Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Synchronous firing patterns of induced pluripotent stem cell-derived cortical neurons depend on the network structure consisting of excitatory and inhibitory neurons

Shoko Iida [a, *], Kenta Shimba [a], Koji Sakai [a], Kiyoshi Kotani [b, c], Yasuhiko Jimbo [a]

[a] The Department of Precision Engineering, School of Engineering, The University of Tokyo, Room 1122, Faculty of Engineering Building 14, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
[b] The Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan
[c] The PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

ARTICLE INFO

Article history:
Received 21 April 2018
Accepted 25 April 2018
Available online xxx

Keywords:
Cerebral cortical neuron
Excitatory/inhibitory balance
Induced pluripotent stem cell
Microelectrode array
Synchronized burst

ABSTRACT

The balance between glutamate-mediated excitation and GABA-mediated inhibition is critical to cortical functioning. However, the contribution of network structure consisting of the both neurons to cortical functioning has not been elucidated. We aimed to evaluate the relationship between the network structure and functional activity patterns in vitro. We used mouse induced pluripotent stem cells (iPSCs) to construct three types of neuronal populations; excitatory-rich (Exc), inhibitory-rich (Inh), and control (Cont). Then, we analyzed the activity patterns of these neuronal populations using microelectrode arrays (MEAs). Inhibitory synaptic densities differed between the three types of iPSC-derived neuronal populations, and the neurons showed spontaneously synchronized bursting activity with functional maturation for one month. Moreover, different firing patterns were observed between the three populations; Exc demonstrated the highest firing rates, including frequent, long, and dominant bursts. In contrast, Inh demonstrated the lowest firing rates and the least dominant bursts. Synchronized bursts were enhanced by disinhibition via $GABA_A$ receptor blockade. The present study, using iPSC-derived neurons and MEAs, for the first time show that synchronized bursting of cortical networks in vitro depends on the network structure consisting of excitatory and inhibitory neurons.

1. Introduction

The cerebral cortex contains both glutamatergic excitatory neurons and GABAergic inhibitory neurons. The balance of excitation and inhibition is critical to maintaining cortical functioning [1–3]. Previous studies show that imbalances in excitation and inhibition are related to neurological disorders [3,4]. The imbalances are also related to abnormality of cortical network structure including excitatory and inhibitory synaptic density and ratio of excitatory to inhibitory neuronal populations [5,6]. However, the contribution of cortical network structure to functioning has not been elucidated because of the difficulty in research using animal models; mutations regarding neuronal development, network structure, and neuron type ratios can produce early mortality [7].

A previous study investigated the relationship between the ratio of excitatory to inhibitory neurons in vitro and neural network activity patterns. Chen and Dzakpasu combined neurons from the hippocampus and striatum, and recorded extracellular potentials using microelectrode arrays (MEAs); they found that activity patterns differed with alterations to the excitatory/inhibitory ratio [8]. However, it has not been determined whether similar phenomena are observed for cortical neurons. Differing results may occur because cortical, hippocampal, and striatal neurons exhibit different gene expression patterns that may affect neuronal activity [9,10].

Pluripotent stem cells (PSCs) can be useful tools to alter cortical network structure in vitro. Various methods of neural induction

Abbreviations: BMI, bicuculline methiodide; Cont, control; Cyc, cyclopamine; ESC, embryonic stem cell; Exc, excitatory-rich population; IBI, inter-burst interval; Inh, inhibitory-rich population; iPSC, induced pluripotent stem cell; MAP2, microtubule-associated protein 2; MEA, microelectrode array; NSC, neural stem cell; PSC, pluripotent stem cell; SAG, smoothened agonist; Shh, sonic hedgehog; VGAT, vesicular GABA transporter.

* Corresponding author.
E-mail address: iida@neuron.t.u-tokyo.ac.jp (S. Iida).

https://doi.org/10.1016/j.bbrc.2018.04.197
0006-291X/

have been reported to form the cortical cells [11,12]. Moreover, a sonic hedgehog (Shh) antagonist or an agonist induces selective differentiation of excitatory or inhibitory neurons, respectively [11,13]. Thus, it is possible to produce excitatory or inhibitory-rich populations using PSCs. Previous studies have also demonstrated that PSC-derived neurons exhibit synchronized bursting activity [14,15], similar to activities observed during in vivo recordings [2] or MEA recordings using dissociated cortical neurons [16,17]. Therefore, PSC-derived neurons are also appropriate for evaluating neuronal network activity.

The present study combined PSC-derived neurons with MEAs to elucidate the contribution of the network structure consisting of excitatory and inhibitory neurons to cortical activity patterns. First, we constructed several types of cortical neuronal populations with different network structures, using selective induction of mouse induced pluripotent stem cells (iPSCs). Second, we analyzed the functional activity patterns of these populations using MEAs.

2. Materials and methods

2.1. Cell culture

The mouse iPSC line (iPS-Stm-FB/gfp-99-1 [18,19]) was provided by RIKEN Bio Resource Center, through the Project for Realization of Regenerative Medicine and the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan. iPSCs were grown on a mytomycin C-treated SNL feeder layer and maintained in DMEM (Thermo Fisher, Waltham, MA, USA) supplemented with 15% v/v fetal bovine serum (Biological Industries, Haemek, Israel), 0.1 mM non-essential amino acids (NEAA; Sigma, St. Louis, MO, USA), 0.1 mM 2-mercaptoethanol (2-ME; Sigma), and 1000 U/mL leukemia inhibitory factor (Wako, Osaka, Japan).

