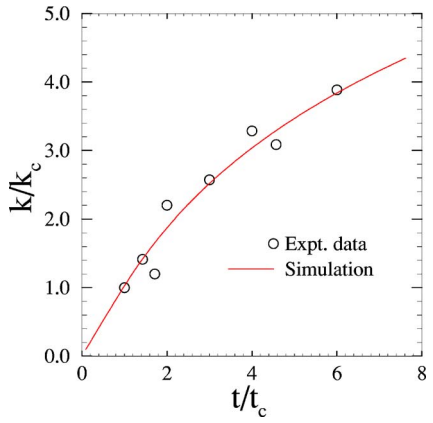

FIG. 4. (Color online) Simulation results for a network grown with connecting probability described by Eq. (24). $N=500$. $k_0/k_c \simeq 3$ obtained by fitting to the experimental data.

reduced growing time t/t_c. The simulation results depend only on k_0/k_c and its value is fitted to achieve best agreement with the experimental data. The simulation results are not sensitive to the values of P_c (as long as $P_c \lesssim 1$) and for sufficiently large N.

The best-fitted value of k_0/k_c is ≈ 3 which is somewhat larger than that obtained from fitting with the mean field theory ($k_0/k_c \simeq 1.5$) in previous sections. This is presumably due to the fluctuation effects, which would cause some sites to be highly connected but then basically inert to new connections. As a result, fluctuations would tend to slow down the growing process which is manifest in a larger k_0/k_c. It is also easy to see that in this model the degree distribution function is non-scale-free and results in a completely connected graph in the asymptotic long time limit.

B. Local intracellular interactions: Exclusion near the cell boundary

The experimental observation of an exponential decay of $P(k)$, i.e., the more connections the system possesses, the less probable it is to form a new connection, can be viewed as a kind of homeostatic feedback in the system. There are many possible detailed mechanisms that could lead to such an observation, but we are interested in more generic factors. One plausible reason for the rapid decrease in $P(k)$ with increasing k may be due to geometric effects arising from the excluded volume interactions between the connecting sites on a neuron. As the neuron attempts to establish a connection with another neuron, its neurite has to grow out and reach the neurites of another neuron. If the mean number of connections is large, the available sites for the incoming and outgoing neurites are blocked by the already connected neurites of other neurons. The unconnected neurite has to overcome a kind of excluded volume interaction. The dominant part of this interaction will be the excluded volume interaction for accepting an incoming neurite and connecting to its cell body. Imagine that the neuron cell body (quasi-2D) already has a large number of neurites attached to its boundary; then it would be very hard to have a new neurite connect, i.e., a single neuron possesses a kind of excluded volume interaction in the local vicinity of the cell body. This problem can be viewed as the one-dimensional random sequential adsorption whose exact solution has been well established [13]. For the adsorption of a dimer on a 1D lattice with a constant deposition rate κ, the probability of having an empty n-tuple is given by

$$\mathcal{P}_n(t) = \exp[-2(1-e^{-\kappa t})]\exp[-(n-1)\kappa t]. \tag{25}$$

κt is the number of trial depositions and is just k in our neuron model. Now for a new neurite to connect to the cell body, it will do so if it finds enough space around it. Suppose a new neurite makes a connection if there is an empty m-tuple on the cell boundary, the probability of accepting a new neurite is given by (taking the cell boundary very much larger than the neurite thickness)

$$P(k) \propto \sum_{n \geqslant m}^{\infty} \mathcal{P}_n(k) \sim \exp[-2(1-e^{-k})]\exp[-(m-1)k]. \tag{26}$$

Since the k dependence in the prefactor in (26) is insensitive to k, one has approximately $P(k) \propto \exp[-(m-1)k]$. This gives a plausible account for the observed exponential decay in the connection growth rate as the number of connection increases. Furthermore, comparing Eqs. (16) and (26), k_0 has the physical meaning related to the range of excluded volume interaction for the neurite near the cell boundary, namely, $1/k_0 + 1 \approx m$.

