Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Temporal relation between neural activity and neurite pruning on a numerical model and a microchannel device with micro electrode array

Yohei Kondo [a], Yuichiro Yada [b, c], Tatsuya Haga [d], Yuzo Takayama [e], Takuya Isomura [f, c], Yasuhiko Jimbo [g, f], Osamu Fukayama [a], Takayuki Hoshino [a, *], Kunihiko Mabuchi [a]

[a] Department of Information Physics and Computing, The University of Tokyo, Tokyo 113-8656, Japan
[b] Department of Mechano-Informatics, The University of Tokyo, Tokyo 113-8656, Japan
[c] Research Fellow of the Japan Society for the Promotion of Science, Japan
[d] Brain Science Institute, RIKEN, Saitama 351-0198, Japan
[e] Biotechnology Research Institute for Drug Discovery, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8565, Japan
[f] Department of Human and Engineered Environmental Studies, The University of Tokyo, Tokyo 113-8656, Japan
[g] Department of Precision Engineering, The University of Tokyo, Tokyo 113-8656, Japan

ARTICLE INFO

Article history:
Received 2 March 2017
Accepted 17 March 2017
Available online xxx

Keywords:
Microchannel
Microelectrode array (MEA)
Critical period
Development
Culture device
Synapse elimination
Neurite pruning

ABSTRACT

Synapse elimination and neurite pruning are essential processes for the formation of neuronal circuits. These regressive events depend on neural activity and occur in the early postnatal days known as the critical period, but what makes this temporal specificity is not well understood. One possibility is that the neural activities during the developmentally regulated shift of action of GABA inhibitory transmission lead to the critical period. Moreover, it has been reported that the shifting action of the inhibitory transmission on immature neurons overlaps with synapse elimination and neurite pruning and that increased inhibitory transmission by drug treatment could induce temporal shift of the critical period. However, the relationship among these phenomena remains unclear because it is difficult to experimentally show how the developmental shift of inhibitory transmission influences neural activities and whether the activities promote synapse elimination and neurite pruning. In this study, we modeled synapse elimination in neuronal circuits using the modified Izhikevich's model with functional shifting of GABAergic transmission. The simulation results show that synaptic pruning within a specified period like the critical period is spontaneously generated as a function of the developmentally shifting inhibitory transmission and that the specific firing rate and increasing synchronization of neural circuits are seen at the initial stage of the critical period. This temporal relationship was experimentally supported by an in vitro primary culture of rat cortical neurons in a microchannel on a multi-electrode array (MEA). The firing rate decreased remarkably between the 18–25 days in vitro (DIV), and following these changes in the firing rate, the neurite density was slightly reduced. Our simulation and experimental results suggest that decreasing neural activity due to developing inhibitory synaptic transmission could induce synapse elimination and neurite pruning at particular time such as the critical period. Additionally, these findings indicate that we can estimate the maturity level of inhibitory transmission and the critical period by measuring the firing rate and the degree of synchronization in engineered neural networks.

1. Introduction

Synapse elimination and neurite pruning are essential processes for refinement of mammalian immature neuronal circuits during development. Excessive connections are eliminated in an activity-dependent manner, and these regressive events intensively occur in the early postnatal days known as the critical period [1–6]. However, what makes the temporal specificity of the critical period is unclear. One of the interesting events that coincide with the

* Corresponding author.
E-mail address: takayuki_hoshino@ipc.i.u-tokyo.ac.jp (T. Hoshino).

http://dx.doi.org/10.1016/j.bbrc.2017.03.082
0006-291X/

critical period is the maturation of GABAergic transmission. Recent studies have shown that GABAergic inhibition plays an important role in synapse elimination and neurite pruning [7,8]. Additionally, in the early postnatal days, the function of GABAergic transmission developmentally shifts from excitatory in immature neurons to inhibitory in adult neurons because the concentration difference between intra- and extracellular chloride ions is time-dependently reversed with the potassium chloride cotransporter (KCC) expression [9–12]. It is expected that this functional shift of GABAergic transmission affects neuronal activity and that the critical period occurs as a result of the activity in maturing inhibitory transmission [13]. In order to confirm these expectations, three indices should be observed: the maturity level of inhibitory transmission, the neural activity, and the neural connections. However, experimentally observing these indices is difficult, especially in regards to determining the first index.

Here, we addressed this issue by using both simulation of synaptic connected neural networks and an experimental approach of quantification of neurite length in vitro. We simulated neural activity and synapse elimination during the maturation of the inhibitory transmission by using a modified Izhikevich's model with time-dependent functional shifting of GABAergic transmission. Moreover, we developed a culturing device with a microchannel and multi-electrode array (MEA) that enables us to observe long-term neural activity and neurite pruning. Using both this device and the mathematical model, we discussed how the activity during the time-dependent shift of the inhibitory transmission leads to synapse elimination and neurite pruning.

