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Electrophysiological and morphological classification of layer 1 neocortical somatosensory neurons

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Neocortical neurons, primarily in cortical layers 2-6, can be classified according to their electrophysiological, morphological, and neuromodulator content. Layer 1 of the neocortex contains a sparse number of non-pyramidal, GABAergic neurons. Previous studies have described two neuron types in layer 1, Cajal-Retzius and neuroglial cells. However, little is known about other neurons existing in layer 1, such as axon-descending cells and various multi-polar neurons. We sought to do a comprehensive examination of layer 1 neurons to identify distinguishing electrophysiological and morphological features of each neuronal type. We used in vitro whole-cell patch-clamp recording with concurrent biocytin labeling for visually identified layer 1 neurons from the primary somatosensory cortex of 12- to 16-day old rats. After recording and histochemical processing, three-dimensional reconstructions of the individual neurons were made. These two techniques, in combination, allowed us to obtain precise physiological and morphological data for each individual neuron. We proposed three new morphological neuron types in addition to two known types, with quantitative qualification. Each cell type had distinctive electrophysiological biases, while each firing type had no morphological bias. Thus five subtypes in each firing pattern could be made. By examining gene expression of these cells, we will further specify isolated classes of neurons in neocortical layer 1 and begin to determine their role in the neocortical microcircuit.

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Plasticity in single-cell-based reconstructed neuronal network pattern

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To examine the plasticity in local neuronal network pattern at single-cell level is significance for doing bionic applications and investigating the fundamental rule of learning and memory. In recent years, patterning neuronal networks in culture using micro-fabrication techniques is done, but detection of the plasticity in patterned neural networks is not proved. We then demonstrated the electrophysiological detection of the plasticity in single-cell-based reconstructed neuronal network pattern. To record the firing at multiple cells simultaneously for long term and topographically control the cells position and their connections, we have developed an on-chip multi-electrodes array (MEA) measurements system with an agarose microchamber (AMC) array. AMC was fabricated by photothermal etching where a portion agarose layer is melted with a 1480 nm infrared laser beam. Using this method, we formed a single-cell-based neural network pattern of Rat hippocampal cells with an AMC array without cells escaping from the electrode positions in the microchamber, and measured the activity change and histeresis using tetanic stimulations. As a result, we detected the network activity changes in the speed of evoked responses and the burst time before and after tetanic stimulation, and that effects of tetanus left for about 24 hours. In addition, the preservation and fadeaway in tetanized activities were suggested from the burst waveform. We hope that these results provide the progress for understanding the plasticity in local neural circuit.

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RNG105 in neuronal RNA granules: Involvement in local translation and synapse formation

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Local translation in neuronal dendrites is an important basis for activity-dependent synaptic modifications, and is responsible for long-term synaptic plasticity. RNA granules, which consist of clusters of ribosomes and RNAs, play key roles in transport of mRNAs to the dendrites and local translational control. RNG105 (RNA granule protein 105) is a novel RNA-binding protein in the RNA granules in neuronal dendrites. RNG105 has ability to bind directly to mRNAs and repress translation. Dissociation of RNG105 from the RNA granule is induced by BDNF (brain-derived neurotrophic factor), a growth factor responsible for synaptic plasticity. The dissociation of RNG105 is related with the induction of local translation of mRNAs located in the RNA granules. These findings suggest that RNG105 is a translational repressor in the RNA granule and becomes dissociated from the granule by synaptic stimulation, which cancels the translational repression of the mRNAs in the RNA granule at the stimulated synaptic area.

We produced RNG105 knockout mice. They were neonatally lethal without any breathing. Although the knockout neurons developed neuronal processes normally, synaptic formation was abnormal and a neural network was fragile in the knockout mice. These results suggest possible involvement of RNG105 in a synaptic function(s).

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On-chip regulation of neural stem cell differentiation

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On-chip regulation of neural stem cell differentiation

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Neural stem cells (NSCs) have an ability to proliferate and differentiate into glia or neuron. It is known that the differentiation of NSCs is affected by two major environmental factors. First is a liquid factor. Second is a physical factor like a cell-cell contact. It is important for neural differentiation to reveal the effects of factors. But each effects are difficult to consider independently. Our purpose is to evaluate the effect of each factors in neural differentiation. It is need for our purpose to use experimental system which enables us to evaluate each factors independently. We use the agarose micro fabricated technique; infrared focused laser melts the agarose. This system realize the number of cell and cell-cell contact controled. We estimate the only effect of liquid factor using this system. NSCs derived from mouse striatum have a tendency to differentiate into neuron under the existence of retinoic acid and forskolin in the culture. Isolated NSC in the agarose micro chamber differentiates into neuron as well as dispersed culture. These results indicates cell-cell contact can be restricted and single NSC differentiation can be traced using the agarose micro fabricated technique. The condition of retinoic acid and forskolin coexisted as a liquid factor is effective for differentiation into neuron.