C. Global intercelluar interactions between neurites

Excluded volume interactions are still significant even at length scales not close to the vicinity of a neuron, especially when the mean connectivity of the network is significant. This is simply because if the substrate of the culture is already densely occupied by other neurites, the growth of a new neurite into this region is expected to be retarded. This growth-suppressing interaction is of a global (intercellular) scale and is especially significant in our quasi-two-dimensional networks. The observed exponential decay of $P(k)$ is also consistent with the above view from the following argument: the probability of a new connection attempt crossing the existing bonds is proportional to $P(k) \times$(bond density). Since the average bond density is proportional to k, so if the mean connectivity is increased by δk, the change in the connecting probability is given by $\delta P \propto -P(k)\delta k$, which results in the exponential decay of $P(k)$. Even though we do not know the strength of such suppressing interactions between the crowded connections, we can still examine such an effect by simulation. Such a suppressing interaction can also be viewed as costing some sort of penalty for neurites crossing each other. For the extreme case of a very strong interaction, the neurites will try to avoid each other and will not cross. Again this kind of suppression of the neuronal crossing can also be viewed as an effective homeostatic feedback mechanism which suppresses long distance connections (which are not economical). If crossing cost no penalty, then there would be no self-control for the growth of connections, i.e., no homeostatic feedback, which

is not favorable for the biological system and also not observed experimentally. The simplest intuitive model for such homeostatic feedback would be simply to impose some crossing restriction in a two-dimensional growing network.

We carried out Monte Carlo simulations to investigate the growth of connections of a 2D network subjected to the constraint that there is strictly no crossing of the connections. Of course, in reality neurite connections can cross but it would presumably cost some sort of energy penalty. In the simulation, neuron sites are initially randomly placed on a plane, then a pair of sites is randomly chosen in an attempt to make a connection; a connection is allowed only if the new connection does not intersect with any other existing connections. The results are then averaged over many different realizations of the initial random neuron positions. The mean connectivity of the network as a function of growing time is monitored. The connecting probability of two previously unconnected neurons is also measured. Figure 5(a) displays the connecting probability $P(k)$ as a function of the mean connectivity k showing an exponential decay of $P(k)$ for $1 \lesssim k$. The purpose of the simulation is just to demonstrate that such crossing penalty interaction in the growth of a two-dimensional network will lead to a strong suppression in the probability of connection as the mean connectivity of the network is increasing. We have also verified that qualitatively similar results are also obtained for finite penalty of connection crossings. More sophisticated growth models incorporating such neurite interactions will be necessary to model the realistic growth dynamics of the experimental neuronal network, which is under current investigations. Nevertheless, our simulation result demonstrates that the intercellular neurite avoiding interactions is a plausible mechanism for the exponential slowing down of the connection growth after the synchronized cluster is formed. Furthermore, Fig. 5(b) also shows that the mean connectivity grows logarithmically in time after some initial stage of growth, again consistent with experimental observations. It should be emphasized that the $P(k)$ discussed here is different from the degree of distribution function $P_{deg}(k)$ that is frequently studied in statistical network theory [14]. The degree distribution functions in the present simulations are also shown in Fig. 5(c) at early and late stages of the network growth. $P_{deg}(k)$ is highly asymmetric and cannot be described by Poisson nor power law distributions.