2. Material & methods

2.1. Neuron model

We used the Izhikevich's model of a simple spiking neuron [14] shown by the following equations.

$$\frac{dv}{dt} = 0.04v^2 + 5v + 140 - u + I_{syn}, \tag{1}$$

$$\frac{du}{dt} = a(bv - u), \tag{2}$$

$$\text{if } v(t) \geq 30\ \text{mV},\ \text{then} \begin{cases} v \leftarrow c \\ u \leftarrow u + d \end{cases}, \tag{3}$$

here v, whose unit is mV, denotes the membrane potential of the neuron, and u denotes its recovery variable. The unit of time corresponds to ms. I_{syn} denotes the total synaptic current.

The dimensionless parameters a, b, c, and d determine firing patterns. If the neuron is excitatory, the parameters are set by the following rules.

$$(a,\ b) = (0.02,\ 0.2), \tag{4}$$

$$(c,\ d) = (-65,\ 8) + (15,\ -6)r^2, \tag{5}$$

here r is a random number between 0 and 1. When $r = 0$, it means regular spiking neurons, and when $r = 1$, it means chattering neurons.

If the neuron is inhibitory, the parameters are set by the following rules.

$$(a,\ b) = (0.02,\ 0.25) + (0.08,\ -0.05)r\ , \tag{6}$$

$$(c,\ d) = (-65,\ 2), \tag{7}$$

here r is a random number between 0 and 1. When $r = 0$, it means low-threshold spiking neurons, and when $r = 1$, it means fast spiking neurons.

2.2. Synaptic model

On the one hand, immature GABAergic transmission is excitatory because intracellular chloride ion concentration is higher than extracellular concentration at the immature neuron, resulting in an efflux of chloride ions that leads to depolarization of the neuron when ligand-gated chloride channels open. On the other hand, the neuron chloride ion concentrate becomes lower due to sufficient expression of the potassium chloride cotransporter (KCC) at a mature neuron, and then the function of GABAergic transmission results in chloride influx, which leads to hyperpolarization of the neuron. This phenomenon can be modeled by a chloride ion equilibrium potential shift. In fact, the chloride ion equilibrium potential shift in rat cerebellar Purkinje neurons has been reported [9].

We therefore introduced the maturity coefficient of inhibitory transmission ($m,\ 0 \leq m \leq 1$) to the conventional synaptic current model proposed by Izhikevich [14] as shown in the following maturing rule.

$$I_{syn} = g_{AMPA}(0 - v) + g_{NMDA}\frac{\left[\frac{v+80}{60}\right]^2}{1 + \left[\frac{v+80}{60}\right]^2}(0 - v) + g_{GABA_A}(-70m - v) + g_{GABA_B}(-90m - v), \tag{8}$$

The term of $-70\ m$ and $-90\ m$ express the equilibrium potential in the GABAergic transmissions by $GABA_A$ and $GABA_B$, respectively, and their units are in mV. If $m = 1$, the equation represents a mature synaptic current equivalent to the conventional Izhikevich's neuron spiking model. Conversely, if $0 \leq m < 1$, the equation represents an immature synaptic current. By increasing the coefficient m from 0 to 1 continuously, we can simulate the functional shift of the GABAergic transmission from excitatory to inhibitory.

2.3. Synaptic plasticity model

We applied the spike-timing dependent plasticity (STDP) model [14] as a synaptic plasticity model described by the following equations.

if $t_{pre} < t_{post}$ Long-Time Potentiation,

$$\text{then}\ \frac{dc_{ij}}{dt} \leftarrow \frac{dc_{ij}}{dt} + A_+ \exp\left(\frac{t_{pre} - t_{post}}{\tau_+}\right), \tag{9}$$

if $t_{pre} > t_{post}$ Long-Term Depression,

$$\text{then}\ \frac{dc_{ij}}{dt} \leftarrow \frac{dc_{ij}}{dt} - A_- \exp\left(\frac{t_{pre} - t_{post}}{\tau_+}\right), \tag{10}$$

here, the variable c_{ij} denotes the synaptic strength between neurons i and j. Parameters $A_+ = 0.004$, $\tau_+ = 15$ ms, and $A_- = 0.004$, $\tau_- = 20$ ms. Long-term depression (LTD) has been reported to promote synapse elimination, and synapses that have low synaptic strength tend to be eliminated selectively [15–17].

Then, we added selectivity for synapse elimination by determining the threshold of synaptic strength ($0 \leq c_{ij} \leq 0.5$) in the above STDP model as the following elimination rule.

Please cite this article in press as: Y. Kondo, et al., Temporal relation between neural activity and neurite pruning on a numerical model and a microchannel device with micro electrode array, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.03.082