We used a previously described method of neural differentiation [13]. iPSCs were dissociated into single cells with 0.25% trypsin-EDTA (Thermo Fisher) and 1.0×10^3 cells/100 μL/well were seeded into 96-well low cell adhesion plates (Sumitomo Bakelite, Tokyo, Japan), in neural induction medium. The neural induction medium consisted of Glasgow minimum essential medium (Sigma) supplemented with 10% knockout serum replacement (Thermo Fisher), 2 mM GlutaMAX (Thermo Fisher), 1 mM sodium pyruvate (Thermo Fisher), 0.1 mM NEAA, and 0.1 mM 2-ME. Six days after neural induction, cell aggregates were trypsinized into single cells and plated onto a culture dish coated with polyethyleneimine (Sigma)/laminin (Thermo Fisher) with N2 medium at a cell density of 4.0×10^3 cells/mm^2. Day 0 was defined as the time point when iPSC derivatives were transferred to a culture dish. The N2 medium consisted of DMEM/F12 + GlutaMAX (Thermo Fisher) supplemented with N2 supplement (Thermo Fisher), 1 mM sodium pyruvate, 5 μg/mL insulin (Sigma), 0.1 mM NEAA, and 0.1 mM 2-ME [12]. The medium was changed to a 1:1 mixture of N2 medium and B27 medium at day 6. The B27 medium consisted of Neurobasal (Thermo Fisher) supplemented with B27 supplement (minus vitamin A; Thermo Fisher), 100 U/mL-100 μg/mL penicillin-streptomycin (Thermo Fisher), and 2 mM GlutaMAX.

For selective differentiation to cortical excitatory and inhibitory neurons, a Shh antagonist and a Shh agonist were added to the media, respectively [13]. For the excitatory neuron-rich population (Exc), 5 μM of the Shh antagonist cyclopamine (Cyc; Wako) was added from day -3 to 2 (3–8 d after induction). For the inhibitory neuron-rich population (Inh), 3 nM of the smoothened agonist (SAG; AdipoGen, San Diego, CA, USA) was added from day -3 to 0 (3–6 d after induction) and 10 nM was added from day 0–2 (6–8 d after induction). For the control condition (Cont), neither Cyc nor SAG was added.

2.2. Immunocytochemistry and quantification

Immunocytochemistry was performed as previously described [20]. Primary and secondary antibodies were used at the following dilutions: anti-nestin (1:1000; rabbit; Sigma), anti-microtubule-associated protein 2 (MAP2; 1:500; mouse; Merck Millipore, Darmstadt, Germany), anti-βIII-tubulin (1:1000; rabbit; Sigma), anti-vesicular GABA transporter (VGAT; 1:1000; guinea pig; Frontier Institute, Hokkaido, Japan), Alexa fluor 488 anti-mouse IgG (1:500; goat; Thermo Fisher), Alexa fluor 546 anti-rabbit IgG (1:500; goat; Thermo Fisher), and Alexa fluor 633 anti-guinea pig IgG (1:500; goat; Thermo Fisher) antibodies. Nuclei were counterstained with 300 nM 4′,6-diamidino-2-phenylindole (DAPI; Sigma).

ImageJ software was used for quantitative analyses. For quantification of the proportions of cells expressing nestin, iPSC derivatives were fixed at day 3 and a minimum of 550 cells from each independent neural induction were counted. This process was repeated three times for each of the three types of neuronal populations (Exc, Inh, and Cont). Data are shown as means of the proportions from three independent experiments.

For quantitative evaluation of GABAergic neuronal differentiation, iPSC derivatives were fixed at day 13 and stained with anti-VGAT antibody. First, five fluorescence images were collected of random 225 μm × 225 μm areas, using a confocal laser scanning microscope (LSM510; Carl Zeiss, Oberkochen, Germany). Next, binary images were created of βIII-tubulin+ and VGAT + regions, with manually determined thresholds. Finally, we calculated the proportion of βIII-tubulin/VGAT + co-expression region to βIII-tubulin + region in the five images. These processes were repeated three times, for each independent neural induction. Data are shown as means of the proportions from three independent experiments.

2.3. Extracellular recordings

Conventional 8 × 8 MEA substrates with electrodes measuring 50 μm × 50 μm and with an interelectrode distance of 250 μm were fabricated [20]. Neural activity was recorded from MEAs, using a sampling frequency of 25 kHz and a previously reported system [21]. Spontaneous activity of iPSC-derived neurons was recorded for 20 min on day 5, followed by recordings every week. The number of samples is described in Supplementary Table I.

2.4. Pharmacological treatment

The $GABA_A$ receptor antagonist, 1(S),9(R)-(-)-bicuculline methiodide (BMI; Sigma), was added to the culture medium at a final concentration of 10 μM. To avoid handling effects, activity recordings were started 5 min after BMI application, and performed for 15 min.

2.5. Data analyses

Neuronal activities (spikes) were detected as follows; for each electrode, the standard deviation (SD) of the amplitude (σ_{amp}) was calculated and negative peaks crossing a $-5\sigma_{amp}$ threshold were detected as spikes.

Synchronized activity of neuronal populations was analyzed by detecting network bursts. Network bursts were defined as episodes with synchronous firing from multiple electrodes [22]. Samples with synchronized bursting were manually selected and used for burst detection. First, spikes with firing rates higher than 0.1 spikes/s were partitioned into 10 ms time bins. Second, the product of the number of spikes and the number of active electrodes was calculated for each bin. Third, the threshold for burst detection was defined as 10 or the 98th percentile of the product for all bins,

Please cite this article in press as: S. Iida, et al., Synchronous firing patterns of induced pluripotent stem cell-derived cortical neurons depend on the network structure consisting of excitatory and inhibitory neurons, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.197