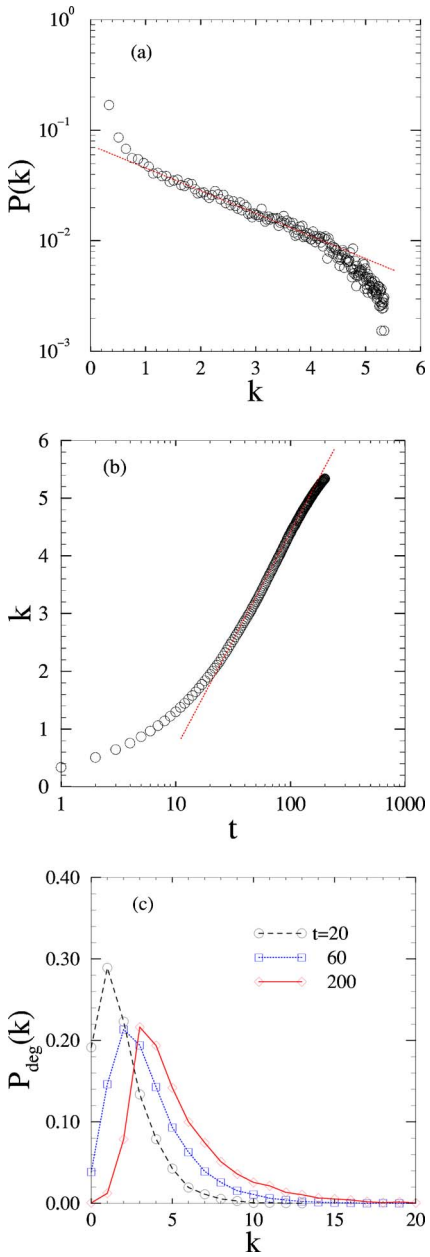

FIG. 5. (Color online) Simulation results for a two-dimensional network grown with the constraints of strictly forbidden bond crossings. $N=200$. (a) The measured probability of making an allowed bond as a function of the average coordination number of the network. (b) Time dependence of the mean degree of the network. t is in units of Monte Carlo steps/neuron. The straight lines are just guides to the eyes to show the exponential decay of $P(k)$ and logarithmic growth of $k(t)$. (c) Degree distribution functions of a growing 2D self-avoiding network.

V. BIOLOGICAL RELEVANCE AND OUTLOOK

In this work, by detailed analysis of the data on the SF of neuronal networks, a growth model describing the growth of neuronal connections is constructed that can explain the experimental observations. In this model, after the network has grown to a stage with reasonable connectivity, the growth is subsequently slowed down. Presumably this would allow the system to maintain a long time span to perform the necessary biological functions. If the system continues to grow rapidly at its initial accelerated pace, it will use up energy too soon, which is biologically unfavorable. Furthermore, if there are too many connections for each neuron, it may exceed the information capacity for a single neuron, resulting in low performance or lack of functioning. Thus, there appears to be an optimal effective number of connections for the best performance of the network. The growth of the neuronal connection network is rather well described, at least phenomenologically, by an early active search regime followed by a retarded diffusive regime. The microscopic mechanism leading to such a growth law is still not fully understood, although various studies have shown that electrical activities,

both spontaneous activity in early embryonic brains and experience-driven activity during the postnatal period, are essential for the growth of neuronal circuits [11]. It is possible that the neuron possesses some biochemical internal feedback device within the cell so that when it receives too many signals from other neurons (too many connections), the neuron will slow down further growth of connections. On the other hand, inhibitory cells would suppress the firing activity and presumably may have some effects on the retardation of connection growth in late stages of the culture. Their precise roles in the growth of neuronal connections is still unclear, but we believe they are deeply related to the homeostatic plasticity of neural development [10]. Hopefully well-controlled electrophysiological measurement can shed some light on this issue. But as illustrated in the present work, the more generic excluded volume and self-avoiding interactions can also be conveniently employed by the network to slow down the connectivity growth. Detailed experiments on the biochemical signaling pathway of the neurons would be able to clarify the picture. Many interesting further experiments are possible: such as using transfection of cell cultures with E-GFP plasmids to follow the growth of several individual neurons and to characterize their lengths and branching over time. Similarly, by immunostaining one can quantitatively measure the number of physical synapses as a function of time. Finally, direct electrophysiological measurements can be analyzed to provide information on the synaptic contributions over time to the cell firing. Some of these experiments are under way and will be reported in future.

Another interesting issue is the topological structure of such neuronal networks. It has been demonstrated that the neuronal network of *C. elegans* resembles a small-world network [15], while it has been suggested that the brain function network is a scale-free network [16]. But the brain function network is derived from fMRI data of the activity correlations between macroscopic regions (which consist of many neurons) of the brain, which is quite different from the direct synaptic connections between neurons. Preliminary studies of our quasi-two-dimensional cultured neuronal network *in vitro* do not indicate that the network is scale-free. More detailed studies on the topological structure of our networks are currently under way.

On the other hand, it is clear that a deeper understanding of the underlying mechanism of SF will be valuable in understanding not only the connectivity of the network, but also the interactions among the neurons during growth [17]. However, the basic mechanism of spontaneous SF is not clear and still under investigations [18]. Presumably, some detailed realistic dynamics of the neurons must be responsible for SF. A possible source of the induction of SF is the noise in the system, which can be due to thermal effects and ion channel activities, and growing network connectivity can provide a further increase in noise level. It has been demonstrated that regularly coupled excitable systems such as the FitzHugh-Nagumo neuron model, can be driven to synchronized states, oscillating with a well-defined frequency under a suitable noise level (coherence resonance) [19]. The synchronization is further enhanced by the many-body coupling effects among each individual element and the heterogeneity in the network [20]. However, it is still not clear how and why the neuronal network would self-tune the noise level of the system to achieve a synchronized state. Presumably some sort of feedback mechanism during the growth process would self-adjust the noise level of the system in an optimal way. This interesting issue awaits further experimental and theoretical investigations.

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